The Proteins of *Xenopus* Ovary Ribosomes

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1. The preparation of ribosomes and ribosomal subunits from *Xenopus* ovary is described. 2. The yield of once-washed ribosomes (buoyant density in caesium chloride $1.601 \text{ g} \cdot \text{cm}^{-3}$; 44% RNA, 56% protein by chemical methods) was 10.1 mg/g wet wt. of tissue. 3. Buoyant density in caesium chloride and RNA/protein ratios by chemical methods have been determined for ribosome subunits produced by 1.0 mM-EDTA or 0.5 M-potassium chloride treatment and also for EDTA subunits extracted with 0.5 M-, 1.0 M- or 1.5 M-potassium chloride. 4. Analysis of ribosomal protein on acrylamide gels at pH 4.5 in 6 M-urea reveals 24 and 26 bands from small and large EDTA subunits respectively. The actual numbers of proteins are greater than this, as many bands are obviously doublets. 5. Analysis of the proteins in the potassium chloride extract and particle fractions showed that some bands are completely and some partially extracted. Taking partial extraction as an indication of possible doublet bands it was found that there were 12 and 20 such bands in the small and large subunits respectively, making totals of 36 and 46 proteins. 6. From the measured protein contents and assuming weight-average molecular weights for the proteins of large and small subunits close to those observed for eukaryote ribosomal proteins it is possible to compute the total numbers of protein molecules per particle. It appears that too few protein bands have been identified on acrylamide gels to account for all the protein in the large subunit, but probably enough for the small subunit.

Detailed analysis of ribosome function and assembly in eukaryotes will be more difficult to gain than the knowledge of prokaryote ribosomes obtained by Nomura (1970) and Kurland (1970). There are several reasons why it will be more difficult to obtain this detailed knowledge. First, eukaryote ribosomes are not easily separated into functional subunits (Martin & Wool, 1969). Secondly, analysis of the prokaryote ribosome has been helped considerably by the large number of ribosome-function and -assembly mutants isolated (Apirion & Schlessinger, 1969); in particular the ribosomal-ribonuclease-minus strains have greatly decreased the technical problems. Similar genetical techniques are much more difficult to apply to eukaryotes. Thirdly, separation of eukaryote ribosomal proteins in quantity is proving much more difficult than for prokaryotes, and fourthly, not only is there more total protein in each eukaryote ribosome but also there is a greater variety of proteins.

It is known that high salt concentrations will remove specific groups of proteins from prokaryote ribosomes (Meselson, Nomura, Brenner, Davern &

Schlessinger, 1964; Spitnik-Elson & Atsmon, 1969) and this has been shown for rat liver ribosomes (Reboud, Hamilton & Petermann, 1969; Grummt & Bielka, 1970; Clegg & Arnstein, 1970). Craven, Voynow, Hardy & Kurland (1969) have shown that some prokaryote ribosomal proteins, called fractional proteins, do not occur on all ribosomes all the time, as they are present in less than one mol/mol of RNA in a given preparation. Gould (1970) has shown that in rabbit reticulocytes some ribosomal proteins may be present more than once as their total molecular weights are not sufficient to account for all the protein observed.

In the present work I have set lower limits on the number of proteins to be isolated from *Xenopus* ovary ribosome subunits. I have also shown that the proteins from the two subunits differ both electrophoretically and in the way they respond to salt treatment. Further, it is likely that by increasing the salt concentration protein is eluted from the two EDTA-derived ribosomal subunits in a continuous, not a discontinuous, manner. By using the elution of proteins by salt it is possible to identify protein bands on acrylamide gels that may contain more than one protein and thus to work

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out an upper limit to the number of proteins present in each subunit. The conclusion is reached that there is a sufficient number of proteins present for them all to be present once only in each small subunit but not for the large subunit.

MATERIALS AND METHODS

Materials. All chemicals were from BDH Chemicals Ltd. (Poole, Dorset, U.K.) and were AnalaR grade except for the following: tris was from Sigma (London) Chemical Co. Ltd., London S.W.6, U.K.; acrylamide, *N,N'*-methylenebisacrylamide and *NNN'*-tetraethylmonoethylenediamine were from Kodak Ltd., Kirkby, Liverpool, U.K.; urea was from May and Baker, Dagenham, Essex, U.K.; dithiothreitol was from Calbiochem, London W.1, U.K.; *N*-ethylmaleimide and 2-mercaptoethanol were from Koch-Light Laboratories Ltd., Colnbrook, Bucks., U.K.; Polyak-RNA was from Penninsular Chem. Research Inc., Gainesville, Fla., U.S.A.

Animals. Mature female *Xenopus laevis laevis* Daudin were purchased from The South African Snake Farm, Fish Hoek, Cape Province, South Africa, and kept six to eight per box (80 cm × 40 cm × 25 cm) in about 15 cm of water. They were fed on liver or heart and the boxes were cleaned out twice weekly.

Solutions. Compositions of buffers were as follows: A, 50 mM-tris-HCl, pH 7.6, -35 mM-KCl-1.5 mM-MgCl₂; B, 0.25 M-sucrose in buffer A; C, 50 mM-tris-HCl, pH 7.6, -35 mM-KCl-1.0 mM-EDTA (disodium salt); D, 50 mM-tris-HCl, pH 7.6, -0.5 M-KCl.

Preparation of ribosomes. Animals were killed by pithing and the ovaries were removed into ice-cold pre-weighed medium A. After reweighing, the ovaries were homogenized in 2-3 vol. of medium B by using a Teflon-glass homogenizer at 1000-2000 rev./min by three complete strokes. The connective tissue was removed by filtration through muslin and the homogenate was centrifuged at 20 000 rev./min for 15 min at 0°C in a Sorvall RC2B centrifuge (8 × 40 rotor). A large pellet of yolk and pigment overlaid by mitochondria was formed, together with a thick pellicle of fat. The opaque, straw-coloured supernatant was withdrawn with a syringe and centrifuged at 35 000 rev./min for 180 min at 4°C in a precooled 8 × 50 rotor of an MSE 65 centrifuge. The tubes were only half-filled, as it was found that this improved the yield. The clear straw-coloured supernatant was removed and the tube walls dried with tissue paper. The pellet was in

two layers, a hard, clear, yellow, lower layer and a loose, opaque, reddish, upper layer. The two layers were usually easily separable and it was found that the upper layer contained over 80% of the ribosomes and was contaminated by a membranous component and ferritin, and the lower layer contained much glycogen.

After rinsing (with medium B) the entire pellet was resuspended in the same medium by gentle homogenization, by using a glass ball made to fit the centrifuge tubes, to give a crude ribosomal solution (approx. 20 mg of ribonucleoprotein/ml). In some experiments the crude ribosomal solution was re-centrifuged at 60 000 rev./min for 60 min in a precooled 8 × 25 titanium rotor in an MSE 65 centrifuge. The washing removed a great deal of protein, including one with an absorption maximum at 420 nm, giving the second supernatant a reddish colour. The hard pellet was a pale straw colour and was resuspended in medium A to give a concentration of 20 mg of ribonucleoprotein/ml. The once-washed ribosomes were called 'pure' ribosomes.

