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Binding of rat ribosomal proteins to yeast 5.8S ribosomal ribonucleic acid

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ABSTRACT

5.8 S RNA-protein complexes were prepared using purified yeast 5.8 S RNA and proteins from the large ribosomal subunit of rat liver. Formation of such hybrid complexes, as measured by Millipore filtration, was dependent on protein concentration. Binding of proteins to the RNA could approach saturation. Such complexes were isolated from sucrose density gradient centrifugation and shown to contain proteins L6, L8, L19, L35 and L35a. These proteins were identified by their molecular weights on polyacrylamide gels containing dodecylsulfate and their mobilities on two dimensional polyacrylamide gels.

INTRODUCTION

The 5.8 S ribosomal RNA is a component of the large ribosomal subunit of eukaryotes (1,2). Although the primary structure of this RNA species from a variety of organisms has been determined (3), little is known about its functional role. It has been suggested that the eukaryotic 5.8 S RNA may be involved in protein synthesis (4). In fact, two of the E. coli 5 S rRNA binding proteins, L18 and L25, have been shown to bind to yeast 5.8 S rRNA but not to yeast 5 S RNA (4). The hybrid complexes have ATPase and GTPase activities similar to that found for the homologous complexes of E. coli 5 S RNA-proteins. Recently, the proteins which bind to rat liver 5.8 S RNA immobilized on Sepharose have been identified (5-9).

This communication describes the isolation and characterization of the hybrid RNA-protein complexes composed of yeast 5.8 S rRNA and rat liver ribosomal proteins. The complexes have been isolated by centrifugation in sucrose density gradients. The proteins present in the complexes have been isolated, their molecular weights determined on SDS-containing polyacrylamide gels and their identities determined on two dimensional polyacrylamide gels. The major rat liver ribosomal proteins found in the yeast 5.8 S RNA-protein complexes are L6, L8, L19, L35 and L35a.

MATERIALS AND METHODS

Preparation of Yeast 5.8 S rRNA

Saccharomyces cerevisiae (ATCC 22244) was used as the source of the 5.8 S rRNA. Cells were grown in low phosphate medium (10) at 28°C for 20 h with vigorous aeration

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in the presence of 15 mCi of carrier-free [^{32}P]phosphoric acid. RNA was prepared from whole cells which were suspended in 10 mM Tris-HCl, pH 7.4, 10 mM EDTA, 0.5% SDS and 4 mg/ml purified bentonite. Equal volume of redistilled phenol was added to the cell suspension. The mixture was shaken for 15 min at 60°C and then for 30 min at room temperature. The aqueous phase was collected by centrifugation for 5 min at 10,000 rpm. The phenol phase was reextracted with equal volume of buffer at room temperature. The combined aqueous phase was extracted twice with water-saturated redistilled phenol. To the final aqueous phase were added 0.1 volume of 4 M sodium acetate, pH 5.0, and 2 volumes of cold ethanol. The RNA was collected by centrifugation and dissolved in 6 M urea. The RNA sample was heated for 5 min at 60°C to release the 5.8 S rRNA and applied to a slab of polyacrylamide gel (10%) as described previously (4) except that 15 mM iodoacetic acid and 0.02% diethyl pyrocarbonate were included in the gel. After electrophoresis, the 5.8 S rRNA was detected by autoradiography and the RNA band was excised and subjected to another cycle of electrophoresis. The RNA was recovered from the gel by phenol extraction (11) and dissolved in Buffer A (30 mM Tris-HCl, pH 7.4, 20 mM $MgCl_2$, 340 mM KCl. 6 mM β-mercaptoethanol).

Fingerprinting Analysis

RNA fragments were digested with RNase T_1 and fractionated by 2-D paper electrophoresis (12).

Preparation of Rat Liver Ribosomal Proteins

Ribosomes were prepared from fresh rat liver essentially as described by Martin and Wool (13). Proteins were extracted from the purified ribosomes with acetic acid (67%) in 10 mM Tris-HCl, pH 7.7, 20 mM β-mercaptoethanol and 33 mM magnesium acetate (14,15). Proteins were precipitated with acetone. The protein precipitate was dissolved in 8 mM urea, 10 mM Tris-HCl, pH 7.2, and 20 mM β-mercaptoethanol, reprecipitated with acetone and dissolved in Buffer A.

