AFFINITY CHROMATOGRAPHY OF *DROSOPHILA MELANOGASTER* RIBOSOMAL PROTEINS TO 5S rRNA

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SUMMARY

The binding of *Drosophila melanogaster* ribosomal proteins to *D. melanogaster* 5S rRNA was studied using affinity chromatography of total ribosomal proteins (TP80) on 5S rRNA linked via adipic acid dihydrazide to Sepharose 4B. Ribosomal proteins which bound 5S rRNA at 0.3 *M* potassium chloride and were eluted at 1 *M* potassium chloride were identified as proteins 1, L4, 2/3, L14/L16, and S1, S2, S3, S4, S5, by two-dimensional polyacrylamide gel electrophoresis. Using poly A–Sepharose 4B columns as a model of non-specific binding, we found that a subset of TP80 proteins is also bound. This subset, while containing some of the proteins bound by 5S rRNA columns, was distinctly different from the latter subset, indicating that the binding to 5S rRNA was specific for that RNA species.

INTRODUCTION

Information concerning the interactions of the RNA and protein components of ribosomes has come from a variety of experimental techniques, including covalent cross-linking of RNA to proteins in ribosomes[1-3], isolation by EDTA treatment of subparticles of ribosomes containing both RNA and protein components[4-6], reconstitution of RNA–protein complexes from their components[7], and affinity chromatography of ribosomal proteins on columns containing RNA covalently bound to Sepharose[8,9]. In particular, affinity chromatography of rat liver proteins on 5S rRNA columns has identified a number of 5S rRNA binding proteins, one of which is the same protein isolated as a complex with 5S rRNA after EDTA treatment of ribosomes[9,10]. The EDTA released 5S rRNA–protein complex from several eukaryotes[11-13] (as well as an analogous reconstituted complex from prokaryotes[14,29]) has GTPase and ATPase activity, while ribosomes depleted of this particle do not[12]. Thus, 5S rRNA and one or more of the proteins which bind it have been suggested to be involved in the GTP hydrolysis center of ribosomes[11-14]. In this communication we have extended affinity chromatography studies to *D. melanogaster* and have found both similarities and differences between its 5S rRNA binding proteins and those of

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rat liver. In addition, we have shown that some *D. melanogaster* ribosomal proteins can also bind poly A–Sepharose 4B columns, but that the pattern of binding to poly A (as a model of nonspecific binding of ribosomal proteins to RNA) is significantly different from the 5S rRNA binding pattern.

MATERIALS AND METHODS

Sephadex G-100 and cyanogen bromide activated Sepharose 4B were purchased from Pharmacia. Polyadenylic acid (6–13S) was obtained from PL Biochemicals. Urea was Fisher ultra pure grade. *E. coli* tRNA was from Grand Island Biological. Bovine serum albumin (fraction V) was from Sigma. *D. melanogaster* stock Oregon R was used for all experiments.

Purification of 5S rRNA and binding to Sepharose 4B

Crude preparations of rRNA were obtained by phenol extraction of 80S ribosomes of *D. melanogaster* embryos (2–22 h old) according to Kurland[15] or from the poly A minus fraction of total RNA extracted from *D. melanogaster* embryos (0–20 h) by the method of Spradling *et al.*[16]. In either case, 5S rRNA was purified from these preparations by chromatography on Sephadex G-100[17], elution from polyacrylamide gels after preparative polyacrylamide gel electrophoresis[18], or a combination of these two steps. The 5S rRNA used in the experiments described here was generally more than 93% pure as determined from densitometric scans of polyacrylamide gels on which it was run (Fig. 1A). The 5S rRNA was identified by its comigration with *E. coli* 5S rRNA (a gift of Dr. S. Altman) (Fig. 1A) and shown not to be 5.8S rRNA by the appearance of a band migrating above it (which must be 5.8S rRNA) after treatment of whole ribosomal RNA with 6 M urea for 5 min at 60°C (Fig. 1B).