Table 1 gives the yields and various properties of the crude and pure ribosomes prepared from *Xenopus* ovary by the above method and is discussed below.

Preparation of ribosome subunits. Ribosome subunits were prepared in an MSE BXIV titanium zonal rotor. A linear 10-40% (w/v) sucrose gradient in the appropriate buffer (buffer C for EDTA subunits and buffer D for KCl subunits) was introduced and followed by a 'cushion' of 45% (w/v) sucrose. The volume of the gradient was usually 400 ml. The sample of ribosomes (20 mg/ml) in medium A was treated either with EDTA (3 mM final concn.) or KCl (0.5 M final concn.), made 3% with sucrose, and then layered on the gradient followed by an overlay of the appropriate buffer. The sample volume was usually 25 ml, and the overlay was usually 200 ml, and as the rotor has a total volume of 675 ml there was usually 50 ml of 'cushion' left in the rotor during the run. The rotor was run at 47 000 rev./min for 180-360 min. It was found that the temperature of the rotor was extremely difficult to control until a two-stage pump had been fitted to the centrifuge. Before this modification was made the temperature of the solution would rise from about 5-6°C on entry to the pre-cooled (-2°C) rotor, to about 18-20°C on exit after 180 min at 47 000 rev./min with the maximum thermostat setting. The temperature was measured by means of a thermistor sited in the fluid lines about 10 cm from the feed head, which was cooled continuously with ice-cold water pumped at 20 ml/min. After modification the temperature would remain constant at the input value

Table 1. *Chemical and physical properties of crude and pure ribosomes from Xenopus ovary*

The results are given as the means ± S.E.M. with the numbers of determinations in parentheses. Values for '% RNA' are calculated from chemical and buoyant-density determinations (see Table 5).

Ribosomes	Yield (mg/g wet wt.)	RNA/protein ratio	Buoyant density (g·cm ⁻³)	<i>E</i> ₂₄₀ / <i>E</i> ₂₆₀ ratio	<i>E</i> ₂₈₀ / <i>E</i> ₂₆₀ ratio	% RNA	
						Chemical	Buoyant density
Crude	11.9 ± 0.45 (22)	0.466 ± 0.01 (5)	1.606 ± 0.002 (3)	0.750 ± 0.006 (22)	0.589 ± 0.009 (22)	32	56
Pure	10.1 ± 0.56 (22)	0.790 ± 0.03 (14)	1.601 ± 0.005 (3)	0.707 ± 0.014 (13)	0.553 ± 0.024 (13)	44	55

(5–6°C) over the 360 min run time at 47 000 rev./min with the maximum thermostat setting.

At the end of the run the sample was recovered [by displacement with 50% (w/v) sucrose] through a variable-path flow cell in a Perkin–Elmer 137 spectrophotometer recording continuously at 260 nm. Typical results are shown in Fig. 1 and recoveries are given in Table 2. The resolution deteriorated when samples larger than 500 mg were run; likewise samples in less than 10 ml showed no improvement in resolution (Eikenberry, Bickle, Traut & Price, 1970).

Recovery of ribosome subunits from zonal fractions. The zonal fractions were dialysed for 2–3 h against either buffer C or buffer D to lower the sucrose concentration and then centrifuged at 35 000 rev./min for 16 h in an MSE 8×50 rotor at 4°C, or at 60 000 rev./min for 2 h at 4°C in an MSE

8×25 rotor. The recovery of the small subunit was usually greater than 60% and that of the large subunit greater than 80%.

Salt treatment of ribosome subunits. EDTA-derived ribosome subunits were produced by zonal centrifugation and dialysed against either buffer A or buffer C for 2–3 h to lower the sucrose concentrations. After dialysis the fractions were centrifuged at 20 000 rev./min for 15 min in a Sorvall RC2B centrifuge 8×40 rotor to remove aggregated material. The material removed at this step was probably membranous, as it contained little nucleic acid and formed a buff-coloured pellet quite unlike the translucent pellet characteristic of glycogen. Moreover, it was noted that the membranous material in the original ribosome preparations is aggregated by EDTA treatment.

The supernatants were divided into four portions and

Fig. 1. (a) Profile of the amount of RNA and protein and the E_{320} from a zonal fractionation of EDTA-treated ribosomes. Ovary (32 g) gave 4200 E_{260} units of ribosomes, which were resuspended in medium C to give a final volume of 20 ml. The ribosomes were layered over a 350 ml linear 6–40% (w/v) sucrose gradient in medium C and followed by a 250 ml overlay of medium C. After 2½ h at 46 000 rev./min the gradient was recovered through a variable-path flow cell in a u.v. spectrophotometer recording continuously at 260 nm. The temperature of the effluent was 7.5°C. Fractions (5×50 ml and 20×20 ml) were collected and the amount of RNA and protein and the E_{320} were measured. The centrifugal field in the sample zone was 100 000g and the maximum centrifugal field was 150 000g. (b) Profile of the amount of RNA and protein and the E_{320} from a zonal separation of 0.5 M-KCl-treated ribosomes in an MSE BXIV rotor. Ovary (50 g) gave 5900 E_{260} units of ribosomes, which were re-suspended in medium D to give a final volume of 26 ml. The ribosomes were layered over a 350 ml linear 20–45% (w/v) sucrose gradient in medium D and followed by a 250 ml overlay. After 2½ h at 46 000 rev./min the gradient was recovered through a variable-path flow cell as described in (a) above. The temperature of the effluent was 9.0°C. Fractions (5×50 ml and 21×20 ml) were collected and the amount of RNA and protein and the E_{320} were measured. The centrifugal field in the sample zone was 100 000g and the maximum centrifugal field was 150 000g. RNA, ○; protein, ●; E_{320} , △.

Table 2. *Ribosome extraction and zonal centrifugation of EDTA-treated ribosomes*

For experimental details see the text. Ovaries from four adult females had a total weight of 81.3g. The yield of ribosomes was 12mg/g wet wt. Zonal fractions 1 and 3 are pooled fractions from the sample zone to the beginning of the 30S subunit zone and from the zone between the 30S and 50S subunit peaks respectively.

Fraction	Total E_{260}	E_{240}/E_{260} ratio	E_{280}/E_{260} ratio	Total RNA (mg)	Total protein (mg)	RNA/protein ratio
Crude ribosomes	9700	0.711	0.581	705	1510	0.467
Pure ribosomes	8190	0.655	0.553	696	849	0.820
Ribosome supernatant	1670	0.801	0.622	21	623	0.003
Zonal fractions						
1	190	0.713	0.556	13	42	0.310
2 (30S)	1520	0.641	0.547	112	115	0.975
3	781	0.645	0.542	48	48	1.000
4 (50S)	3260	0.635	0.540	304	297	1.025
Total	5751	—	—	477	512	0.930
Recovery (%)	70	—	—	68	60	—

made 0.5, 1.0 or 1.5M with respect to KCl, one portion remaining untreated. The concentration of ribonucleoprotein during the salt treatment was 1.4–1.8mg/ml for the small subunit and 2.7–3.1mg/ml for the large subunit. The treatment was performed at 0°C in ice for 30 min. The solutions were then layered over 10ml of 20% (w/v) sucrose in the appropriate buffer and centrifuged at 35000rev./min for 16h at 4°C in an MSE 8×50 rotor. The supernatants were recovered by syphoning and the pellets rinsed with buffer B before resuspension. Some 70–80% of the particles derived from the large subunit were recovered in the pellet, but only 10–20% of those from the small subunit; 50–60% of the latter were in the 20% sucrose 'cushion'. EDTA-derived particles, KCl-derived particles and KCl-treated EDTA particles, after storage at –20°C or after recovery by centrifugation, formed large aggregates, which made the suspensions opaque, but they could be clarified by heating them to 37°C for 5–10 min. Attempts to measure the sedimentation coefficients of these particles were unsuccessful because of the presence of large numbers of small aggregates (dimers, trimers etc.). The results of a salt-treatment experiment giving the recoveries, the RNA/protein ratios, and the u.v.-absorption ratios are given in Table 3.

