APPEARANCE OF A KIDNEY-SPECIFIC RIBOSOMAL PROTEIN DURING MOUSE EMBRYONIC DEVELOPMENT

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SUMMARY

Ribosomes were prepared from adult mouse liver and kidney and the protein components examined by SDS polyacrylamide gel electrophoresis. A kidney specific protein was identified and was found to be associated with the large ribosomal subunit. In vitro labelling of 11- and 14-day embryonic kidneys and subsequent analysis of the ribosomal proteins indicated a change in the ribosomal population during development. The kidney specific protein was synthesized during the first four days of kidney organogenesis.

INTRODUCTION

An increasing amount of work is being devoted to the analysis of eukaryotic ribosome structure (1,2). The question of tissue and/or organ specificity of some ribosomal proteins is controversial and results supporting (3,4) and contradicting (5) the occurrence of some tissue specificity have been reported. In viral/bacterial systems, viral invasion of the cell can trigger the new ribosomal proteins and the modification of existing ones (6), and ribosomal proteins appear to affect the initiation and translational efficiency of some messenger RNAs (7). If eukaryotic ribosomal proteins can play a similar role, and if they differ from tissue to tissue, they may be implicated in the control of differentiation.

We have investigated the ribosomal proteins from mouse kidney and liver, and have found differences among the higher molecular weight proteins. The synthesis of a kidney-specific ribosomal protein during the embryonic development of the kidney is also described.

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MATERIALS AND METHODS

Animals: Randomly bred CF_1 strain mice were used and embryonic kidney rudiments were incubated in vitro as previously described (8). Ribosomal proteins were labelled for 6 hr at 37° using either L-2(n)-^{3}H- or ^{35}S-methionine at a concentration of 50 μc/ml.

Ribosome preparation: Adult livers or kidneys were disrupted in 5 vol. of buffer A (0.05 M Tris-HCl (pH 7.6), 0.1 M NH_4Cl, 0.005 M magnesium acetate, 0.005 M 2-mercaptoethanol and 0.25 M sucrose) using a Ten-Broeck all-glass homogenizer and then centrifuged at 12,000 g for 10 min. The supernatant fraction was made 1% with Triton X-100 and centrifuged at 50,000 rpm in a type 65 Spinco rotor for 90 min. The resulting pellets were resuspended in buffer B (0.05 M Tris-HCl (pH 7.6), 0.24 M KCl, 0.01 M magnesium acetate, 0.005 M 2-mercaptoethanol and 0.25 M sucrose) and centrifuged at 12,000 g for 10 min. The supernate was then centrifuged at 50,000 rpm for 75 min and the pelleted ribosomes were resuspended in buffer B, cleared by low speed centrifugation, loaded over a 6 ml cushion of buffer B, containing 17% sucrose, and centrifuged at 50,000 rpm for 3 hr. The final pellets were suspended in buffer C (0.05 M Tris-HCl (pH 7.6), 0.05 M KCl, 0.01 M magnesium acetate and 0.25 M sucrose), cleared by low speed centrifugation and the ribosomes were precipitated using the ethanol-magnesium method (9) and stored at -20°. Embryonic rudiments were sonicated then homogenized together with carrier adult kidney and the ribosomes prepared as above. Ribosomal subunits were prepared using buffer A, in which the magnesium acetate was replaced by 0.01 M EDTA, for the formation of the gradients (10).

Electrophoresis: Polyacrylamide gels containing 11% acrylamide, 0.28% N,N-methylene bis-acrylamide and 0.1% sodium dodecyl sulfate (SDS) in 0.1 M sodium phosphate buffer, pH 7.2 were used. Proteins suspended in 0.02 M phosphate buffer containing 1% SDS, 1% 2-mercaptoethanol, and 15% sucrose were heated at 65° for 10 min, cooled and loaded on the gels in a volume of 50-100 μl. The electrophoresis buffer comprised 0.1 M sodium phosphate, pH 7.2

and 0.5% SDS, and electrophoresis was carried out at a constant current of 8 mamps per tube for 6 hr. Occasionally 14% acrylamide gels were run for 11 hr at 8 mamps per tube. Gels were stained in 1% fast green, destained by diffusion and scanned at 600 nm using a Gilford linear transport and recording spectrophotometer.

Determination of radioactivity: Individual protein bands were cut out, decolorized and solubilized by incubation at 50° for 12 hr in 30% H_2O_2, then heated to 100° for 1 min, cooled, mixed with 5 ml of a liquid scintillation cocktail (3a70; RPI Corp., Elk Grove Village, Illinois, U.S.A.), chilled, dark adapted and counted in a Nuclear Chicago Mark II liquid scintillation counter.