Drosophila 5S rRNA or poly A were covalently linked to cyanogen bromide activated Sepharose 4B through an adipic acid dihydrazide linker by the method of Burrell and Horowitz[8]. Recovery of the fraction of 5S rRNA not reacted (generally 10–30%) and subsequent electrophoresis showed that the conditions of this reaction left 5S rRNA intact (Fig. 1C).

Protein purification and affinity chromatography

D. melanogaster 80S ribosomes were isolated and total 80S proteins (TP80) extracted as previously described[19]. In some experiments TP80 was further purified by one or two precipitations with acetone. TP80 was initially dissolved in a small volume of 6 M urea and then diluted with 19 volumes of buffer D (0.3 M potassium chloride, 20 mM magnesium chloride, 20 mM Tris–HCl, 6 mM 2-mercaptoethanol, pH 7.4) and loaded (typically in 100–350 μl) on columns (typically 200–500 μl) of 5S rRNA (107–113 μg) or poly A (164–217 μg) Sepharose 4B equilibrated with buffer D and allowed to enter the bed slowly (typically *ca.* 0.3–0.6 ml/h). The columns were washed (typically) with 10–17 volumes of buffer D and then 6–12 volumes of buffer E (1 M potassium chloride, 5 mM EDTA, 20 mM Tris–HCl, 6 mM 2-mercaptoethanol, pH 7.4). In some experiments the buffer E elution was followed by an elution with 6–12 volumes of buffer U (1 M or 2 M potassium chloride, 5 mM EDTA, 20 mM Tris–HCl, 6 M urea, pH 7.4). Columns were run at room temperature.
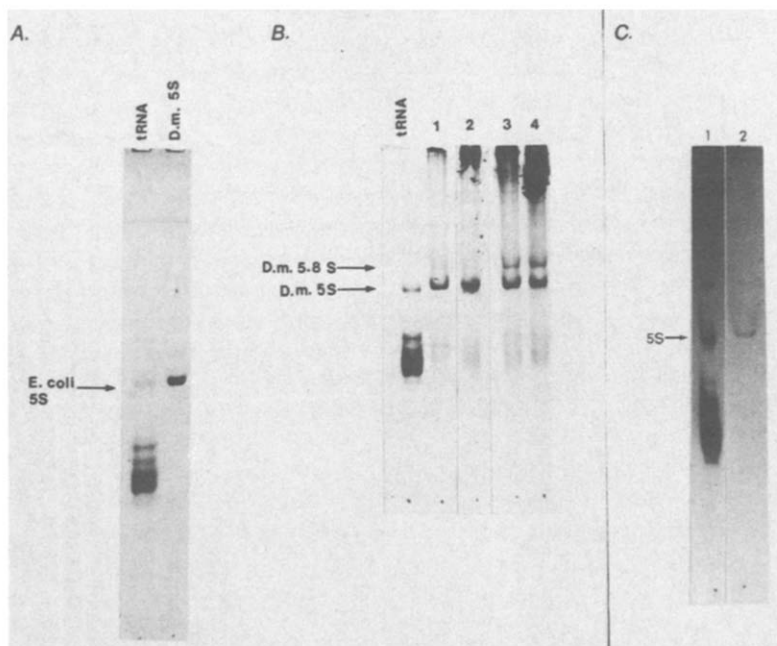
Fig. 1. Purification of *D. melanogaster* 5S rRNA. (A) Purified *D. melanogaster* 5S rRNA (right lane) run on a polyacrylamide gel and stained as described by Peacock and Dingman[28]. Also shown (left lane) are a sample of *E. coli* tRNA and the position of *E. coli* 5S rRNA in this gel system. (B) Total *D. melanogaster* rRNA treated with 6*M* urea at 60°C for 5 min (lanes 3 and 4) or not treated (lanes 1 and 2) run on a polyacrylamide gel and stained as described above. tRNA is *E. coli* tRNA. (C) Fraction of *D. melanogaster* 5S rRNA unreacted with activated Sepharose 4B (lane 2) and *E. coli* tRNA standard (lane 1) run on a polyacrylamide gel and stained as described above.

