A ribosomal protein that is immunologically conserved in archaebacteria, eubacteria and eukaryotes

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A ribosomal protein which exhibits cross-reaction between organisms belonging to the eubacterial, archaebacterial and eukaryotic groups was studied by immunoblotting analysis. It was identified as the equivalent of the *E. coli* ribosomal protein L2.

Eubacteria *Archaebacteria* *Eukaryote* *Ribosomal protein L2* *Immunoblotting* *Evolution*

1. INTRODUCTION

Cross-reactions between ribosomal proteins from archaebacteria, eubacteria and eukaryotes have been analysed by means of the immunoblotting technique [1]. It was found that the immunological relatedness was group-specific and therefore closely paralleled the relationship delineated by the 16 S rRNA oligonucleotide data [2]. The number of cross-reacting proteins was higher and the strength of their cross-reaction more intense when the organisms under comparison belonged to the same primary kingdom [1]. Only very few cross-reacting proteins were detected when organisms from different kingdoms were compared.

There was, however, one striking exception: A ribosomal protein of approx. 30 kDa cross-reacted intensively in the immunoblots of most of the organisms from all 3 primary kingdoms [1]. Since the strong immunological conservation of this protein points to an important structural and/or functional role in the ribosome, we have determined its identity.

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2. MATERIALS AND METHODS

2.1. *Organisms and growth conditions*

The following organisms were used:

Eubacteria: *Escherichia* (*E.*) *coli; Pseudomonas* (*Ps.*) *aeruginosa; Rhodopseudomonas* (*Rps.*) *sphaeroides; Clostridium* (*C.*) *butyricum*, DSM 552; *Streptomyces* (*Str.*) *griseus*.

Archaebacteria: *Methanobacterium* (*Mb.*) *thermoautotrophicum*, DSM 1054; *Mb. bryantii*, DSM 863; *Mb. formicicum*, DSM 1312; *Methanococcus* (*Mc.*) *vannielii*, DSM 1224; *Methanospirillum* (*Msp.*) *hungatei*, DSM 864; *Methanosarcina* (*Ms.*) *barkeri*, DSM 1232; *Halobacterium* (*Hb.*) *marismortui; Sulfolobus* (*S.*) *solfataricus*, DSM 1616; *Thermococcus* (*Tc.*) *celer*, DSM 2476; *Desulfurococcus* (*D.*) *mobilis*, DSM 2161; *Thermoproteus* (*Tp.*) *tenax*, DSM 2078.

Eukaryotes: *Saccharomyces* (*Sacch.*) *cerevisiae* A364A; *Podospora* (*P.*) *anserina*.

Growth conditions have been described [3].

2.2. *Preparation of ribosomes, ribosomal subunits and ribosomal proteins*

The preparation of ribosomes and ribosomal subunits has been described [3–5]. Ribosomal pro-

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tein L2 of *E. coli* was isolated as in [6]. Cleavage with cyanogen bromide was performed as in [7].

2.3. *Immunological techniques*

(i) Rabbits were immunized with purified ribosomal protein L2. IgG was prepared either by gel filtration or by chromatography on staphylococcal protein A–Sepharose; specific antibodies were purified by affinity chromatography on CNBr-activated agarose to which pure protein L2 had been coupled [8].

(ii) Immunoblotting analysis: The immunoblotting procedure employed was essentially that of [9] and [10] with the modifications in [1]. In principle, total ribosomal proteins from 50 S subunits (TP50) or 70 S ribosomes (TP70) were separated either on SDS-polyacrylamide gradient gels (15–25%) as in [11] or on two-dimensional polyacrylamide gels as in [12]. The separated ribosomal proteins were electrophoretically transferred to nitrocellulose sheets. After staining with amido black, the sheets were reacted with a suitably diluted antibody solution and then washed extensively to remove unspecifically adsorbed antibodies. Cross-reacting antibodies were detected by treatment of the nitrocellulose with *Staphylococcus aureus* A protein (iodinated with ^{125}I) and by subsequent autoradiography.