RESULTS

The ribosomal proteins analysed by SDS polyacrylamide gel electrophoresis were stained with fast green to allow quantitation of the protein present in the resolved bands (11) and enable the calculation of the approximate number of proteins per band (12). Kidney ribosomal proteins are resolved into 28 distinct bands (Figure 1). For convenience of description, the bands have been placed into one of four groups. Section A contains 7 bands, ranging in molecular weight from 61,000-170,000; all of the bands appear to represent proteins present in fraction amounts (i.e., at less than one copy per ribosome). Band A 4 is the most prominent and is sometimes further resolved into two. Section B contains 7 bands, of 40,000 to 58,000 in molecular weight; two of the bands appear to contain two protein equivalents per ribosome each; the remainder approximately one. Section C is less well resolved, but contains 12 distinct bands, ranging in molecular weight from 17,000-37,000. Individual bands contain from 2 to 7 protein equivalents per ribosome. Section D has two bands, 12,200 and 15,500 in molecular weight, with approximately 3 protein equivalents in each.

The kidney ribosomal preparation has a protein, number 5 in section B of the gel (Fig 2A), that clearly has no counterpart in the liver preparation (Fig 2B). This differential is illustrated in Figure 2, where band 3 is re-

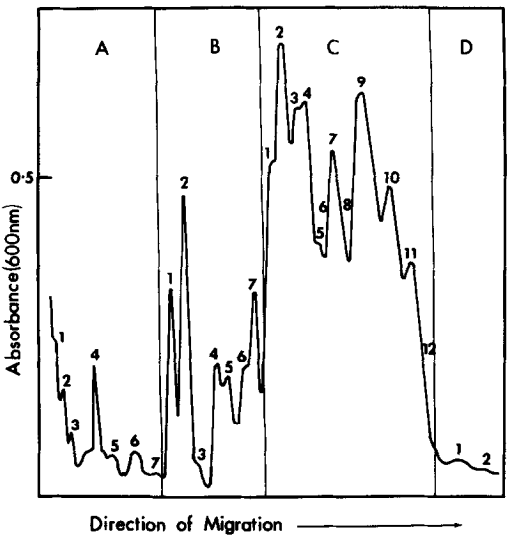

Figure 1: Electrophoretic analysis of mouse kidney ribosomal proteins on SDS-polyacrylamide gels. The figure shows a scan of the stained gel obtained using a linear transport of a Gilford recording spectrophotometer. The scan has been divided into sections (A,B,C,D). The numbers above the peaks in each sections represent the nomenclature used in the text and other figures. The direction of migration is from left (cathode) to right (anode).

solved into two by using 14% gels run for 11 hr and the other bands are clearly separated. There is also a reproducible quantitative difference in band B 1, kidney having approximately one protein equivalent per ribosome in this band and liver having approximately two. However, we wish to concentrate on kidney specific band B 5.

To test whether kidney band B 5 is truly ribosomal, or a minor cell sap contaminant or an mRNA-associated protein (13,14), and to assign this band and the other proteins to appropriate ribosomal subunits, the ribosomes were dissociated into subunits using EDTA-containing gradients. Thus, mRNA, tRNA (15) and 5S RNA with its associated protein (16) are lost, and dissociation eliminated additional proteins found only on 80S ribosomes (17). Hence, some true ribosomal proteins are probably lost from EDTA-subunits but the remaining proteins are strictly ribosomal. All the proteins detectable in the B region of the total ribosomes (Figure 2) are found associated with the large subunits (Figure 3) with the exception of a part of B 7 for kidney and a part of B 6 for

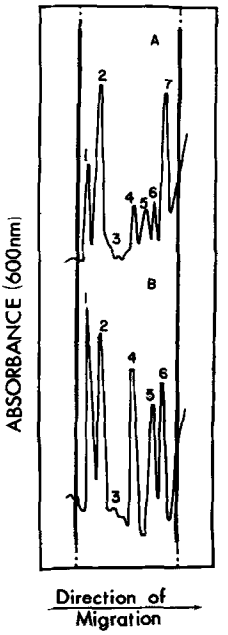

Figure 2: Comparison of mouse kidney (A) and liver (B) ribosomal proteins by SDS-polyacrylamide gel electrophoresis on 14% gels. Only the B section of the gels (see Figure 1) is presented in the figure. Other conditions are as in Figure 1.