Polyacrylamide gel electrophoresis

TP80 samples and fractions from affinity columns were precipitated with 15% trichloroacetic acid, the precipitated protein washed with acetone, and analyzed on discontinuous SDS polyacrylamide gels[20] in one dimension or in two-dimensional urea polyacrylamide gels[21].

Protein and RNA assays

Protein determinations were made by the method of Bradford[22] or by measuring $A_{280\ nm}$ (for *D. melanogaster* TP80 the two methods gave virtually identical results using bovine serum albumin as a standard in the Bradford determinations and assuming 1 $A_{280\ nm}$ = 1.0 mg/ml of protein). RNA was quantitated by the conversion 1 $A_{260\ nm}$ = 40 μg/ml.

RESULTS

When TP80 is chromatographed on a 5S rRNA Sepharose 4B column, as described in Materials and methods, with binding of proteins in buffer D and elution with buffer E only, a profile such as that shown in Fig. 2A is seen. Of the total amount of protein coming off the column under these conditions, *ca.* 95% is in the
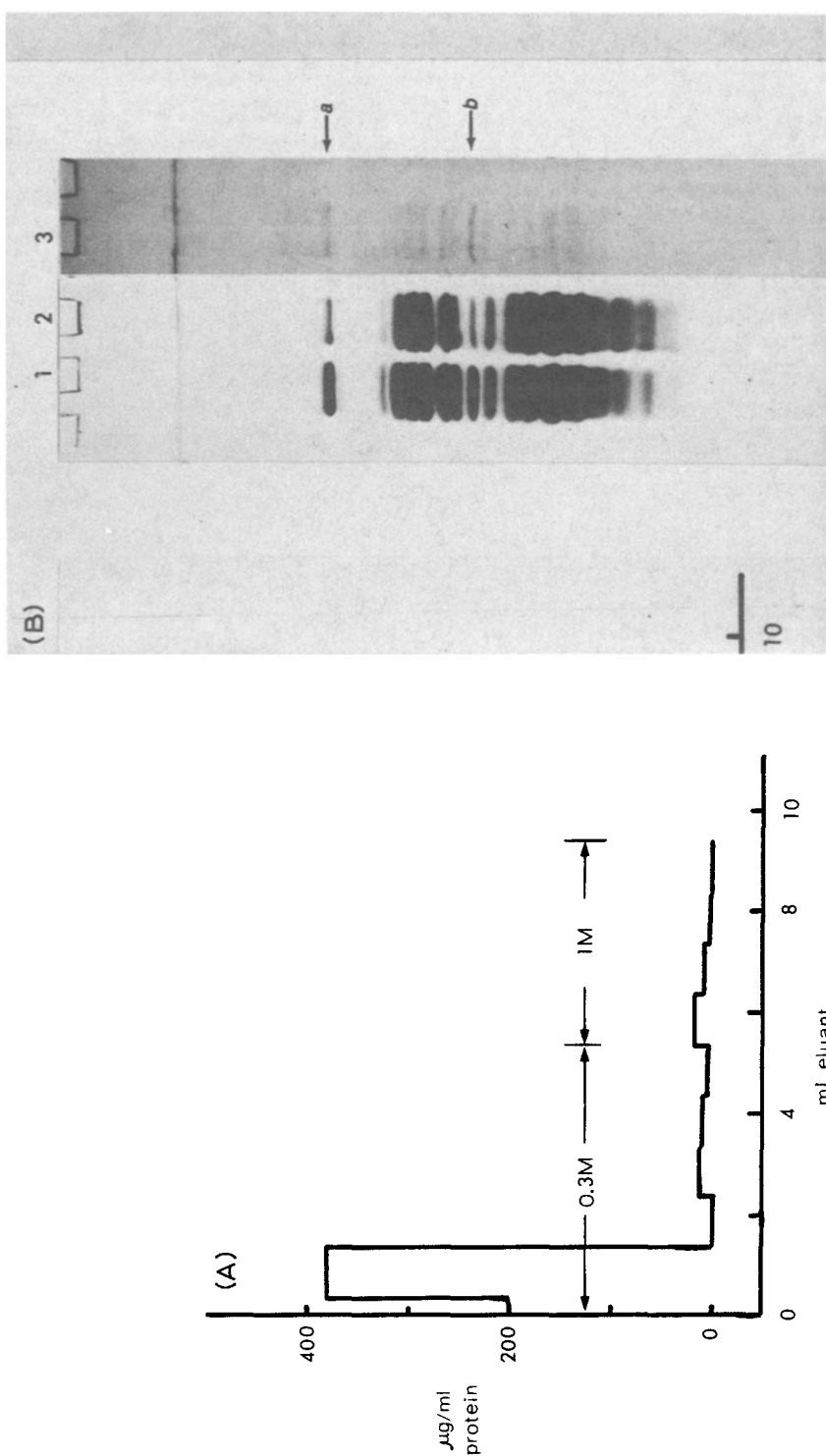
Fig. 2. Chromatography of *D. melanogaster* TP80 on a 5S rRNA Sepharose 4B column. (A) **Protein content of column fractions** *versus* elution volume. Elution with buffer D (0.3 *M*) and buffer E (1 *M*) as indicated. (B) One-dimensional polyacrylamide gel of fractions from the column shown in Fig. 2A: lane 1, the TP80 sample used as the load for the column; lane 2, 0.15 ml of each of fractions 1 (0.35-ml total volume) and 2 (1.0-ml total volume) from the buffer D flow-through; lane 3, 0.2 ml of each of fractions 1 (1.0-ml total volume) and 2 (1.0-ml total volume) from the buffer E elution. Column contained 107 μg of 5S rRNA.