Extraction of ribosomal proteins. (a) By the 66% acetic acid method (Leboy, Cox & Flaks, 1964). The ribosome or subunit preparation was mixed with 2 vol. of acetic acid at 4°C and stirred for 16–18h. The precipitate (RNA) was removed by centrifugation at 20000rev./min for 30 min in a Sorvall RC2B 8×40 rotor. The supernatant was dialysed against many changes of water and the protein was recovered by freeze-drying.

(b) By the 2M-LiCl-6M-urea method (Moore, Traut, Noller, Pearson & Delius, 1968). The ribosome preparation was precipitated with 1 vol. of ethanol and recovered by centrifugation (3000rev./min for 30 min at 4°C in an MSE Mistral centrifuge). The pellet was dissolved in freshly deionized 6M-urea and then made 2M with solid LiCl. The solution was left at 4°C for 16–18h and the precipitate was removed by centrifugation at 40000rev./min for 30 min in an MSE 10×10 rotor at 4°C. The supernatant was dialysed exhaustively against 50mM-sodium acetate buffer pH 4.5 in 6M-urea.

(c) By the 4M-guanidine-HCl method (Cox, 1968). The ribosomes were recovered by ethanol precipitation and dissolved in 4M-guanidine-HCl at 4°C and the pH was adjusted to 5.0 with a drop of 3.0M-sodium acetate buffer, pH 5.0. A precipitate formed on standing and was removed by centrifugation at 40000rev./min for 30 min in an MSE 10×10 rotor at 4°C. Proteins were recovered from the supernatant by precipitation with 8 vol. of ethanol at –20°C overnight and centrifugation at 20000rev./min for 30 min in a Sorvall RC2B 8×40 rotor. The precipitated proteins were dissolved in 6M-urea and the pH was adjusted to 5.0 with 3.0M-sodium acetate, pH 5.0.

Reduction of ribosomal proteins. Hardy, Kurland, Voynow & Mora (1969) have shown that interchain disulphide bonds may be the cause of at least one anomalous band in electrophoretic separations of *Escherichia coli* ribosomal proteins. Accordingly all protein preparations were reduced. Two methods were used, reduction by dithiothreitol followed by blocking with *N*-ethylmaleimide, or reduction by 2-mercaptoethanol and its inclusion at all subsequent stages.

(a) Reduction by dithiothreitol. When a protein preparation was first dissolved in urea at pH 8.4 with 20mM-tris-acetate buffer it was treated with 10mM-dithiothreitol for 30 min at 20°C, and then by 10mM-*N*-ethylmaleimide at pH 7.4 for 30 min at 20°C. The pH was adjusted to 5.0 with 3.0M-sodium acetate buffer, pH 5.0, and the proteins were dialysed against 50mM-sodium acetate, pH 5.0, in 6M-urea.

(b) Reduction by 2-mercaptoethanol. The protein was dissolved in 10mM-2-mercaptoethanol in 6M-urea-20mM-tris-acetate, pH 8.4,–12mM-methylamine at 20°C for 1 h, then dialysed against 50mM-sodium acetate (pH 5.0)–6M-urea–1mM-2-mercaptoethanol.

Electrophoresis of ribosomal proteins. For this 10g of acrylamide, 0.25g of *N,N'*-methylenebisacrylamide and 100mg of ammonium persulphate were dissolved in 50mM-sodium acetate-acetic acid buffer, pH 4.5, in 6M-urea and made up to 100ml. The mixture was filtered (Sartorius membrane filter; 0.45µm) and degassed; then 0.15ml of *NNN,N'*-tetraethylmonoethylenediamine was added with swirling. Portions (7.5ml) were pipetted into 18cm×0.8cm tubes and over-layered with degassed water. The tubes were pre-run with 0.66M-2-alanine–

Table 3. Salt fractionation of ribosome subunits produced by zonal centrifugation of EDTA-treated ribosomes in an MSE BXIV rotor

Subunits were prepared by zonal centrifugation in an MSE BXIV rotor. Portions were treated with various concentrations of KCl at 0°C for 30 min and layered over 20% (w/v) sucrose in the appropriate buffer. After centrifugation the tube contents were fractionated by siphoning to give two fractions plus a pellet. Fraction 1 corresponds to the volume of sample layered on the sucrose 'cushion', which is recovered as fraction 2.

Sample	Total E_{260}	E_{240}/E_{260} ratio	E_{280}/E_{260} ratio	Total RNA (mg)	Total protein (mg)	RNA/protein ratio
30S subunit	305	0.628	0.521	23	27	0.850
30S, 0.5M-KCl-treated						
Fraction 1	26.4	0.948	0.651	0.17	6.25	0.026
Fraction 2	89.0	0.674	0.539	6.38	7.75	0.870
Pellet	156.0	0.588	0.497	9.66	7.73	1.250
Recovery (%)	89			70	80	
30S, 1.0M-KCl-treated						
Fraction 1	22.0	0.948	0.763	0.162	15.25	0.011
Fraction 2	116	0.647	0.522	7.13	3.81	1.870
Pellet	164.9	0.564	0.489	9.98	5.21	1.910
Recovery (%)	99			75	93	
30S, 1.5M-KCl-treated						
Fraction 1	27.7	0.962	0.745	0.03	20.70	0.001
Fraction 2	89.0	0.647	0.539	8.45	3.56	2.37
Pellet	181.5	0.572	0.484	10.98	3.87	3.07
Recovery (%)	97			84	104	
50S subunit	635	0.632	0.530	56.0	56.5	0.992
50S, 0.5M-KCl-treated						
Fraction 1	14.4	1.110	0.820	0.18	12.8	0.014
Fraction 2	15.3	0.900	0.702	0.21	9.2	0.025
Pellet	646.0	0.621	0.519	58.8	37.9	1.545
Recovery (%)	106			106	104	
50S, 1.0M-KCl-treated						
Fraction 1	17.4	1.400	1.085	0.213	25.3	0.009
Fraction 2	22.0	1.010	0.732	0.257	9.4	0.027
Pellet	682.0	0.619	0.506	57.0	23.0	2.480
Recovery (%)	107			102	102	
50S, 1.5M-KCl-treated						
Fraction 1	13.3	1.460	1.145	0.140	25.3	0.006
Fraction 2	19.2	1.045	0.758	0.203	8.7	0.023
Pellet	646.0	0.609	0.496	57.0	18.2	3.130
Recovery (%)	106			102	92	

acetic acid buffer, pH 4.5, in 6M-urea at 4 mA/gel (constant current) for 3–6 h. The electrode buffer was changed and the samples were underlayered in 6M-urea–10% sucrose, Methylene Blue being added as a tracking dye. The gels were electrophoresed at 2 mA/gel (constant current) for 16–18 h, by which time the dye had migrated to the end of the gel. It was found that the biggest single factor in the reproducibility of separations was a rapid gelling time, which was 20–30 min under these conditions.