(iii) To check the specificity of the immunoblotting reaction, a control serum was prepared as follows: 1 ml of the anti-L2 serum diluted 1:5 in buffer A (10 mM Tris–Cl, pH 7.4, 0.2% SDS, 0.5% Triton X-100, 0.9% NaCl, 1% bovine serum albumin) was incubated with 10 μg purified L2 protein from *E. coli* for 1 h at 37°C. After a further 60 min at 0°C the immunoprecipitate formed was removed by centrifugation; the supernatant was used as control serum.

3. RESULTS AND DISCUSSION

A first indication for the identity of the cross-reacting protein was obtained when ribosomal proteins from *E. coli* that had been separated by two-dimensional electrophoresis were reacted using the immunoblotting technique with antisera directed against total ribosomal proteins from *Mb. bryantii* or from yeast. There was a distinct reaction of protein L2 from *E. coli* with these sera (not shown).

Further experiments were then carried out with antisera specific for *E. coli* L2 or with specific antibody purified from these sera by immunoaffinity chromatography. These antibodies were used to study the immunological relationship of the *E. coli* L2 protein to ribosomal proteins of representative organisms from eubacteria, archaebacteria and eukaryotes.

Fig.1A shows the results obtained with one-dimensionally separated ribosomal proteins from different eubacteria. Although belonging to very distant taxonomic groups, ribosomes from all these organisms contained one protein which reacted with almost equal intensity with anti-L2 from *E. coli*. Comparison with the migration of a molecular mass standard (not shown) revealed that the molecular mass of the cross-reacting proteins was close to 30 kDa. The immunoblotting data shown in fig.1A support and extend results from immuno-double-diffusion experiments in which a cross-reacting of antibodies against *E. coli* L2 was demonstrated with ribosomal proteins from several *Bacillus* species [13]. Authors in [18] have determined an L2 homologue in ribosomes from spinach chloroplasts by immunological methods. Independently, cross-reaction of *E. coli* L2-specific antibodies with a ribosomal protein from spinach chloroplasts has been observed [20,21], using the same antisera as those used here.

Fig.1B gives the results obtained with proteins from various archaebacteria, separated by one-dimensional electrophoresis. Immunological relationship was detected with one protein from members of the order Methanobacteriales (*Mb. thermoautotrophicum, Mb. formicicum* and *Mb. bryantii*) and with a ribosomal protein from *Mc. vannielii* (order Methanococcales). Weak cross-reactions were obtained with one ribosomal protein from *Msp. hungatei, Ms. barkeri* (both order Methanomicrobiales) and from *Hb. marismortui* (not shown). The antibodies either did not react or else reacted only very weakly with ribosomal proteins from *S. solfataricus, Tp. tenax, D. mobilis* and *Tc. celer* (not shown).

When ribosomal proteins of cytoplasmic ribosomes from eukaryotes were tested, the antibodies reacted with one ribosomal protein from *Sacch. cerevisiae* and from *P. anserina* (fig.1C) but showed no reaction with the ribosomal proteins of rat liver or chicken liver. Antibody preparations specific for *E. coli* protein L2, raised in a total of