buffer D flow-through and *ca.* 5% in the buffer E elution, which shows up as a small but reproducible peak. One-dimensional SDS polyacrylamide gel electrophoresis of fractions from the column shown in Fig. 2A show that, as compared with the profile of the load of the column (TP80), the profile of proteins not bound in buffer D is deficient in certain bands (*e.g.* a and b) which are enriched in the profile of the proteins eluted with buffer E (Fig. 2B). Raising the potassium chloride concentration in buffer E to 2 *M* does not change the results.

When TP80 is chromatographed on a poly A–Sepharose 4B column (made with approximately the same amount of RNA as the 5S rRNA column shown in Fig. 2) (Fig. 3), a large number of the total TP80 bands are bound to the column in buffer D and eluted in buffer E. The profile of the buffer E wash is very similar to the profile of the buffer D flow-through. In addition, there are some bands (*e.g.* a and b) which are in TP80 and are bound to the poly A column in buffer D and not eluted in buffer E (Fig. 3); one of these (band a in Fig. 3) is identifiable from the standard *D. melanogaster* TP80 one-dimensional gel profile[19] as proteins 1 and L4.
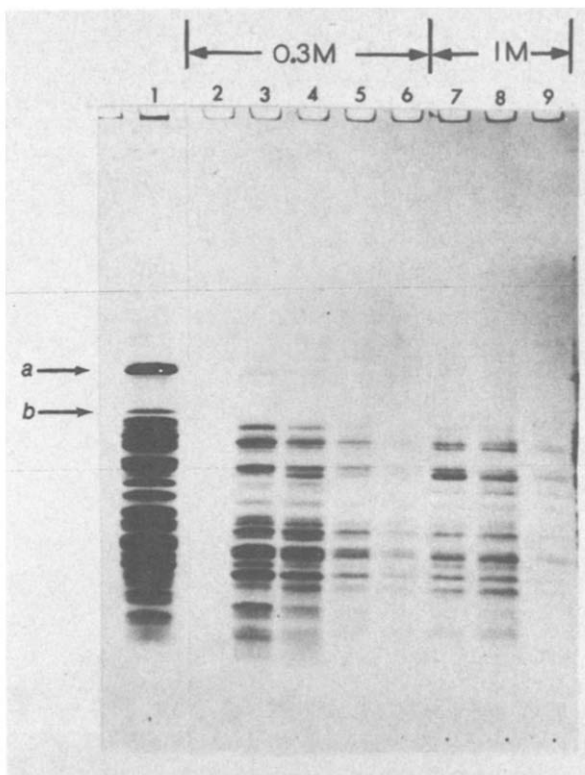