The gels were removed and stained with 1% Amido Black in methanol–acetic acid–water (4:1:5, by vol.). They were destained in the same solvent without the dye.

Analytical sucrose gradients. Ribonucleoprotein particles were run in 10–45% (w/v) linear sucrose gradients in medium A, and RNA preparations were run in 5–20% (w/v) linear sucrose gradients in 10 mM-sodium acetate, pH 5.0,–0.1 mM-EDTA. The gradients were pumped

out by displacement either through an Isco density-gradient fractionator or through a flow cell in a Perkin-Elmer UV 137 spectrophotometer recording at 260 nm.

Extraction of RNA. RNA was extracted by the cold sodium dodecyl sulphate–phenol method of Brown & Littna (1964). As well as polyvinyl sulphate at 100 µg/ml, macaloid (a gift from Dr J. R. Tata) and polytak RNA were used as ribonuclease inhibitors at a concentration of 100 µg/ml. RNA preparations were stored in ethanol at –20°C until required.

Base-ratio analysis. The method of Katz & Comb (1963) was used and the extinction coefficients given by them were used for computation of the molar ratios.

Determination of buoyant density in caesium chloride. Ribonucleoprotein particles were dialysed into a buffer (50 mM-triethanolamine–HCl, pH 8.6,–50 mM-KCl) and fixed for 24 h in 6% (v/v) (neutralized) formaldehyde. The

fixed particle preparation was mixed with the light CsCl solution and 5 ml linear gradients were prepared between the heavy and the light solutions; the resulting CsCl gradient contained a gradient of fixed particles in the opposite direction. The gradients were centrifuged at 35000 rev./min for 18 h in a Spinco L2 centrifuge at 6°C in an SW39 rotor.

The gradients were collected in 35–40 seven-drop fractions. Every fourth tube was used for measurement of refractive index (n) at 20°C, from which the density was calculated according to the equation $\rho = (10.860 \times n) - 13.942$ g/ml (Meselson *et al.* 1964). The other fractions were diluted with 1 ml of deionized water and the E_{260} values read with a Unicam SP.500 spectrophotometer. The gradients and E_{260} values were plotted together and the buoyant density of the peak was calculated by extrapolation.

Determination of RNA. RNA was determined by the method of Fleck & Munro (1962) by using $E_{1\text{cm}}^{1\%}$ at 260 nm = 250 to calculate the mass of RNA.

Determination of protein. The method of Lowry, Rosebrough, Farr & Randall (1951) was used, with bovine serum albumin as standard.

Determination of ribonucleoprotein. For the calculation of the mass of ribonucleoprotein from the $E_{1\text{cm}}^{1\%}$ at 260 nm = 100 was used.

RESULTS

Extraction of ribosomes from *Xenopus* ovary. The absolute amount of ribosomes in a mature *Xenopus* ovary, measured by an isotope-dilution experiment, was 15 mg of 80S ribosomes/g wet wt. of ovary. Table 1 shows that it is possible to extract 12 mg of crude ribosomes and 10 mg of pure ribosomes/g wet wt. of mature *Xenopus* ovary, which represents a recovery of 80 and 66% respectively of the total ribosome content. The weight of ovary in a mature female varies considerably; 120 g females from the wild (age unknown) have 10–20 g ovaries, whereas 100 g laboratory-reared females 3–4 years old have 5–10 g ovaries. It is thus possible to obtain 50–200 mg of pure ribosomes from the ovaries of a single female toad.

Measurements of RNA and protein (Table 1) show that for every 100 mg of RNA in crude ribosomes there is 212 mg of protein, and for every 100 mg of RNA in pure ribosomes there is 127 mg of protein. Thus the washing procedure removes 40% of the crude ribosome pellet protein. If the protein removed by washing was ribosome-bound then it might be possible to detect a buoyant-density difference between crude and pure ribosomes. The buoyant densities of crude and pure ribosomes were found to be identical (Table 1). This is taken to mean that the protein removed by washing was not bound to the crude ribosomes in a form fixable by formaldehyde treatment. The RNA/protein ratio calculated from the buoyant density of 80S ribosomes (1.04) is much higher than the ratio (0.790) obtained by chemical means, and may indicate that

further non-ribosome-bound protein exists in the pure ribosome pellet. Indeed, for every 100 mg of RNA in ribosomes of buoyant density $1.583 \text{ g} \cdot \text{cm}^{-3}$ there should be 97 mg of protein; however, the amount measured for pure ribosomes was 127 mg, which means that 24% of the pure ribosome pellet protein is not bound to ribosomes in a form fixable by formaldehyde.

Extraction of ribosomal proteins. Three methods for the extraction of ribosomal proteins were tried, the 66% acetic acid method (Leboy *et al.* 1964), the lithium chloride-urea method (Moore *et al.* 1968), and the guanidine-HCl method (Cox, 1968). In the first experiment ribosomes labelled *in vivo* for 9–10 months with ^3H - or ^{14}C -labelled amino acids were prepared from *Xenopus* ovary by zonal centrifugation. The ribosomes were extracted first by 66% acetic acid and then by lithium chloride-urea. The lithium chloride-urea treatment removed protein that remained bound to the RNA after the acetic acid treatment. It was shown by electrophoresis that these lithium chloride-urea-soluble proteins were distinct from the acetic acid-soluble proteins.

In a second experiment ovaries were labelled for 5 days *in vitro* with ^3H]lysine and ^{14}C]lysine separately and the ribosomes were extracted with guanidine-HCl or lithium chloride-urea each with or without $\frac{1}{2}$ vol. of ethanol. The results show that with guanidine-HCl plus ethanol 25–32% of the radioactivity is lost in the pellet, but guanidine-HCl minus ethanol extracts 97% of the radioactivity whereas with lithium chloride-urea plus ethanol 7–8% of the radioactivity is lost in the pellet; lithium chloride-urea without ethanol extracts 98% of the radioactivity. It is concluded that the guanidine-HCl and lithium chloride-urea procedures are equally efficient methods of extracting ribosomal protein and are both more efficient than the 66% acetic acid method. Electrophoresis showed that qualitatively there is little difference between the guanidine-HCl and lithium chloride-urea methods.

Purity of ribosome subunits produced by zonal centrifugation. Routine purity checks were not undertaken because it was established in the initial experiments that EDTA-derived ribosome subunit preparations contained 100% small or large subunits, and potassium chloride-derived subunit preparations contained 100% small subunits but only 90% large subunits, the latter being contaminated with 10% of small subunits.