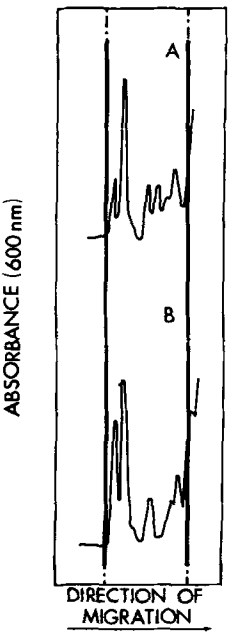

Figure 3: Comparison of mouse kidney (A) and liver (B) ribosomal proteins derived from the large ribosomal subunits by SDS-polyacrylamide gel electrophoresis on 11% gels. The ribosomal subunits were prepared by gradient centrifugation in the presence of EDTA. Only the B section of the gels is presented. Other conditions as in Figure 1.

liver, which partition with the small subunit. The kidney specific band B 5, therefore, is not removed as a result of EDTA treatment and is assignable to the large subunit.

Eleven-day and 14-day embryonic kidney rudiments were labelled with L-2(n)-^{3}H and ^{35}S-methionine, respectively, the tissues combined and ribosomes isolated using adult kidney tissue as carrier. The ratio of the percent of the ^{3}H counts to the percent ^{35}S counts for all the kidney ribosomal protein bands resolved by SDS-acrylamide gel electrophoresis is given in Table 1. Significant departures from unity in the ratio (greater than 16%, established in separate experiments using double labelled material from the same developmental stage) indicate changes in the methionine content of the band, presumably reflecting the concentration of such proteins during development. Eleven-day ribosomes have greater amounts of protein, or protein with different methionine content, at positions A 4, C 5 and D 2, while the 14-day ribosomes have greater

Table 1. Comparison of radioactivity distribution in ribosomal proteins of 11- and 14-day old embryonic kidney by the double label procedure, using SDS-acrylamide gels.

Peak number	R(%^{3}H/%^{35}S)	Peak number	R(%^{3}H/%^{35}S)
A 1 + 2	0.98	C 1	0.93
A 3	1.02	C 2	1.01
A 4	1.23	C 3	1.00
A 5	0.95	C 4	0.93
A 6 + 7	0.90	C 5	1.17
		C 6	1.00
B 1	0.92	C 7	0.91
B 2	0.68	C 8	1.07
B 3	0.87	C 9	0.80
B 4	0.97	C 10	0.96
B 5	0.64	C 11	1.12
B 6	0.88	C 12	1.06
B 7	1.00		
		D 1	1.09
		D 2	1.21

$$R = \frac{^{3}H \text{ cpm in band} \times 100}{\text{total } ^{3}H \text{ cpm in gel}} \Big/ \frac{^{35}S \text{ cpm in band} \times 100}{\text{total } ^{35}S \text{ cpm in gel}}$$

Incubation time: 6 hr *in vitro*, in the presence of radioactive methionine; ^{3}H-labelled 11-day embryonic kidney and ^{35}S-labelled 14-day kidney. The tissues were combined before isolation of the ribosomes. The underlined values for R indicate a significant departure from random variation in the ration. Other conditions are as in Figure 1 and in the Experimental section.

amounts of protein at B 2, B 5 and C 9 (Table 1). It should be particularly noted that the kidney specific protein B 5 is absent, or present at low concentration, in 11-day embryonic ribosomes and increases in concentration in 14-day ribosomes indicating a temporal correlation between the synthesis of a kidney specific ribosomal protein and kidney differentiation.

DISCUSSION

The description and characterization of kidney band B 5 constitutes a clear differential in ribosomal proteins between kidney and liver that is not affected by variations in isolation conditions and cannot be attributed to a variety of contaminants. This kidney specific protein is found associated with the large subunit after dissociation of the ribosomes by EDTA. Such a localization is of interest since differences between normal and tumor ribosomes appear to involve the appearance of additional large-subunit proteins in the latter (18). Furthermore, variations in eukaryote ribosomal size appear to be due generally to variations in the size of the large subunit (19); apparently, there has been scope for substantial evolutionary divergence in this subunit, while the small subunit has been more conservative.

The character of the ribosomal population appears to change from 11-days to 14-days of gestation, with the apparent loss of some proteins and the acquisition of others, including kidney band B 5. Such changes are correlated with the time of initiation of kidney differentiation (8). The occurrence and developmental history of band B raises the question of the functional role of such proteins. The possibility that they may influence the translational efficiency of particular mRNA's have been reported for a number of systems (20, 21), while the capacity of ribosomes to affect such differentials has also been documented (7,22). Experiments to determine specific functions for eukaryote tissue-specific ribosomal proteins are clearly desirable.

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