Identification by Affinity Chromatography of the Rat Liver Ribosomal Proteins That Bind to Escherichia coli 5 S Ribosomal Ribonucleic Acid*

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The eukaryotic and prokaryotic ribosomal proteins that bind to Escherichia coli 5 S rRNA were identified by affinity chromatography. The E. coli ribosomal proteins that associated with the nucleic acid were L5, L18, and L25 confirming earlier findings using the same and different procedures. The rat liver ribosomal proteins that associated with E. coli 5 S rRNA were L6, L7, L19, L25a, and S9; several of those proteins also bind to rat liver 5 S rRNA (L6, L19) and to 5.8 S rRNA (L5, L19, and S9).

The large subunit of eukaryotic ribosomes contains two small ribonucleic acids, 5 S and 5.8 S, whereas prokaryotic ribosomes have only 5 S rRNA. There are reasons to suspect that prokaryotic 5 S rRNA is functionally homologous with eukaryotic 5.8 S rather than 5 S rRNA (1, 2). 5.8 S rRNA is part of the transcript of rDNA with 18 and 28 S rRNA, just as prokaryotic 5 S rRNA is part of a transcription unit with 16 and 23 S rRNA; the genes for eukaryotic 5 S rRNA, on the other hand, are located at a separate site in the nucleus. What is perhaps most significant is that all prokaryotic 5 S rRNAs have the sequence G-A-A-C (near position 45) (2-4) — that sequence is complementary to G-T-U-C in the T-U arm of all tRNAs except eukaryotic initiator-tRNA; eukaryotic 5 S rRNA has instead the conserved sequence G-A-U-C that is complementary to G-A-U-C found only in eukaryotic initiator-tRNA. 5.8 S rRNA has instead the conserved sequence G-A-A-C that is complementary to G-T-C in eukaryotic elongator tRNA. It is assumed that the G-T-C of tRNA associates with a complementary sequence in prokaryotic 5 S (5, 6), and with eukaryotic 5.8 S rRNA, during binding of aminocarboxyl-tRNA to the ribosomal A site (1, 2). The fragment T-C-C-G inhibits factor-dependent binding of aminocarboxyl-tRNA to prokaryotic (7, 8) and eukaryotic ribosomes (9); the P site binding of initiator-tRNA is not affected. A consistent construct is that in eukaryotes 5.8 S rRNA forms part of the ribosomal A site and participates in the binding of elongator tRNAs; that 5 S rRNA forms part of the P site and takes part in the binding of initiator-tRNA.

The putative functional homology of prokaryotic 5 S rRNA and eukaryotic 5.8 S rRNA appears apparent support from the finding that Escherichia coli ribosomal proteins L18 and L25, which are the 5 S rRNA binding proteins in the ribosome of the bacterium (10-14), form a specific ribonucleoprotein complex with yeast 5.8 S rRNA (1), although it was subsequently discovered that the E. coli proteins did not bind to the yeast nucleic acid when it was immobilized on Sepharose.

EXPERIMENTAL PROCEDURES

The experimental procedures have been described before (15, 16).

RESULTS

Affinity Chromatography of E. coli Ribosomal Proteins on E. coli 5 S RNA-Sepharose—5 S rRNA was prepared from E. coli 70 S ribosomes by extraction with phenol and sodium dodecyl sulfate (16). The rRNA was purified by chromatography on DEAE-cellulose and by filtration through Sephacryl S-200 (Fig. 1). The pure E. coli 5 S RNA was coupled, after oxidation with periodate of its 3'-hydroxyl to an aldehyde, to Sepharose 4B through an adipic acid dihydrazide spacer (14-16, 18). In separate experiments, mixtures of all the proteins of one of the subunits of E. coli or rat liver ribosomes were chromatographed (in binding buffer) on 5 S RNA-Sepharose columns at 22°C (Fig. 2). In these experiments, the 5 S rRNA is in excess, the ribosomal proteins are in limiting amounts. The proteins that were bound to 5 S rRNA were eluted with buffer containing a high concentration of salts and EDTA (dissociation buffer), collected, and identified by a combination of one-dimensional electrophoresis in polyacrylamide gels containing sodium dodecyl sulfate and electrofocusing in two dimensions in gels containing urea. The combination of the two procedures is important. There are pairs of ribosomal proteins that cannot be identified by either method alone; however, a certain identification can always be made by combining the two (15).