Three criteria of purity were used; fractions were re-run on analytical gradients, the RNA was extracted and run on analytical gradients, and the base ratio of the RNA was measured. Fig. 2 shows the percentage of each EDTA- or potassium chloride-derived subunit in particular fractions

across a zonal gradient and indicates that a constant proportion of small potassium chloride-derived subunits contaminated the large subunit fractions. Analysis of the RNA from subunit preparations agrees with the analysis of the ribonucleoproteins in the purity of each preparation. Titration of the RNA to pH 1.0 with hydrochloric acid followed by neutralization reveals hidden breaks in the RNA. Sucrose gradients of RNA from ribosome subunits before and after titration revealed that the RNA of the large subunit was more readily damaged than the RNA of the small subunit. Base ratios determined by the method of Katz & Comb (1963) on RNA derived from zonal preparations of small and large ribosome subunits are given in Table 4 and are consistent with the published values (Brown & Weber, 1968; Loening, Jones & Birnstiel, 1969), although determined in a different way.

Properties of EDTA- and potassium chloride-derived subunits. The RNA and protein composition and buoyant density of ribosome subunits prepared by zonal centrifugation were determined and are given in Table 5; the chemical and buoyant-density results are in reasonable agreement. The two subunits are demonstrably different in overall chemical composition and buoyant density, and also in their response to treatment with 0.5M-potassium chloride. Table 5 shows that the EDTA- and potassium chloride-derived small ribosome subunits are indistinguishable on the basis of chemical composition (46 and 47% RNA respectively) and buoyant density (42% RNA each). However, the EDTA-derived large ribosome subunit has an RNA content of 50% both by chemical and buoyant-density determination, whereas the potassium chloride-derived large ribosome subunit has 60–65% RNA.

Fig. 2. Purity of 20 ml fractions produced by zonal centrifugation (see Fig. 1 and text for details). Samples of ribonucleoprotein particles were analysed as described in the text. The areas under the peaks were measured by planimetry and the percentage of each particle was calculated, (a) for a zonal gradient run in EDTA and (b) for a zonal gradient run in 0.5M-KCl. 30S subunit, ○; 50S subunit, ●.

Table 4. Base composition of RNA extracted from EDTA-derived ribosome subunits separated by zonal centrifugation in an MSE BXIV rotor

The results are given as the mean of two determinations $\pm$ S.E.M.

Subunit	Composition (mol %)				
	UMP	CMP	AMP	GMP	C+G
Small	24.7 $\pm$ 0.3	22.8 $\pm$ 0.7	21.1 $\pm$ 0.2	31.3 $\pm$ 0.5	54.2
Large	19.7 $\pm$ 0.1	27.0 $\pm$ 0.2	17.0 $\pm$ 0.1	36.4 $\pm$ 0.2	63.4

Table 5. Some chemical and physical properties of various subunits of *Xenopus* ovary 80S ribosomes

'% RNA' is calculated from the reciprocal of the buoyant density by the formula $\% \text{ RNA} = 100(\bar{v}_p - \bar{v}_{\text{os}})/(\bar{v}_p - \bar{v}_r)$ assuming the partial specific volume of RNA ($\bar{v}_r$) and protein ($\bar{v}_p$) to be 0.531 and 0.738 respectively; $\bar{v}_{\text{os}}$ is the reciprocal of the buoyant density in CsCl (Hamilton & Ruth, 1969). The results are given as the mean $\pm$ s.e.m. with the number of experiments in parentheses.

Particle	RNA/protein ratio	Buoyant density ($\text{g} \cdot \text{cm}^{-3}$)	E_{240}/E_{260} ratio	E_{280}/E_{260} ratio	% RNA		% of protein lost	
					Chemical	Buoyant density	Chemical	Buoyant density
Pure 80S	0.790 $\pm$ 0.029 (14)	1.583 $\pm$ 0.009 (48)	0.707 $\pm$ 0.014 (13)	0.553 $\pm$ 0.024 (13)	44	51	0	0
30S, EDTA	0.849 $\pm$ 0.032 (21)	1.540 $\pm$ 0.007 (13)	0.630 $\pm$ 0.004 (16)	0.523 $\pm$ 0.004 (16)	46	42	0	0
30S, KCl	0.892 $\pm$ 0.028 (13)	1.538 $\pm$ 0.012 (10)	0.740 $\pm$ 0.011 (9)	0.558 $\pm$ 0.011 (9)	47	42	4	0
50S, EDTA	0.985 $\pm$ 0.030 (23)	1.582 $\pm$ 0.005 (13)	0.637 $\pm$ 0.003 (16)	0.525 $\pm$ 0.004 (16)	50	51	0	0
50S, KCl	1.533 $\pm$ 0.061 (16)	1.655 $\pm$ 0.006 (10)	0.645 $\pm$ 0.015 (9)	0.501 $\pm$ 0.002 (9)	60	65	33	44

The conclusion is that treatment with EDTA or 0.5M-potassium chloride produces indistinguishable small subunits, but that treatment with 0.5M-potassium chloride produces a large subunit with 33–44% less protein than the EDTA-derived large ribosome subunit. Thus the protein removed from *Xenopus* ovary ribosomes by 0.5M-potassium chloride comes from the large subunit. Disc-gel electrophoresis of proteins from EDTA- and potassium chloride-derived ribosome subunits and proteins soluble in potassium chloride, is consistent with this interpretation, and so is the observation that the precipitation of potassium chloride-soluble proteins during dialysis to remove the salt is prevented by the presence of potassium chloride-derived large ribosome subunits but not by the small subunits.

Treatment of EDTA-derived subunits with 0.5M-potassium chloride gave particles (EK) of different chemical composition and buoyant density to those produced by treatment with EDTA or potassium chloride alone. The small EK subunit shows a loss of 30–54% of the protein in the EDTA or potassium chloride-derived small subunit, but the EK large ribosome subunit contains approximately the same amount of RNA and protein as the potassium chloride-derived large subunit (Table 6). Conversely EDTA treatment of potassium chloride-derived ribosome subunits gives particles (KE) of buoyant density comparable with that of the untreated potassium chloride-derived subunits. The conclusion is that pretreatment with EDTA allows more protein to be removed from the small subunit by 0.5M-potassium chloride, presumably by causing unfolding of the particle that reveals proteins capable of being removed by 0.5M-potassium chloride. In contrast, pretreatment of the large subunit with EDTA causes no change or a slight decrease in the amount of protein removed by 0.5M-potassium chloride, presumably either because unfolding by EDTA reveals no more proteins capable of being removed by 0.5M-potassium chloride or because EDTA causes no more unfolding than already occurs in 0.5M-potassium chloride. The fact that EDTA treatment of potassium chloride-derived subunits causes no change in their buoyant density suggests that the role of EDTA in protein removal by salt is a passive but irreversible one as it may be removed before salt treatment without affecting the result. Salt treatment of EDTA-treated potassium chloride-derived ribosome subunits confirms this, as the particles (KEK) have buoyant densities similar to those of the EK particles (Table 6).

Salt fractionation of EDTA-derived ribosome subunits. EDTA-derived ribosome subunits were exposed to three different concentrations of potassium chloride in an attempt to determine

Table 6. *Some chemical and physical properties of various salt-extracted ribosome particles from Xenopus ovary*

Particle nomenclature is as follows: 30 denotes small subunit, 50 denotes large subunit, first letter denotes type of subunit [EDTA (E) or KCl (K)], second and third letters denote subsequent treatment in their order and superscripts are molar concentrations. The results are given as the mean $\pm$ s.e.m. with the number of determinations in parentheses. '% RNA' was calculated as described in Table 5.