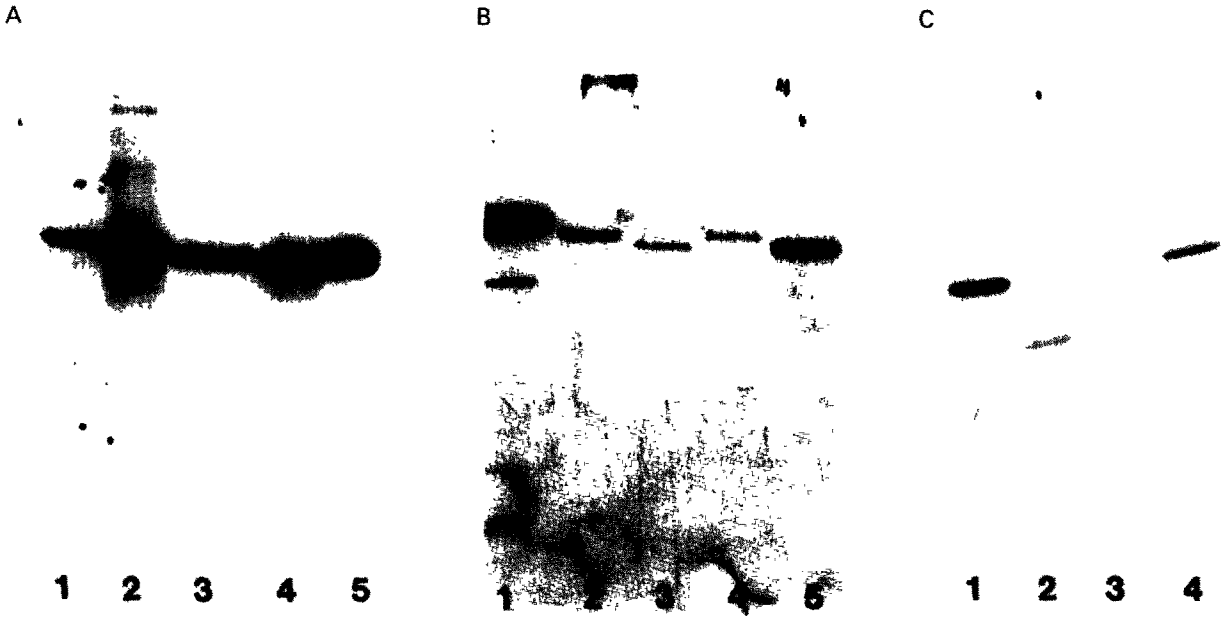

Fig.1. (A) Autoradiography of an immunoblotting experiment with antibodies directed against ribosomal protein L2 from *E. coli* which were purified by immunoaffinity chromatography. The following antigens were separated on an SDS gradient gel: (1) total ribosomal proteins (TP70) from *St. griseus*; (2) TP70 from *C. butyricum*; (3) TP70 from *Rps. sphaeroides*; (4) TP70 from *Ps. aeruginosa*; (5) TP50 from *E. coli*. (B) Immunoblotting experiment with antibodies pooled from 7 different antisera specific for *E. coli* L2. The antigens used were: (1) TP50 from *E. coli*; (2) TP50 from *Mb. thermoautotrophicum*; (3) TP50 of *Mb. bryantii*; (4) TP70 from *Mb. formicicum*; (5) TP50 from *Mc. vannielii*. The weaker (fast migrating) band in lane 1 could be a proteolytic L2 product; it was not seen in other preparations (see A, lane 5). (C) Immunoblotting experiment as in A. The antigens used in this experiment were: (1) TP60 from *Sacch. cerevisiae*; (2) TP60 from rat liver; (3) TP60 from chicken liver; (4) TP80 from *P. anserina*.

7 different animals, all gave similar results to those described above.

Next, the size of the L2-equivalent proteins in relation to the other large subunit proteins was examined. Fig.2 gives the one-dimensional separation of large subunit proteins from *E. coli, Saccharomyces cerevisiae, Methanococcus vannielii*, and *Methanobacterium bryantii*; the protein immunologically homologous to *E. coli* L2 is indicated. L2 is the largest protein in the *E. coli* 50 S subunit and also in the 50 S subunits of other eubacteria (not shown). The homologous protein from yeast and from archaebacteria (fig.2) is significantly smaller. Moreover, one or more proteins are present in the large subunit of archaebacteria and of yeast possessing a distinctly higher molecular mass.