Fig. 3. Chromatography of *D. melanogaster* TP80 on poly A–Sepharose 4B. One-dimensional SDS polyacrylamide gel of fractions from a run of *D. melanogaster* TP80 on a poly A–Sepharose 4B column. Lane 1 contains the TP80 sample used as the load for this column; lanes 2–9 contain, from left to right, column fractions in order of elution, with buffers D (0.3 *M*) and E (1 *M*) as indicated. Lane 2 contains all of a 0.1-ml fraction, lane 3 contains 0.2 ml of a 1.25-ml fraction, and lanes 4–9 each contain 1.25 ml (of 1.25-ml total volume) of each fraction. Column contained 172 μg of poly A.

While many of the same TP80 bands are bound by both poly A and 5S rRNA in buffer D and eluted in buffer E, the enrichment of certain bands in the buffer E eluent and the one-dimensional gel profile of the buffer D flow-through of the 5S rRNA and poly A columns are clearly different from each other.

Metspalu et al.[10] have found that rat liver ribosomal protein L5 is bound to 5S rRNA columns even at 1 M LiCl and can be eluted with 8 M urea/4 M LiCl. We attempted, in some experiments, to see if any D. melanogaster proteins exhibited the same behavior by washing 5S rRNA columns with buffer U after the buffer E wash. This wash released proteins from the column which, by one-dimensional gel profile, were seen to be a complete complement of TP80, the load for the column. The same is true if a poly A column is washed with buffer U after washing with buffer E. The binding of TP80 to the column until eluted with buffer U seems to be a property of the Sepharose 4B resin itself and not the poly A or 5S rRNA as total TP80 can be eluted from a column made from adipic acid dihydrazide Sepharose 4B (not linked to any RNA) with buffer U after the normal buffer E wash (Fig. 4). In contrast to the poly A and 5S rRNA columns, however, the buffer D flow-through from the adipic acid dihydrazide Sepharose 4B control is identical to TP80. In addition, only a small amount (compared with poly A and 5S rRNA columns) of low molecular weight TP80 proteins (which are not the major bands eluted with buffer E from either 5S rRNA or poly A columns) is eluted from such a column with buffer E (Fig. 4).
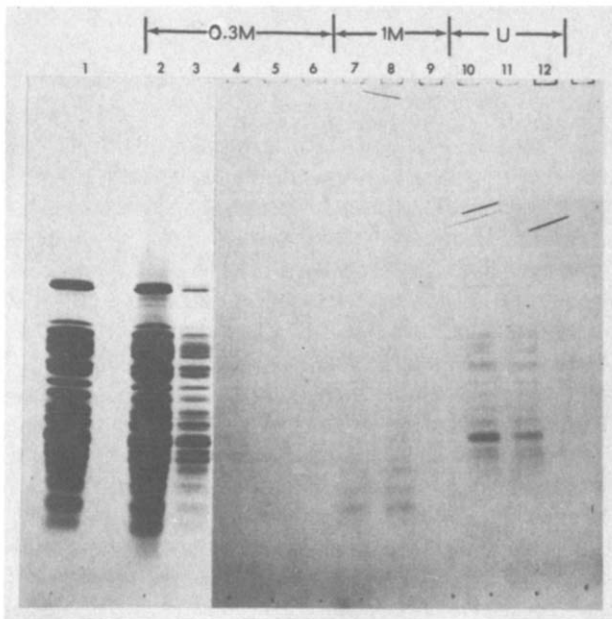