Particle	RNA/protein ratio	Buoyant density (g $\cdot$ cm $^{-3}$)	E_{240}/E_{260} ratio	E_{280}/E_{260} ratio	% RNA		% of protein lost	
					Chemical	Buoyant density	Chemical	Buoyant density
30 EDTA	0.849 $\pm$ 0.032 (21)	1.540 $\pm$ 0.007 (13)	0.630 $\pm$ 0.004 (16)	0.523 $\pm$ 0.004 (16)	46	42	0	0
30 EK $^{0.5}$	1.209 $\pm$ 0.048 (5)	1.633 $\pm$ 0.018 (5)	0.587 $\pm$ 0.007 (5)	0.503 $\pm$ 0.007 (5)	55	61	31	54
30 EK $^{1.0}$	2.262 $\pm$ 0.276 (6)	1.735 $\pm$ 0.008 (6)	0.580 $\pm$ 0.007 (6)	0.498 $\pm$ 0.006 (6)	69	80	62	82
30 EK $^{1.5}$	3.260 $\pm$ 0.652 (5)	1.751 $\pm$ 0.013 (5)	0.580 $\pm$ 0.008 (5)	0.495 $\pm$ 0.007 (5)	77	82	75	84
30 KE	—	1.548 (1)	—	—	—	44	—	9
30 KEK	—	1.646 (1)	—	—	—	64	—	60
50 EDTA	0.958 $\pm$ 0.030 (23)	1.582 $\pm$ 0.005 (13)	0.637 $\pm$ 0.003 (16)	0.525 $\pm$ 0.004 (16)	50	51	0	0
50 EK $^{0.5}$	1.226 $\pm$ 0.061 (5)	1.624 $\pm$ 0.009 (7)	0.633 $\pm$ 0.008 (5)	0.521 $\pm$ 0.001 (5)	56	60	21	34
50 EK $^{1.0}$	2.005 $\pm$ 0.016 (6)	1.658 $\pm$ 0.019 (4)	0.619 $\pm$ 0.003 (6)	0.513 $\pm$ 0.001 (6)	67	70	51	55
50 EK $^{1.5}$	2.678 $\pm$ 0.191 (5)	1.679 $\pm$ 0.076 (4)	0.625 $\pm$ 0.006 (5)	0.512 $\pm$ 0.010 (5)	73	75	63	65
50 KE	—	1.663 (1)	—	—	—	67	—	48
50 KEK	—	1.694 (1)	—	—	—	73	—	61

whether specific groups of proteins could be removed. The results of RNA, protein and buoyant density determinations on the series of ribonucleo-protein particles generated by such treatment are given in Table 6. They show that for the small subunit 30–54%, 62–82% and 75–84% of the protein in the untreated subunit is removed by

0.5M-, 1.0M- and 1.5M-potassium chloride respectively; and the corresponding values for the large subunit are 21–30%, 51–74% and 63–80%. Thus it is possible to remove increasing quantities of protein from both large and small EDTA-derived subunits by increasing the salt concentration.

Fig. 3. (a)–(f).

Fig. 3. Buoyant density in CsCl of formaldehyde-fixed ribonucleoprotein particles. See the Materials and Methods section for details of the method used. (a) 30S EDTA-derived subunit; (b) 80S ribosome; (c) 50S EDTA-derived subunit; (d) 0.5M-, (e) 1.0M- and (f) 1.5M-KCl-treated EDTA 30S subunits; (g) 0.5M-, (h) 1.0M- and (i) 1.5M-KCl-treated EDTA 50S subunits.

Determination of buoyant density in caesium chloride gradients of formaldehyde-fixed salt-treated EDTA-derived ribosome subunits (Fig. 3) shows that as the salt concentration increases there is an increase in the peak width at half height. If the peak width is an indication of heterogeneity in the ribonucleoprotein population then we conclude that salt treatment, while causing a shift in the mean buoyant density of the ribonucleoprotein population, also causes an increase in the heterogeneity of this population. The heterogeneity is probably based on the elution of protein in a continuous rather than in a discontinuous fashion, resulting in a continuous change in the mean buoyant density of the ribonucleoprotein population. There is no evidence for discrete protein-deficient particles being produced by salt treatment of EDTA-derived subunits.

Description of the proteins from the small and large EDTA-derived subunits. Disc electrophoresis of ribosomal proteins reveals a reproducible pattern of bands from both large and small EDTA-derived subunits. The proteins resolve into two major regions designated A and B (Fig. 4; Plate 1). The bands of region B are reproducible from preparation; to preparation however, some minor slow-moving bands (labelled a, b, c etc. in Fig. 4) in

region A are reproducible in different separations of the same preparation, but are not quantitatively reproducible from one preparation to the next (Plate 1).

Proteins from the large EDTA-derived subunit are resolved into eight components in region A and 18 in region B, whereas proteins from the small EDTA-derived subunit are resolved into six components in region A and 18 in region B, making a total of 26 and 24 components respectively. It is apparent that many of the bands are doublets; seven in the large and four in the small subunit, which would bring the totals to 33 and 28 components respectively, and there may be other cryptic double bands.

Nature of the protein eluted by salt treatment of EDTA-derived ribosomal subunits. The result of disc-gel electrophoresis of the proteins from one salt-fractionation experiment is shown in Plate 2. Division of the bands into three classes according to whether they are completely, partially or not eluted by a particular salt concentration (Table 7) reveals that from the EDTA-derived small ribosome subunit 0.5M-potassium chloride removes eight bands completely, 11 partially and leaves five bound; 1.0M-potassium chloride removes 18 bands completely, five partially and leaves one bound;

Table 7. *Extraction of individual protein bands by potassium*

Densitometer traces (cf. Fig. 4) of negative photographs of stained gels were used to determine whether bands were lost. The results are based on the combined results of three separate experiments. A band was considered lost if between 25 and 75% of stain had been lost; and to remain bound if less than 25% of the stain had been lost. Bands that remained bound at all salt concentrations, were used as internal reference standards against which the staining of the

Band	KCl concentration	...	0.5 M	1.0 M	1.5 M	Band	0.5 M	1.0 M	1.5 M
30A1			E	—	—	30B1	E	—	—
30A2			P	P	P	30B2	P	P	P
30A3			E	—	—	30B3	P	P	P
30A4			E	—	—	30B4	E	—	—
30A5			P	P	E	30B5	P	E	—
30A6			B	P	E	30B6	E	—	—
						30B7	P	E	—
						30B8	P	E	—
						30B9	E	—	—
						30B10	B	B	B
						30B11	B	E	—
						30B12	E	E	—
						30B13	B	E	—
						30B14	B	E	—
						30B15	P	E	—
						30B16	P	E	—
						30B17	P	E	—
						30B18	P	E	—

and 1.5 M-potassium chloride removes 20 bands completely, three partially and leaves one bound. The EDTA-derived large ribosome subunit loses five bands completely, 11 partially and 10 remain bound in 0.5 M-potassium chloride; 1.0 M-potassium chloride removes 10 bands completely, 11 partially and leaves five bound; and 1.5 M-potassium chloride removes 14 completely, 11 partially and leaves one bound.