Formation of Yeast RNA-Rat Liver Protein Complexes

Yeast 5.8 S [^{32}P]-rRNA was dissolved in Buffer A and heated at 37°C for 15 min. Ribosomal proteins were added to the reaction mixture and incubated for 15 min at 37°C and 15 min at 4°C. The extent of complex formation was determined by filtration on Millipore filters (HA, 0.45 μm) as described previously (16).

Density Gradient Centrifugation on RNA-Protein Complexes

Typically, a reaction mixture containing 5.8 S [^{32}P]-rRNA (0.5-1.0 x 10^6 cpm) from yeast and rat liver ribosomal protein (200-300 equivalent units) was incubated as described for the formation of complexes and applied to a sucrose density gradient. One equivalent unit of protein corresponds to the amount of proteins obtained from 1 A_{260} unit of rat liver ribosomes (4). The amount of rat liver protein used for each experiment was predetermined by kinetic experiments such that saturation amounts of protein were used. The sucrose density gradient (5-15%) with a 50% sucrose cushion was prepared essentially as described (4) except that all sucrose solutions were made up in

Buffer A. Centrifugation was conducted at 26,000 rpm (100,000 x g) at 4°C for 48 h in a Spinco SW-27 rotor. One-ml fractions were collected from the bottom of the tube. The amounts of radioactivity and of protein in each fraction were determined by Cerenkov counting and by spectrophotometric measurement at 280 Å, respectively.

Isolation and Iodination of Proteins from Complexes

Fractions containing the 5.8 S rRNA-protein complexes were dialyzed against 15 mM Tris-HCl, pH 7.4, 10 mM $MgCl_2$, 30 mM NH_4Cl and 6 mM β-mercaptoethanol and concentrated by filtration. Proteins were extracted with 67% acetic acid in 0.1 M $MgCl_2$ (14) and dialyzed against 6% acetic acid extensively at 4°C. Proteins were labeled with [^{125}I]-iodine in the presence of chloramine T, dialyzed against the above buffer and precipitated with acetone.

Polyacrylamide Gel Electrophoresis in Sodium Dodecyl Sulfate

[^{125}I]-Labeled rat liver proteins were analyzed by polyacrylamide gel (15%) electrophoresis in sodium dodecyl sulfate as described by Laemmli (17). Molecular weight standard proteins used were bovine serum albumin (68,000), pyruvate kinase (57,000), glyceraldehyde phosphate dehydrogenase (36,000) and cytochrome c (11,700). The standard proteins were detected by staining the gel with Coomassie Blue and the radioactive proteins by autoradiography.

Two-Dimensional Polyacrylamide Gel Electrophoresis

Rat liver ribosomal proteins were identified by 2-D gel electrophoresis (15,18). Protein spots were excised and counted in a gamma counter.

Protein Determination

The concentration of protein was determined as described by Bradford (19). Bovine serum albumin was used as a standard.

RESULTS

Purity of the yeast 5.8 S rRNA used in this study was checked by electrophoresis on polyacrylamide gels and by RNA fingerprinting. The yeast 5.8 S rRNA showed a single band on gels (data not shown). The RNA was digested with RNase T_1 and the products analyzed by two dimensional paper electrophoresis. The fingerprint obtained was similar to that published by Rubin (11).

To determine the extent of interactions between yeast 5.8 S RNA and rat liver proteins from the large ribosomal subunits, RNA was incubated with proteins under conditions known to promote formation of RNA-protein complexes (4). The complexes were analyzed by Millipore membrane filtration. The extent of complex formation was proportional to the amount of proteins added (Fig. 1). A plateau of complex formation was approached at about 10 equivalent units of proteins; at the present, the exact stoichiometry of proteins bound could not be determined. For comparison, data obtained using yeast RNA and E. coli 50 S ribosomal proteins is also shown (Fig. 1).

To facilitate identification of those rat liver ribosomal proteins which were bound to