Fig. 4. Chromatography of D. melanogaster TP80 on adipic acid dihydrazide Sepharose 4B. D. melanogaster TP80 was chromatographed on adipic acid dihydrazide Sepharose 4B (not linked to RNA) as described in Materials and methods. Lane 1, TP80 load for this column; lanes 2–12, column fractions in order of elution, with elution buffers D (0.3 M), E (1 M), and U as indicated. Lanes 2 and 10–12 each contain 0.2 ml (of 1.0-ml total volume) of each fraction; lanes 3–9 each contain 1.0 ml (of 1.0-ml total volume) of each fraction. Column contained ca. 1.1 and 1.7 times as much activated Sepharose 4B as columns in Figs. 2 and 3, respectively.
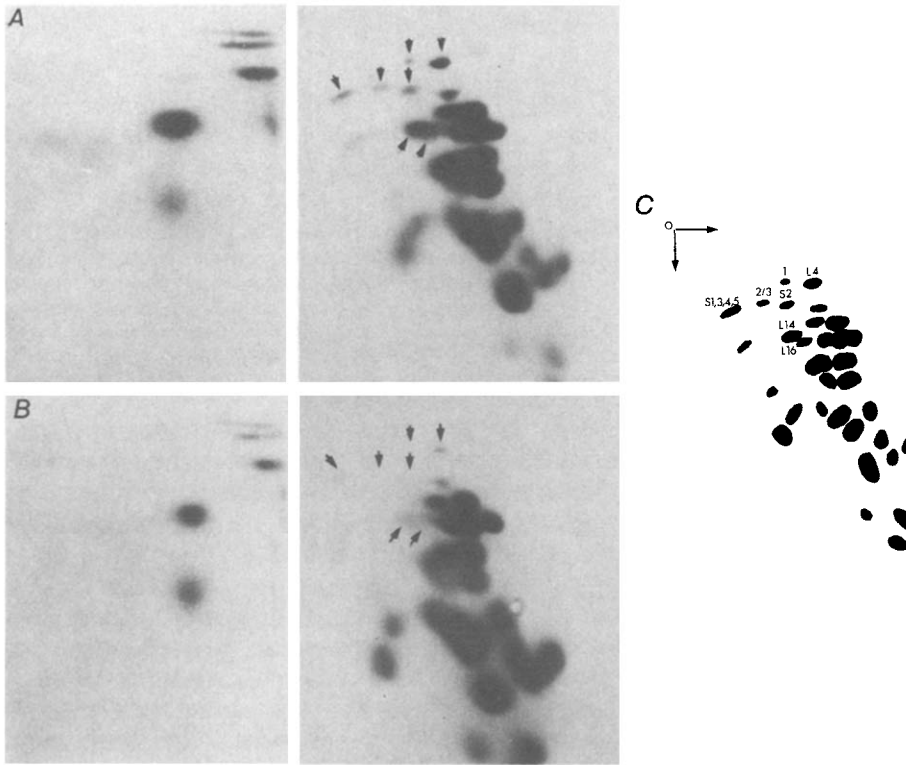
Fig. 5. Two-dimensional polyacrylamide gel electrophoretic analysis of chromatography of TP80 on 5S rRNA-Sepharose 4B columns. Two-dimensional gels were run as described in Materials and methods. (A) Acidic (left) and basic (right) proteins of *D. melanogaster* TP80. (B) Acidic (left) and basic (right) proteins of the buffer D flow-through of TP80 chromatographed on 5S rRNA–Sepharose 4B. (C) Schematic of the basic proteins in A with labeling of proteins consistently diminished in buffer D flow-throughs of 5S rRNA columns. Origins and directions of electrophoresis are as indicated.

(Similar results have been reported for *E. coli* ribosomal proteins[23].) Thus, for both 5S rRNA and poly A columns, the buffer D flow-through and buffer E elutions, but not the buffer U elution, appear to represent specific binding of certain TP80 proteins to 5S rRNA and poly A rather than to adipic acid dihydrazide Sepharose 4B.