Plate 2 shows that all the bands extracted by salt are at least qualitatively recoverable in the supernatant. From the results available at present it is not possible to show quantitative recovery of individual bands. If one considers the two bands 50A8 and 30B10, which remain bound to RNA at all salt concentrations, as markers, then one can say that contamination of supernatant protein preparations by these bands is not detectable, and thus that there is no detectable contamination of the supernatant by particle-bound proteins.

Effect of various salts on the removal of protein from EDTA-derived ribosome subunits. EDTA-derived ribosome subunits were prepared and treated with 1 M-sodium chloride, 1 M-potassium chloride or 1 M-lithium chloride at 0°C for 30 min. The particles were recovered by centrifugation at 60 000 rev./min for 2 h at 0°C in an MSE 8 × 25 rotor. Protein was extracted from the pellet and supernatant fractions by the guanidine-HCl method and run in acid-urea acrylamide gels (Plate 3). The results show that each salt extracts the same proteins qualitatively,

except that one band from the small subunit and two bands from the large subunit are not extracted by potassium chloride but are extracted by lithium chloride and sodium chloride. It is concluded that there are minor variations in the type of protein eluted by each salt.

DISCUSSION

It is well known that discrete groups of proteins may be removed from prokaryote ribosomes by salt treatment (Spitnik-Elson & Atsmon, 1969; Itoh, Otaka & Osawa, 1968). Spitnik-Elson & Atsmon (1969) have shown that the variables listed below alter the nature of the protein removed by salt from *Escherichia coli* ribosomes: the type of univalent cations, the concentration of univalent cations, the concentration of ribosomes, the temperature of salt treatment, the concentration of bivalent cations and the duration of salt treatment. Particularly relevant to the present work is their observation that EDTA pretreatment allows protein extraction to proceed rapidly (complete within 30 min) at 0°C, thus decreasing the number of variables to three. In this paper I have considered the effects of different salts and concentrations on EDTA-derived ribosome subunits at low concentrations (1.5–3.5 mg/ml) but have not considered the effect of ribosome concentration.

Table 8 compares the RNA contents of ribosome subunits from various sources determined in different laboratories. It can be seen, by comparison with

EXPLANATION OF PLATE I

Acid-urea separation in acrylamide gels of three different preparations of EDTA-derived ribosome subunits. Gels 1, 3 and 5, 30S subunit; gels 2, 4 and 6, 50S subunit. Protein equivalent to 0.5mg of subunit was separated at 2mA/gel for 17h. Possible doublet bands are indicated. The line indicates the division between regions A and B.

EXPLANATION OF PLATE 2

Salt fractionation of proteins from EDTA-derived subunits of *Xenopus* ovary ribosomes. Proteins were extracted by the guanidine-HCl method from either the particle or soluble fractions of 30S and 50S particles after extraction with KCl. Protein equivalent to 0.5mg of subunit was separated on acid-urea gels at 2mA/gel for 16h. (a), 30S series; (b), 50S series. In both (a) and (b): gels 1, 0.5M-KCl; 2, 1.0M-KCl; and 3, 1.5M-KCl-extracted particle fraction; gels 4 and 9, 30S EDTA-derived subunit; gels 5 and 10, 50S EDTA-derived subunit; gels 6, 0.5M-KCl; 7, 1.0M-KCl; and 8, 1.5M-KCl-soluble protein fraction.

EXPLANATION OF PLATE 3

Acrylamide gel separation of proteins extracted from 30S and 50S subunits by different salts. Protein equivalent to 0.5mg of subunit was separated at 2mA/gel for 18h. (a), 30S series; (b), 50S series. Gels 1 and 2, proteins from 80S ribosomes; gel 3, 30S EDTA-derived subunit; gel 4, 50S EDTA-derived subunit. Gels 5 and 8, after 1.0M-Li⁺ treatment; gels 6 and 9, after 1.0M-Na⁺ treatment; and gels 7 and 10, after 1.0M-K⁺ treatment; particle-bound and soluble protein fractions respectively. Bands extracted by Li⁺ and Na⁺ but not by K⁺ are indicated by the lines.

chloride from EDTA-derived subunits of Xenopus ovary ribosomes

individual bands were extracted (E), partially extracted (P), or remained bound (B) at given KCl concentration to have been extracted if more than 75% of the stain had been lost; to have been partially extracted if Bands 30B10 and 50A8, which never appeared in the salt-soluble fractions, and were considered to remain other bands could be compared. A and B refer to the regions of the gels where the bands are found (Fig. 4).

Band	KCl concentration	...	0.5M	1.0M	1.5M	Band	0.5M	1.0M	1.5M
50A1	E	—	—	—	50B1	E	—	—	—
50A2	E	—	—	—	50B2	P	P	E	E
50A3	B	B	P	—	50B3	P	P	P	P
50A4	E	—	—	—	50B4	P	E	—	—
50A5	B	B	P	—	50B5	P	P	P	P
50A6	E	—	—	—	50B6	P	P	P	P
50A7	P	E	—	—	50B7	B	B	P	P
50A8	B	B	B	—	50B8	P	P	E	E
					50B9	P	P	P	P
					50B10	B	B	P	—
					50B11	P	E	—	—
					50B12	P	E	—	—
					50B13	B	P	E	E
					50B14	B	P	P	P
					50B15	P	E	—	—
					50B16	B	P	E	E
					50B17	B	P	P	P
					50B18	B	P	P	P

the results in the present paper, that *Xenopus* ovary ribosome subunits apparently contain slightly less RNA per unit of protein than rat liver ribosomes. Loss of protein during treatment of EDTA-derived ribosomal subunits with 0.5M salt is approximately the same as given for rat liver subunits by Reboud *et al.* (1969), although they used lithium chloride. Clegg & Arnstein (1970) used 0.5M-potassium chloride to obtain subunits from rat liver ribosomes, the smaller of which has lost 41% of its protein compared with the EDTA-derived small subunit; on the other hand similar treatment of *Xenopus* ribosomes produced a small subunit with the same compositions as the EDTA-derived particle, whereas the large subunit had lost 33–44% of its protein compared with the EDTA-derived particle.

Clegg & Arnstein (1970) have shown that treatment of rat liver ribosomes with potassium chloride removes protein from the small subunit at 0.2M and from the large subunit at 0.6M in the presence of 0.5mM-Mg²⁺ (Table 8). Discontinuous changes both in the sedimentation of unfixed particles and in the buoyant density in caesium chloride of formalin-fixed particles were correlated with removal of protein. Analysis by acrylamide-gel electrophoresis showed that both complete and partial dissociation of certain bands by salt treatment was found.