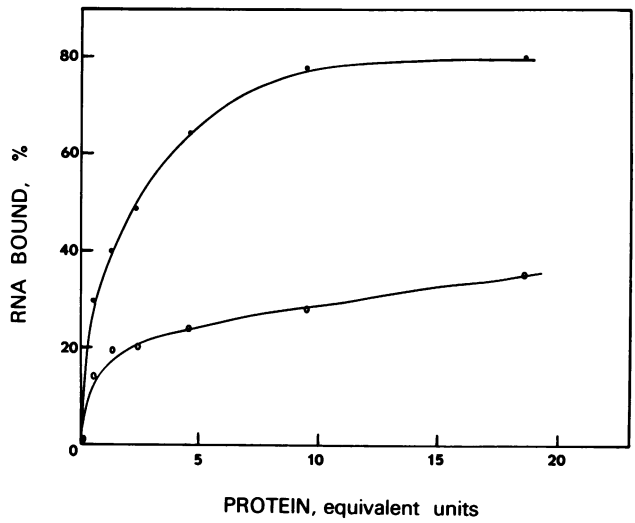

Figure 1. Binding of proteins from the large ribosomal subunits of rat liver and E. coli to yeast 5.8 S RNA (2000 cpm) was preincubated at 37°C for 15 min followed by additions of varying amounts of rat liver proteins (●) or E. coli proteins (○) for an additional 15 min at 37°C and 15 min at 4°C. Complexes were measured by Millipore filtration.

the yeast RNA, the complexes were first sedimented in a sucrose density gradient to remove unbound proteins. Proteins were isolated from the complexes, labeled with [^{125}I] and analyzed on polyacrylamide gels. Figure 2 shows a typical sucrose density gradient profile. The uncomplexes proteins sedimented close to the top of the gradient (Fig. 2A). Free 5.8 S RNA sedimented as a single peak away from the proteins (Fig. 2B). When proteins were incubated with [^{32}P] RNA, one major radioactive peak was detected (Fig. 2C).

Pooled material from the peak (fractions 17-22, Fig. 2C) was extracted to remove the RNA; the resulting proteins were iodinated and analyzed by one-dimensional polyacrylamide gel electrophoresis in the presence of dodecyl sulfate. Five radioactive protein bands were detected by autoradiography with molecular weights of 33,000, 28,000, 24,000, 18,000 and 16,000 (Fig. 3). It is unlikely that any of these radioactive bands could have originated from the radioactive RNA used in the formation of the RNA-protein complexes, since the amount of detectable [^{32}P] radioactivity in the acetic acid extracted protein fraction before iodination was less than 100 cpm. With the published molecular weights for rat liver ribosomal proteins (20), we can tentatively identify those protein candidates that fall into each molecular weight category (Table I).

To identify more precisely the bound proteins, the [^{125}I] labeled proteins extracted from the complexes were analyzed by 2-dimensional polyacrylamide gels with trace amounts of carrier large subunit proteins to provide background for the identification of proteins (Fig. 4a). Each protein spot was excised and the amount of radioactivity

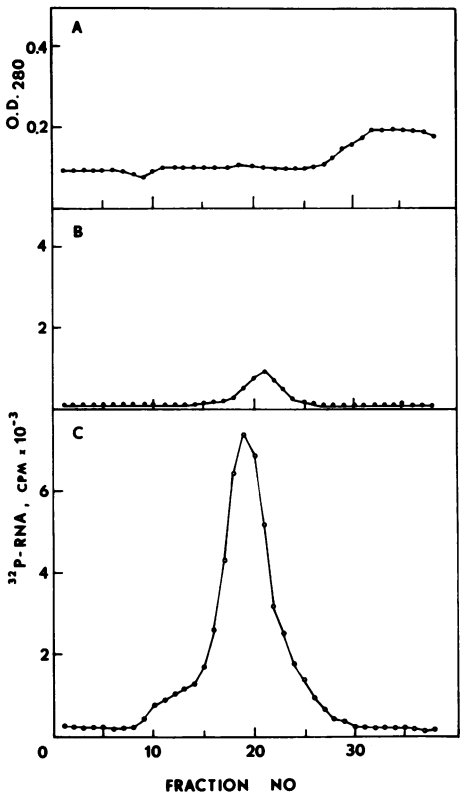

Figure 2. Sucrose density profile of 5.8 S RNA-protein complexes. (A) Unbound rat liver ribosomal proteins. (B) Free yeast 5.8 S RNA (4 x 10^4 cpm). (C) Hybrid yeast RNA-rat liver protein complexes (5 x 10^5 cpm RNA and 220 equivalent units of proteins).

determined. Five major radioactive spots were detected (Fig. 4b). Based on their electrophoretic mobilities on 2-dimensional gels, together with their molecular weights, these proteins are L6, L8, L19, L35 and L35a.

To be certain that all isolated proteins from the large ribosomal subunits of rat liver could be iodinated under our conditions, total proteins from the large ribosomal subunits were treated under identical conditions and analyzed on two-dimensional polyacrylamide gels. A comparison of the Coomassie Blue stained electrophoretogram and the autoradiogram of the same electrophoretogram indicated that all proteins were iodinated (data not shown).

