SPECIES SPECIFICITY OF RIBOSOMAL PROTEINS IN EUCARYOTIC AND PROCARYOTIC ORGANISMS

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Summary: Immunological analysis of ribosomal proteins from E. coli, rat liver, yeast (S. Cerevisiae) and plant (mung bean cotyledon) show no cross reactions. Electrophoresis by the split-gel technique in polyacrylamide also indicates that the proteins from eucaryotic (80S) and procaryotic (70S) ribosomes are unique for each species.

In bacteria, the chemical nature of the ribosomal proteins (RP) has been shown to vary from species to species (1). The results of similar studies on animal RP appear to be contradictory (2, 3). While it is established that RP from different tissues of the same animal are essentially identical, those from different animals (e.g. rat and rabbit) recently have been reported to be similar in one case (3) and characteristically different in another (2).

We wish to report here studies on comparative immunological and electrophoretic analyses of RP from Escherichia coli, yeast, plant (mung bean cotyledon) and rat (liver). The aim was to investigate whether or not the RP within and between these eucaryotic and procaryotic organisms contain any common components.

Preparation of antisera: Ribosomes were treated with equal volume of 4M lithium chloride and 8M urea, left overnight at 4° and then centrifuged. The supernatant solution was dialysed against 0.05M Sodium pyrophosphate (5), pH 8.5 and used as antigen. Male white rabbits were injected with 5 - 10 mg of the antigen in 1ml emulsified with 1ml of Freund's adjuvant. A booster dose of 5mg was given to each rabbit after 1 month. Animals were bled during the 6 - 10th week when the maximum titre was obtained.

Results and Discussion: The results of Ouchterlony immunodiffusion analysis are shown in Figure 1. Simultaneous exposure of antisera to plant, rat, E. coli

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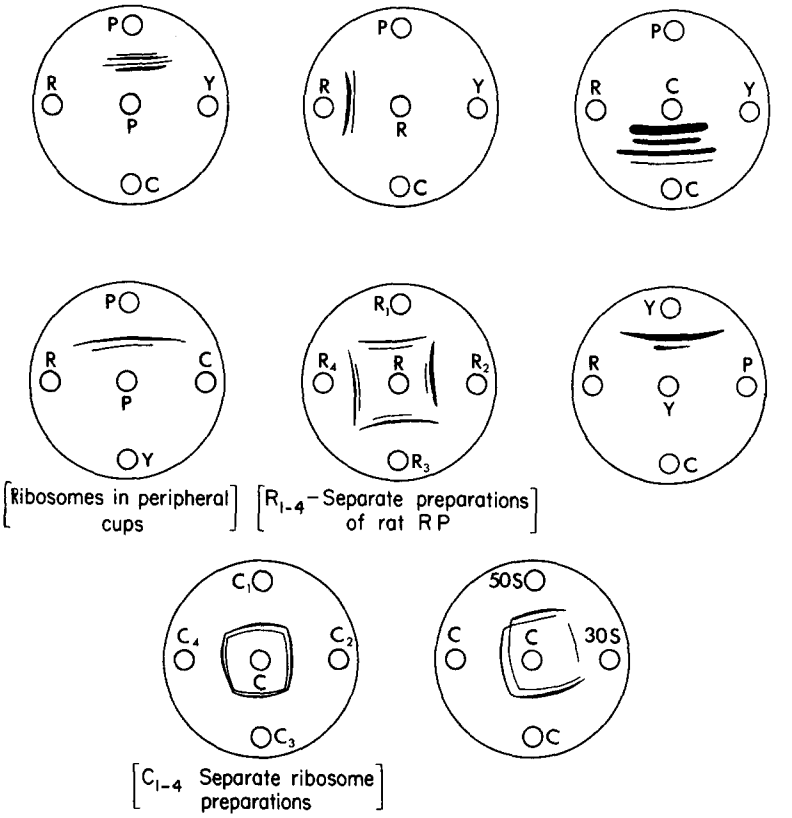