The TP80 proteins which bind 5S rRNA in buffer D and are eluted with buffer E can be identified by a comparison of their two-dimensional gel profiles with that of TP80 (Fig. 5). The TP80 proteins consistently missing in the buffer D flow-through, and thus which apparently bind 5S rRNA strongly, are 1, L4, (also identified from one-dimensional gels), 2/3, L14/L16, and S1, S2, S3, S4, S5. These proteins are also seen, along with several other TP80 proteins, when the TP80 which bind 5S rRNA in buffer D and are eluted with buffer E are run on a 2-D gel (data not shown). If we assume that the TP80 profile represents equimolar amounts of all the ribosomal proteins, then those proteins which are seen in the buffer E elution but are not significantly reduced in the buffer D flow-through appear not to bind 5S rRNA as strongly as proteins 1, L4, 2/3, L14/L16, and S1, S2, S3, S4, and S5.

DISCUSSION

Our results show that both poly A and *D. melanogaster* 5S rRNA Sepharose 4B columns will bind a number of *D. melanogaster* total ribosomal proteins in 0.3 *M* potassium chloride (buffer D), at least some of which can be eluted with 1 *M* potassium chloride (buffer E). That poly A can bind a large number of ribosomal proteins indicates that many *D. melanogaster* TP80 proteins may have a non-specific binding capacity for nucleic acids. In this connection, there has been a report[24] that poly U Sepharose 4B columns will bind certain *E. coli* ribosomal proteins (which also bind *E. coli* 5S rRNA) in a salt concentration dependent manner. The overlap of the sets of proteins which bind to 5S rRNA and poly A that we see may indicate that some of those that bind to 5S rRNA bind non-specifically; the clear difference in the proteins not bound in buffer D and those bound in buffer D and eluted in buffer E between the two columns, however, indicates that there is some specificity in the binding of *D. melanogaster* proteins to 5S rRNA.

The possibility that the ribosomal proteins which bind to 5S rRNA are a mixture of those binding specifically and non-specifically notwithstanding the ribosomal proteins which bind 5S rRNA include both large and small subunit proteins. Studies of binding of rat liver ribosomal proteins to rat liver 5S rRNA have generally been done with total protein from 60S ribosomal subunits, but Ulbrich and Wool[9] have reported that no 40S ribosomal subunit proteins bind 5S rRNA when they are chromatographed on 5S rRNA Sepharose 4B columns. The binding of small subunit proteins of *D. melanogaster* TP80 to *D. melanogaster* 5S rRNA in our experiments may represent differences between the two systems, non-specific binding to 5S rRNA, or secondary binding of small subunit proteins to large subunit ribosomal proteins which initially bind the 5S rRNA on the column. This latter explanation is supported by experiments which show 40S subunit binding to preformed complexes of 5S rRNA and 60S subunit proteins on affinity columns[25] as well as other evidence which indicates that 5S rRNA is located near the 40S-60S subunit interface in eukaryotic ribosomes[26,27]. One of the *D. melanogaster* 5S rRNA binding proteins is 2/3 (the subunit location of which has not yet been determined), which has analogous mobility in the two-dimensional gel system used by us to rat liver protein L5. Rat liver protein L5 has been shown to be associated with 5S rRNA in subribosomal particles[3] and to bind rat liver 5S rRNA bound to Sepharose 4B[10]. In addition, Metspalu *et al.*[10] found binding to rat liver 5S rRNA of rat liver proteins L1 and L2; these two proteins have analogous two-dimensional gel mobilities to *D. melanogaster* proteins 1 and L4, two of the proteins which we find bind to *D. melanogaster* 5S rRNA. *D. melanogaster* proteins 1 and L4 also bind to poly A, however, and many be an example of proteins which bind 5S rRNA nonspecifically. The other *D. melanogaster* proteins which bind *D. melanogaster* 5S rRNA (S1, S2, S3, S4, S5, and L14/L16) do not have analogous two-dimensional gel mobilities with any rat liver ribosomal proteins, including those which have been shown to bind rat liver 5S rRNA[9,10], and it is not known whether there is any homology between them and the rat liver 5S rRNA binding proteins.

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