DISCUSSION

The present study demonstrates the formation of hybrid complexes containing yeast 5.8 S RNA and rat liver ribosomal proteins. Such complexes are sufficiently stable to allow isolation and subsequent characterization.

A comparison of the rat liver proteins which bind to the rat liver (homologous) and the yeast (heterologous) 5.8 S RNA reveals that several proteins do bind to both

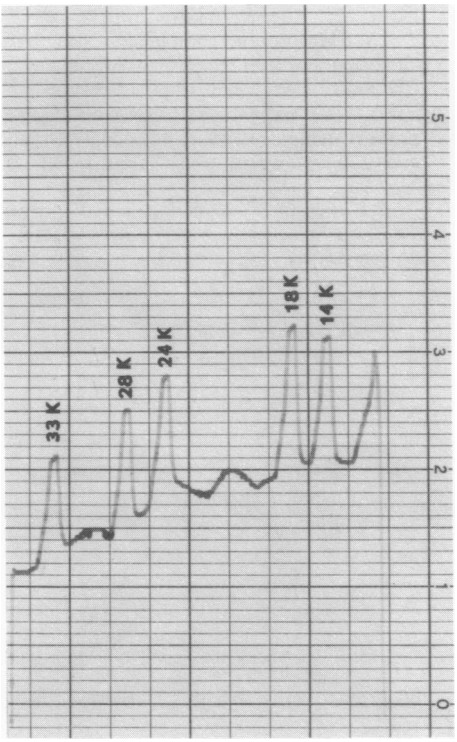

Figure 3. A scan of the autoradiogram of SDS-polyacrylamide gel of the [^{125}I]-labeled proteins extracted from the hybrid RNA-protein complexes. The calculated molecular weights of these proteins using the molecular weight standard proteins described in Materials and Methods are indicated.

RNA species (Table 2), even though different methods of detection of RNA-protein complex formation have been used. By affinity chromatography, Ulbrich et al. (5) originally reported that rat liver 5.8 S RNA immobilized by its 3'-end to Sepharose 4B would bind rat liver ribosomal proteins L6, L8, L19, S9 and S13. Small amounts of proteins L14, L17, L18, L27/L27', L35', S11, S15, S23/S24, and S26 were also bound. More recently, these investigators reported that isolated proteins L6, L8, and L19 would

Table 1. Protein candidates from the large ribosomal subunit of rat liver that bind to yeast 5.8 S RNA.

Molecular Weight[1]	Proteins
32,000	L5, L6
28,000	L8
25,000-24,000	L9, L10, L13', L15, L18, L19
19,000-17,000	L12, L16, L23', L25, L27, L27', L28, L35
16,500-15,000	P1, L20, L22, L23, L31, L33, L34, L36', L35a

[1]Molecular weights of rat liver proteins were determined by electrophoresis in gels containing dodecyl sulfate as reported by Wool (20).