Finally, the L2 equivalent proteins were identified in the two-dimensional electropherograms of large subunit proteins from yeast and two methanogens. Fig.3 shows the homologous reaction and, as an example, the immunoblot delivered by TP50 from *Methanococcus vannielii*. Only one protein reacted in both cases. Fig.4 then identifies in stained two-dimensional separations of TP50 the proteins reacting with anti-L2 (*E. coli*) antibodies. The L2-equivalent protein in yeast is protein YL6 in the nomenclature of [14], that of *Methanococcus vannielii* is ML7 (for nomenclature see [5]). It is interesting to compare the relative spatial positions of the L2-equivalents in the 4 electropherograms of fig.4: The eubacterial L2 protein (see also [15,16]) belongs to the most basic group of large subunit proteins; the archaebacterial and yeast equivalents are, on the other hand, relatively less basic.

In view of the known tendency of immunoblotting experiments to deliver unspecific reactions (discussion [1]) all the experiments presented were also performed with sera from which specific anti-

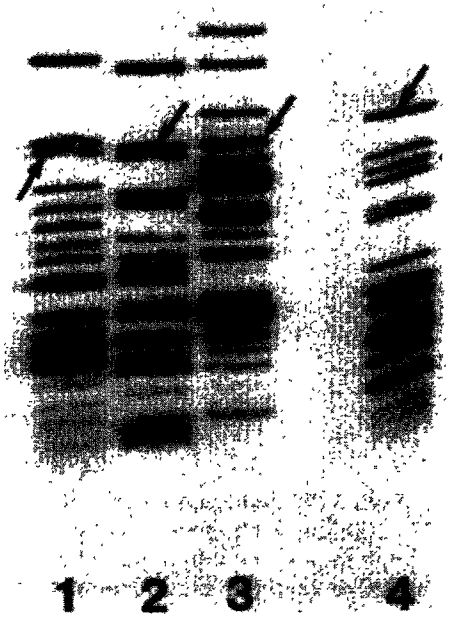

Fig.2. Total ribosomal proteins from the 50 S (60 S) subunit of (1) *Mb. bryantii*; (2) *Mc. vannielii*; (3) *Sacch. cerevisiae* and (4) *E. coli* were separated on an SDS gradient gel and transferred electrophoretically to a nitrocellulose sheet. The proteins were stained with amido black. The 'L2-proteins' determined by immunoblotting are indicated with arrows.

L2 immunoglobulins had been removed by preabsorption with purified *E. coli* ribosomal protein L2. In each case the cross-reaction disappeared.

We next tried to answer the question whether the cross-reaction observed is due to a single strongly conserved determinant on the L2 proteins, or whether it is due to many different determinants whose amino acid sequences are sufficiently conserved to allow reaction with *E. coli* anti-L2 antibodies. To answer this, the L2-equivalent proteins were excised from two-dimensional electropherograms and cleaved with CNBr. The resulting peptides were separated on one-dimensional SDS gels and their cross-reaction with anti-L2 from *E. coli* was studied by immunoblotting. Immunoblots of CNBr-peptides of *E. coli* L2 showed a number of reacting peptides, and the size and number of these peptides was approximately consistent with that expected from the positions of methionine in the L2 sequence [17]. Immunoblots

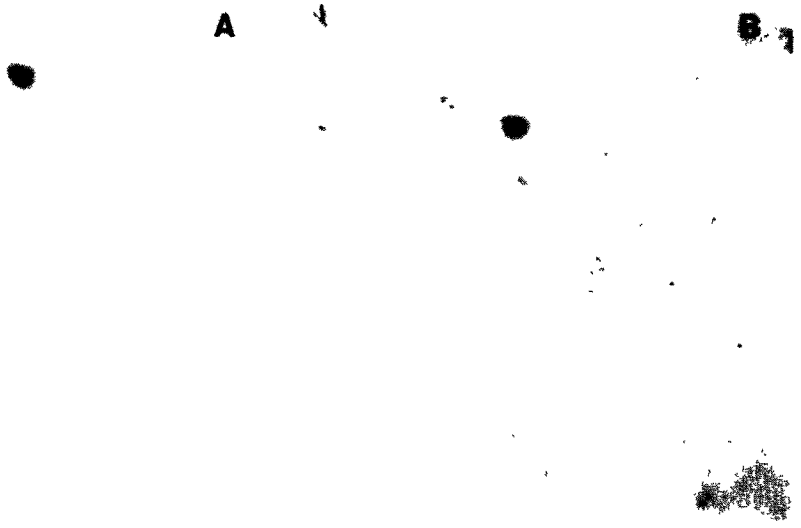

Fig.3. Autoradiogram of an immunoblot showing the reaction of anti (*E. coli L2*) antibodies with electrophoretically separated TP50 from *E. coli* (A) and *Mc. vannielii* (B).