Fig. 1. Ouchterlony Immunodiffusion Test for Ribosomal Proteins. Center cup = Antisera; Peripheral cups = antigen as ribosomal proteins unless indicated;
C = E. coli Y = Yeast (S. Cerevisiae) P = Plant (mung bean cotyledon) R = Rat (liver)

or yeast ribosomal proteins to all these antigens reveal specific reaction only with the corresponding RP. In most cases two or three precipitin lines were observed with a maximum of four in the case of plant and E. coli RP. Substitution of ribosomes for ribosomal proteins gave similar specific reactions, but the number of precipitin lines was reduced. Presumably some of the determinants in the RP may be buried inside the ribosomal surface. Ribosomes or RP from different preparations and of different concentrations were used for optimal antigen-antibody reaction but the number of precipitin lines did not increase. RP prepared from the separated subunits of the E. coli ribosomes showed one major and one minor precipitin line for the 50S subunit

and one minor precipitin line (not apparent in the picture) for the 30S subunit.

The nature of the protein components of E. coli, rat and plant ribosomes were also analyzed by polyacrylamide gel electrophoresis. Figure 2 shows the migration patterns and comparison of these RP by split-gel electrophoresis. The electrophoretic pattern for RP from each source displayed a large number of characteristic bands. Using the split-gel technique, definite differences were observed between RP from two eucaryotic organisms, rat and plant both containing 80S ribosomes, as well as between one eucaryotic (80S ribosomes) and one procaryotic (70S ribosomes) organism, viz. rat and E. coli.

The detection of only a few precipitin lines in the Ouchterlony test is surprising in view of the large number of proteins shown to be present in each preparation by electrophoresis. The possibility that only a few of the total RP are immunogenic is very unlikely in view of their large molecular weight [e.g. 10,000 - 40,000 for E. coli RP (4)]. Alternatively the RP from each source might contain a set of common determinants so that antigenically they can be divided into only a few groups. This would suggest their origin from a comparatively small number of structural genes by gene duplication and somatic mutation. The results obtained here are similar to those for Neurospora ribosomes (5).

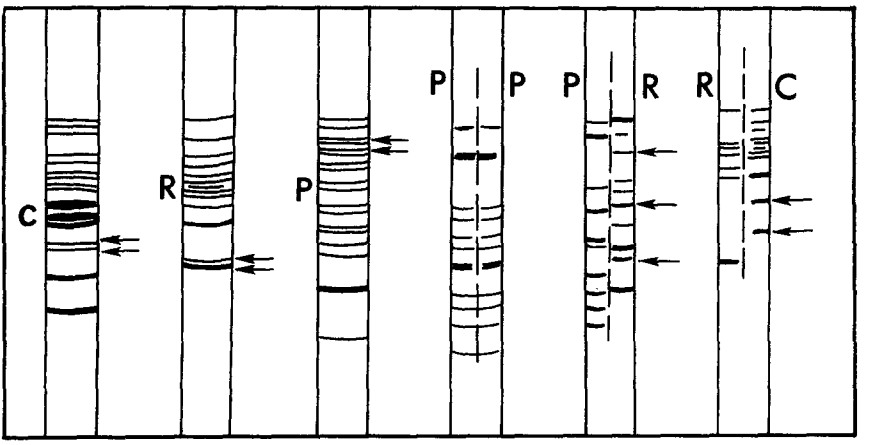

Fig. 2. Polyacrylamide Gel Electrophoretic Patterns of Ribosomal Proteins. Letter designations as in Fig. 1. Characteristic proteins are indicated by arrows; on the right, three diagrams indicate split-gel electrophoresis.

Parisi et al (6, 7) have demonstrated that for active polypeptide synthesis, ribosomes of a given size (70S or 80S) must be supplied with polymerizing enzymes derived from a species which contain ribosomes of the respective size. These findings imply the existence of a common recognititon site in all 70S ribosomes and a different one in the 80S ribosomes, capable of interacting with the polymerizing enzymes from appropriate sources. Our results indicate that the ability of ribosomes of appropriate size to function interchangeably is unrelated to their protein content.

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