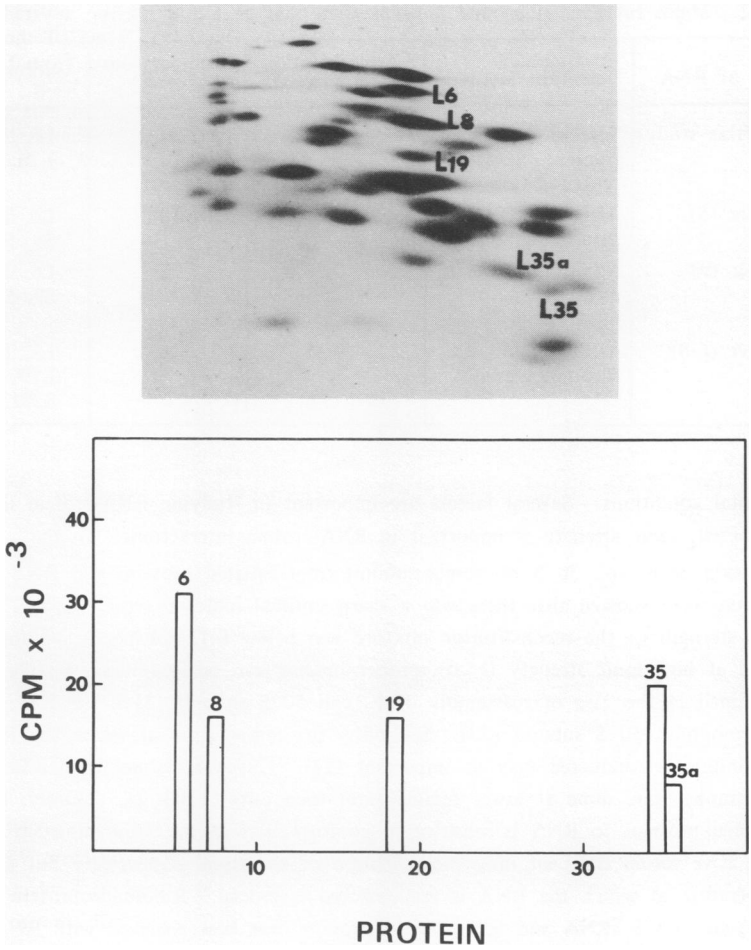

Figure 4. Electrophoresis pattern of rat liver large ribosomal subunit proteins that were bound to yeast 5.8 S RNA. Proteins extracted from RNA-protein complexes were labeled with [^{125}I] and subjected to two-dimensional gel electrophoresis in the presence of a trace amount of non-radioactive marker proteins. (A) Coomassie Blue stained pattern. (B) Amount of radioactivity in each protein spot.

bind to rat liver 5.8 S RNA as measured by nitrocellulose membrane filtration (9). We have not tested the binding of proteins from the small ribosomal subunits. On the other hand, Metspula et al. (6) originally reported that L5, L6, L7 and L18 would bind to rat liver RNA. Their most recent report (8) indicated that protein L19 and not L18 would bind to the RNA. Hence, some of the differences might be attributed to the difficulties in a precise identification of the ribosomal proteins.

Some of the differences in results may be explained on the basis of variable

Table 2. Major rat liver ribosomal proteins that bind to 5.8 S rRNA

Source of RNA	Detection Method	Concentration of KCl, M	Temperature °C	Proteins Bound
Yeast (this study)	Sucrose density gradient centrifugation	0.34	37	L6, L8, L19, L35, L35a
Rat liver (5)	Affinity chromatography	0.30	18	L6, L8, L19, S9, S13
Rat liver (9)	Nitrocellulose membrane filtration	0.30	22	L6, L8, L19, S9, S13
Rat liver (6-8)	Affinity chromatography	0.15	4	L5, L6, L7, L19, S6, S14, S23/24

experimental conditions. Several factors are important in studying RNA-protein interactions. First, ionic strength is important in RNA-protein interactions. In the reconstitution of E. coli 30 S ribosomal subunit from isolated proteins and RNA, Traub and Nomura (18) showed that there was a sharp optimal ionic strength (I = 0.37). If the ionic strength of the reconstitution mixture was below 0.1, nonspecific binding can occur and at high ionic strength (>1.0), proper binding can be inhibited. Similar data were obtained in the case of reassembly of E. coli 50 S subunit (21,22) and B. stearothermophilus 50 S subunit (23). Secondly, the temperature at which the RNA-protein binding is conducted may be important (24). Experiments involving affinity chromatography were done at lower temperatures than ours (Table 2). Thirdly, binding of ribosomal proteins to RNA is sensitive to conformation of the RNA molecule (25, 26). We have found that not only preincubation of the RNA is necessary but also the temperature at which the RNA is preincubated is crucial. Ribonucleoprotein formation between yeast 5.8 S rRNA and heterologous proteins was best achieved with RNA preincubated at 37°C; RNA not incubated or incubated at higher temperature did not interact with proteins as well. Such an observation is in agreement with the concept that proper ribonucleoprotein formation is sensitive to conformation of the RNA molecule. It is possible that either binding of heterologous ribosomal proteins to 5.8 S rRNA is more sensitive to conformational alterations or the covalent linkage of the 3'-end of the RNA molecule to Sepharose has placed certain conformational constraints on the RNA molecule. Interactions of RNA and proteins in solution (verse in solid phase as in affinity chromatography) may be more akin to that in vivo. Nonetheless, it is interesting that at least several proteins, L6, L8, and L19, do bind to both yeast and rat liver 5.8 S rRNA, particularly in view of the fact that the primary structures of yeast and rat liver (Noviboff hepatoma ascites cells) 5.8 S rRNA are rather different (11,24). There are, however, several conserved RNA regions consisting of 10-15 nucleotides in length. Whether these regions are actually involved in RNA-protein interaction awaits further investigation.

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