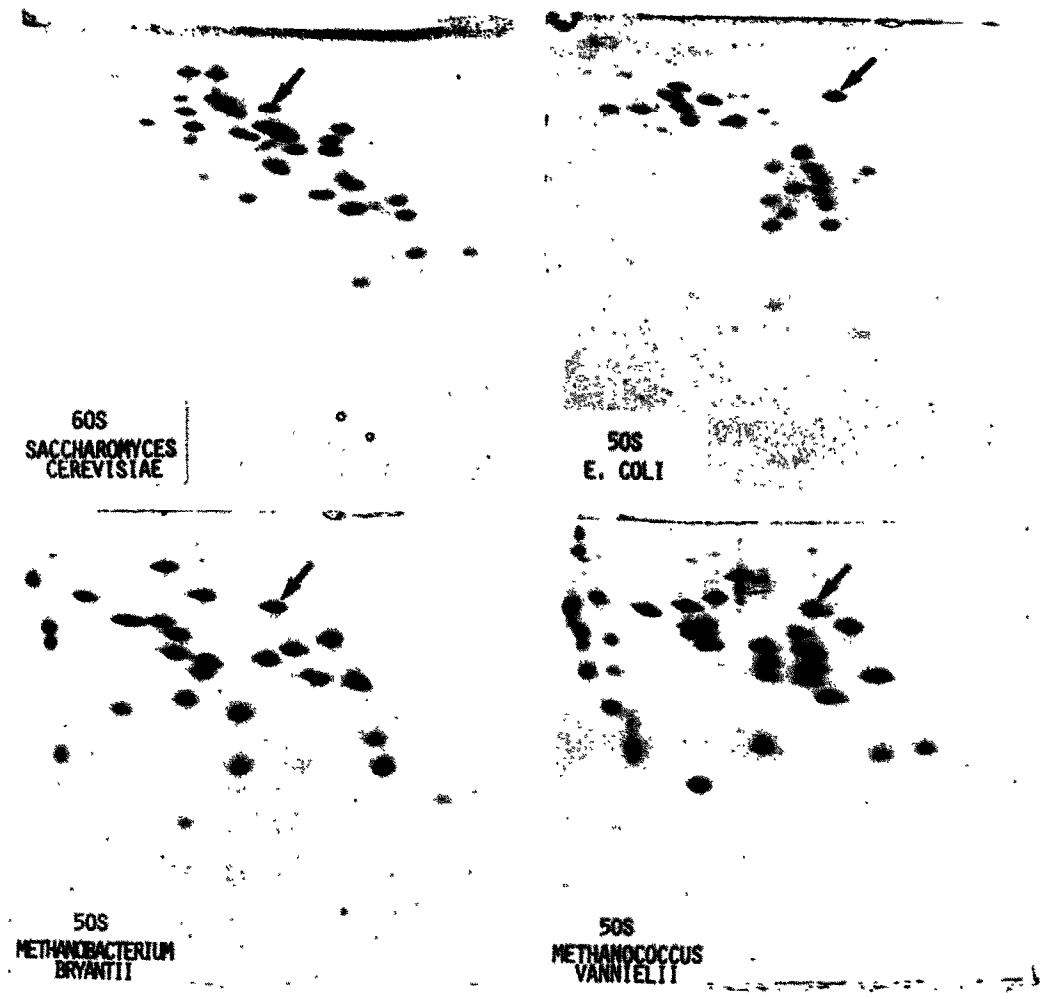

Fig.4. Two-dimensional electropherograms of total ribosomal proteins of the large subunit from *E. coli, Mb. bryantii, Mc. vannielii* and *Sacch. cerevisiae*. Proteins cross-reacting with antiserum directed against *E. coli* L2-protein are marked by arrows.