The principal difference in experimental technique between these experiments and the ones described in the present paper, apart from the source

of material, lies in the treatment of 80S ribosomes in the presence of Mg²⁺ for lengths of time that will not allow the dissociation to go to completion. In the work described here I have treated EDTA-derived ribosome subunits with three concentrations of potassium chloride for times which allow dissociation to go to completion. I have observed, by chemical estimation, a continuous loss of protein that is correlated with a continuous shift in buoyant density in caesium chloride of the formaldehyde-fixed particles. However, such crude parameters may obscure observation of the release of specific proteins or groups of proteins at particular ionic strengths. I therefore attempted to determine by electrophoresis in acid-urea acrylamide gels whether some proteins are more readily extracted by potassium chloride than others. The situation is complicated by the fact that some bands in the acrylamide-gel separation of protein from untreated ribosome subunits are obviously composed of more than one protein and these may be removed at different salt concentrations, giving the appearance of partial extraction of some bands. However, it may be concluded from the evidence presented that sequential extraction of specific ribosomal proteins by potassium chloride does occur.

One other point of difference between the results of Clegg & Arnstein (1970) and those presented here lies in the response of 80S ribosomes to EDTA and to 0.5M-potassium chloride. Clegg & Arnstein (1970) show that with rat liver the small ribosome

Fig. 4. Densitometer traces of negative photographs of acid-urea acrylamide gel separations of proteins isolated from (a) EDTA-derived 30S subunits and (b) EDTA-derived 50S subunits by the guanidine-HCl method. Approx. 250 μ g of protein was used. The gels were run at 2 mA/gel for 16 h. The gap between regions A and B is a prominent feature of these electrophoretic separations and provides a convenient central reference point for comparison of one gel with another.

subunit loses protein during 0.5M-potassium chloride treatment compared with 1.0M-EDTA treatment, whereas the large ribosome subunit does not lose protein until the concentration of potassium

chloride is 0.6M (Table 8). In this paper I have shown that for *Xenopus* ovary it is the large ribosome subunit that loses protein in 0.5M-potassium chloride, whereas the 0.5M-potassium chloride-

Table 8. Comparison of the results in the present paper with those given in other papers

The RNA contents were determined either by chemical methods or from the buoyant density in CsCl of the formaldehyde-fixed particles. The KCl-derived subunits were produced by treatment of 80S ribosomes with 0.5 M-KCl. The ES^{0.5} particles were produced by treatment of the EDTA-derived subunits with 0.5 M of the appropriate salt. The percentage protein loss was calculated from the chemical and buoyant-density determinations.

Author(s) Animal Tissue Salt	Reboud <i>et al.</i> (1969)		Clegg & Arnstein (1970)				This paper <i>Xenopus</i> Ovary Potassium chloride					
	...	Rat	Rat	Liver	Potassium chloride	Potassium chloride	Potassium chloride	Potassium chloride	Potassium chloride			
	...	Liver	Potassium chloride	Potassium chloride	Potassium chloride	Potassium chloride	Potassium chloride	Potassium chloride	Potassium chloride			
	% RNA		% of protein lost		% RNA		% of protein lost		% RNA		% of protein lost	
Particle	Chemical	Buoyant density	Chemical	Buoyant density	% RNA	Buoyant density	Chemical	Buoyant density	% RNA	Buoyant density	Chemical	Buoyant density
30S, EDTA	50	53	0	0	47	0	46	42	46	42	0	0
30S, KCl	—	—	—	—	60	41	47	42	47	42	4	0
30S, ES ^{0.5}	71	—	59	—	—	—	55	61	55	61	31	54
50S, EDTA	55	55	0	0	57	0	50	51	50	51	0	0
50S, KCl	—	—	—	—	57	0	60	65	60	65	33	44
50S, ES ^{0.5}	59	—	15	—	—	—	56	60	56	60	21	34

Table 9. *Calculation of numbers of proteins of a given number-average molecular weight to be expected in Xenopus ovary ribosome subunits and comparison with the maximum and minimum numbers observed by electrophoresis*

The total mass of protein/particle was calculated from the chemical and buoyant-density results in Tables 5 and 6, assuming the molecular weights for *Xenopus* 18S and 28S rRNA to be 0.65×10^6 and 1.5×10^6 respectively (Loening *et al.* 1969). The observed maximum number of proteins/particle was calculated from the results in Table 7, assuming that partial extraction indicates the presence of another protein in the band. The minimum number was calculated from the same results, assuming that partial extraction does not indicate the presence of another protein. The best fit of observed with predicted numbers of proteins for the small subunit was obtained by assuming a number-average molecular weight of 30 000 for the proteins; and for the large subunit by assuming a number-average molecular weight of 47 500 for the proteins. This means that some proteins of the large subunit must be present more than once per particle, as it is likely that the number-average molecular weight for eukaryote ribosomal proteins lies between 20 000 and 30 000 for both subunits (Hamilton & Ruth, 1967; Gould, 1970; H. Pratt & R. A. Cox, personal communication).

Particle	Observed $\times 10^6$ daltons protein		Number of proteins											
			Assumed						Observed					
	Number-average mol.wt. ...		25 000		30 000		35 000		Max.		Min.		Mean	
	Chemical	Buoyant density	Chemical	Buoyant density	Chemical	Buoyant density	Chemical	Buoyant density						
30S EDTA	0.76	0.89	30	36	25	30	22	25	36	24	36	30		
30S EK ^{0.5}	0.54	0.41	22	16	18	14	15	12	17	16	16	16		
30S EK ^{1.0}	0.29	0.16	12	6	10	5	8	5	6	6	6	6		
30S EK ^{1.5}	0.18	0.14	7	6	6	5	5	4	4	4	4	4		
Number-average mol.wt. ...			45 000		47 500		50 000							
50S EDTA	1.72	1.64	38	36	36	35	35	33	46	26	36	36		
50S EK ^{0.5}	1.38	1.14	31	25	29	24	28	23	30	21	25	25		
50S EK ^{1.0}	0.85	0.73	19	16	18	15	17	15	20	16	18	18		
50S EK ^{1.5}	0.63	0.57	14	13	13	12	13	11	12	12	12	12		

derived small ribosome subunit retains the same protein content as the EDTA-derived subunit. It may be that this difference results from the different tissues and animals used.

Gould (1970) has calculated that there is not a sufficient variety of proteins of a given average molecular weight from the rabbit reticulocyte ribosome to make up the required total protein mass. I have made similar calculations (Table 9) by assuming a number-average molecular weight of 30 000 for *Xenopus* ribosomal proteins. This is close to the value of 26 000 determined by equilibrium centrifugation (Hamilton & Ruth, 1967) for total rat liver ribosomes and to the value of 28 000–30 000 determined electrophoretically by Gould (1970) for reticulocyte ribosomal proteins. The calculation predicts 27 proteins in the EDTA-derived small ribosome subunit and 49 in the large subunit. The predicted value falls within the range of proteins observed for the small EDTA-derived subunit (24–36 proteins); but not for the large subunit (24–46 proteins). I am forced to conclude that either the proteins of the large subunit have a number-average molecular weight greater than 30 000 or that some of the proteins are present more than once per large subunit. As the proteins of the large subunit do not migrate in acid-urea gels noticeably more slowly than those of the small subunit it seems unlikely that they are very different in number-average molecular weight. I therefore suggest that although there may not be more than one copy of each protein in the small ribosome subunit, there is probably more than one copy of some proteins in the large subunit. The present evidence does not allow me to decide whether some proteins are present in fewer than one copy per ribosome.

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