In the first experiment, the proteins of the large subunit of E. coli ribosomes were chromatographed on an E. coli 5 S RNA-Sepharose column (Fig. 2). The proteins that bound to the 5 S RNA, about 5.5% of the total, were collected and a preliminary identification was made after separation by electrophoresis on polyacrylamide gels containing sodium dodecyl sulfate (19, 20). The stained gels were scanned at 540 nm (Fig. 3), and the approximate molecular weights of the E. coli proteins were estimated by comparison with rat liver ribosomal proteins. There were three peaks: the first, $M_z \approx 22,900$, comprises 22% of the bound protein; the second, $M_z \approx 14,000$,
Affinity Chromatography of Rat Liver Ribosomal Proteins on E. coli 5 S rRNA-Sepharose—The proteins of the 60 S subunit of rat liver ribosomes were chromatographed on Sepharose to which E. coli 5 S rRNA had been coupled (Fig. 2). The fraction that was retained, about 8.4% of the total, was eluted, and an initial identification of the proteins was made by eluted a gel affinity column. 

The per cent of individual proteins in the fraction that was bound to the 5 S rRNA-Sepharose was estimated by weighing the individual peaks in the tracing obtained by scanning the polyacrylamide-sodium dodecyl sulfate gel at 540 nm (see Figs. 3, 5, and 6).
E. coli 5 S rRNA Binding Proteins

FIG. 3. Electrophoresis on polyacrylamide gels containing sodium dodecyl sulfate of E. coli 50 S ribosomal subunit proteins separated by affinity chromatography on E. coli 5 S rRNA-Sepharose. TP50, a mixture of 30 µg of all the E. coli 50 S ribosomal subunit proteins; A, 30 µg of E. coli 50 S subunit proteins that did not bind to 5 S rRNA-Sepharose (Peak A in Fig. 2, bottom); B, 4 µg of the E. coli 50 S subunit proteins that were bound to 5 S rRNA-Sepharose (Peak B in Fig. 2, bottom). The proteins that were bound to 5 S rRNA-Sepharose (E) were scanned at 540 nm.

trophoresis (Fig. 4). That procedure confirmed the identity of L6 and L7; it established that the other two proteins were L19 (not L14) and L35a (not L22). Thus, four rat liver 60 S ribosomal subunit proteins bind to E. coli 5 S rRNA—L6, L7, L19, and L35a. On the other hand, no E. coli ribosomal proteins bind to rat liver 5 S rRNA (16).

The proteins of the 40 S subunit of rat liver ribosomes were next passed through the E. coli 5 S rRNA-Sepharose column (Fig. 2). There was only one prominent band on the scan of the sodium dodecyl sulfate gel (Fig. 5) and it could be aligned with the protein S9; that identification was confirmed by two-dimensional gel electrophoresis (Fig. 4).

DISCUSSION

The E. coli large ribosomal subunit proteins L5, L18, and L25 have been shown by a variety of procedures (10-13), including affinity chromatography (14, 18), to bind specifically to homologous 5 S rRNA. We have confirmed the finding: when E. coli ribosomal proteins were chromatographed on E. coli 5 S rRNA affinity columns, L5, L18, and L25 were bound in a ratio of approximately 1:2:1. The ratio was determined by scanning the sodium dodecyl sulfate gels after the proteins were separated by electrophoresis (Fig. 5); it may be important that the amount of L5 that was bound to 5 S rRNA would be underestimated if the assessment was from inspection of two-dimensional gels (Fig. 4) as is usually the case. The finding...
reinforces the impression that the binding of ribosomal proteins to ribosomal nucleic acids immobilized on Sepharose columns reflects their association in the ribosome. Hence, the procedure can be used to identify the proteins that interact with specific species of ribosomal nucleic acids.

A number of rat liver ribosomal proteins (L6, L7, L19, L35a, and S9) bind to E. coli 5 S rRNA. Lind et al. (25) also found that rat liver ribosomal proteins bind to E. coli 5 S rRNA but the identity of the proteins was not fully documented. The finding is surprising since no E. coli ribosomal proteins bind to rat liver 5 S rRNA (16, 25, 26), nor to rabbit reticulocyte (14), nor yeast 5 S rRNA (1, 14). There is no immediate obvious explanation of the paradox. Wrede and Erdmann (1) had reported that E. coli L18 and L25 formed a specific complex with yeast 5.8 S rRNA which provided a possible reconciliation of the results: the idea being that the functionally homologous nucleic acids were prokaryotic 5 S and eukaryotic 5.8 S rRNAs. However, no E. coli proteins were bound to rat liver 5.8 S rRNA fixed on Sepharose (15), nor to a yeast 5.8 S rRNA affinity column. The only heterologous binding heretofore convincingly demonstrated is of E. coli L18 and L25 to Bacillus stearothermophilus 5 S rRNA and of B. stearothermophilus B-L5 and B-L22 to E. coli 5 S rRNA (10).