from CNBr-peptides of the L2-equivalents from yeast and from methanogenic organisms yielded either no cross-reaction at all or only a weak reaction with one or two cleavage products. Although we cannot exclude the possibility that sequential and/or conformational determinants are destroyed by the CNBr-treatment, our interpretation of these results is that the immunological relatedness of the L2 proteins is due to the sum of many determinants which individually are only weakly reactive with the antibody.

In summary, under the assumption that our antisera contain antibodies against all ribosomal proteins, L2 appears to be one of the most strongly conserved proteins of the ribosome. Homologous counterparts have now been identified by immunological means in several eubacteria, in the methanogen/halophile branch of archaebacteria, in the cytoplasmic ribosomes of lower eukaryotes and in chloroplasts [18,20,21]. L2, therefore, offers itself as a suitable tool for sequence studies to gain information on the evolution of the ribosome. Its conservation may indicate an important structural or functional role; it is interesting in this con-

nection that biochemical evidence exists that in *E. coli* L2 may be a component of the peptidyltransferase center [19].

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REFERENCES

[1] Schmid, G. and Böck, A. (1984) System. Appl. Microbiol. 5, 1–10.
[2] Balch, W.E., Fox, G.E., Magrum, J., Woese, C.R. and Wolfe, R.S. (1979) Microbiol. Rev. 43, 260–296.
[3] Schmid, G. and Böck, A. (1981) J. Bacteriol. 147, 282–288.
[4] Schmid, G. and Böck, A. (1982) Mol. Gen. Genet. 185, 498–501.
[5] Schmid, G. and Böck, A. (1982) Zbl. Bakt. Hyg., I. Abt. Orig. C 3, 347–353.
[6] Hindennach, I., Kaltschmidt, E. and Wittmann, H.G. (1971) Eur. J. Biochem. 23, 12–16.
[7] Stöffler-Meilicke, M., Epe, B., Wolley, P., Lotti, M., Littlechild, J. and Stöffler, G. (1984) Mol. Gen. Genet., in press.
[8] Tischendorff, G.W. and Stöffler, G. (1975) Mol. Gen. Genet. 142, 193–208.
[9] Towbin, H., Staehelin, T. and Gordon, J. (1979) Proc. Natl. Acad. Sci. USA 76, 4350–4354.
[10] Howe, J.G. and Hershey, J.W.B. (1981) J. Biol. Chem. 256, 12836–12839.
[11] Laemmli, U.K. (1970) Nature 227, 680–685.
[12] Geyl, D., Böck, A. and Isono, K. (1981) Mol. Gen. Genet. 181, 309–312.
[13] Tischendorf, G.W., Geisser, M. and Stöffler, G. (1973) Mol. Gen. Genet. 127, 147–156.
[14] Otaka, E. and Osawa, S. (1981) Mol. Gen. Genet. 181, 176–182.
[15] Geisser, M., Tischendorf, G.W. and Stöffler, G. (1973) Mol. Gen. Genet. 127, 129–145.
[16] Fahnestock, R.S., Strycharz, W.A. and Marquis, D.M. (1981) J. Biol. Chem. 256, 10111–10116.
[17] Kimura, M., Mende, L. and Wittmann-Liebold, B. (1982) FEBS Lett. 149, 304–312.
[18] Dorne, A.M., Eneas-Filko, J., Heizmann, P. and Mache, R. (1984) Mol. Gen. Genet. 193, 129–134.
[19] Schulze, H. and Nierhaus, K.H. (1982) EMBO J. 5, 609–613.
[20] Bartsch, M. (1984) PhD. Thesis, Freie Universität, Berlin.
[21] Bartsch, M. (1984) J. Biol. Chem., submitted.