There is evidence that prokaryotic 5 S rRNA participates in the binding of aminoacyl-tRNA to the ribosomal A site (2, 5-7, 27-29); it is not certain whether it also functions in the binding of the initiator tRNA to the P site (compare Refs. 5 and 27). The idea that the function of prokaryotic 5 S rRNA is split among two eukaryotic nucleic acids, 5 S and 5.8 S rRNA, is attractive, albeit far from being substantiated. The
thesis is that eukaryotic 5.8 S rRNA binds elongator tRNAs, which is supported by the finding that the fragment T-Ψ-C-G prepared from tRNA inhibits the binding of aminoacyl-tRNA but not initiator-tRNA to eukaryotic ribosomes, and that eukaryotic 5 S rRNA serves in the association of eukaryotic initiator-tRNA with ribosomes. The assumption is that eukaryotic 5.8 S is closer in function to prokaryotic 5 S (see the introduction for the arguments on that score) and that eukaryotic 5 S rRNA evolved separately. The need for eukaryotic 5 S rRNA, if it does indeed participate in the binding of initiator-tRNA, may derive from the special nature of the initiation process in eukaryotes. There is increasing evidence that while most of the partial reactions of protein synthesis are very similar, those of initiation are different in eukaryotes and prokaryotes (30, 31). Most noteworthy in the present context is that in eukaryotes, unlike in prokaryotes, the binding of Met-tRNA obligatorily precedes the binding of mRNA (32) and may participate in the selection of the first AUG codon.

It is noteworthy that the rat liver ribosomal proteins that bind to E. coli 5 S rRNA are not a random set but rather include those that associate with rat liver 5 S or 5.8 S rRNA or both. L6 and L19 bind to 5 S and 5.8 S rRNA; L35a binds to both also but only in trace amounts; L7 binds to 5 S in small amounts but not at all to 5.8 S; S9 binds in significant amounts only to 5.8 S rRNA. The similarities in the primary sequences of the small nucleic acids in prokaryotic and eukaryotic ribosomes are no greater than to be expected by chance and hence it is unlikely they share a common ancestor (33). However, it may be some functionally relevant conformation rather than sequence per se that is critical in the binding of proteins to nucleic acids; moreover, it may be possible to derive structurally similar binding sites from different primary sequences. One still cannot account for the failure of E. coli ribosomal proteins to bind to rat liver 5 S or 5.8 S rRNAs in the reciprocal experiments.

The possibility that the binding of rat liver ribosomal proteins to E. coli 5 S rRNA is an artifact cannot be excluded with certainty, but does not seem likely. First, the usual precautions were taken; for example, no rat liver or E. coli ribosomal proteins were bound to Sepharose-hydrazine; and many basic proteins did not bind to 5 S rRNA-Sepharose. We have inspected the properties of the binding proteins—pl, number of basic and acidic residues, hydrophobicity, aromatic amino acids—and do not find anything to distinguish them from many ribosomal proteins that do not bind to 5 S or 5.8 S rRNA. Thus, the association was likely to be the result of an affinity of the proteins for the nucleic acid rather than of ion exchange. Perhaps it is more relevant that in exactly the circumstances in which the rat liver proteins bind to E. coli 5 S rRNA, only 3 of 53 E. coli proteins (L5, L18, and L25) associate with the nucleic acid and they are the very proteins demonstrated to form a complex by a variety of other procedures (10–13). If the binding of E. coli ribosomal proteins to E. coli 5 S rRNA reflects an authentic interaction, then the heterologous association should also derive from a fit between binding sites on the RNA and the protein. It is important that the rat liver ribosomal proteins that bind to E. coli 5 S rRNA (L6, L7, L19, L35a, and S9) are a small subgroup of the total that includes most of the proteins that bind to rat liver 5 S or 5.8 S rRNA or both (see above). Of the proteins that bind to rat liver 5 S or 5.8 S rRNA, only L8 and S13 (5.8 S rRNA binding proteins) do not bind to E. coli 5 S rRNA.

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