Ribosomes from *Xenopus laevis* Ovaries and the Polyuridylic Acid-Directed Biosynthesis of Polyphenylalanine

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Single ribosomes account for 90% or more of the ribosome fraction of *Xenopus laevis* ovaries. These ribosomes have low endogenous activity in a cell-free protein-synthesizing system but synthesize polyphenylalanine in the presence of poly U under conditions comparable with those reported for mammalian or bacterial systems. These observations agree with the view that the ribosome is deficient in mRNA.

Compared with ribosomes from other sources ribosomes from unfertilized ova have low endogenous activity in cell-free protein-synthesizing systems, probably because of a dearth of mRNA (for reviews see Tyler, 1967; Davidson, 1968). Such ribosomes should respond to added mRNA and the biosynthesis of polyphenylalanine under the direction of poly U provides a convenient assay that is commonly accepted. The aim of this work was to show that although ribosomes from *Xenopus laevis* ovaries have low endogenous activity (Ford, 1966) they actively synthesize polyphenylalanine in the presence of poly U under conditions of pH, temperature and ionic composition comparable with those found for ribosomes from mammalian or bacterial sources. An assay for the biological activity of *X. laevis* ribosomes is necessary for structural studies such as the reconstitution of active ribosomes from subparticles or the reconstitution of active subparticles from rRNA and protein subunits.

EXPERIMENTAL

Chemicals. Glutathione was obtained from C. F. Boehringer und Soehne G.m.b.H., Mannheim, Germany; ATP (dipotassium salt) from Nutritional Biochemicals Corp., Cleveland, Ohio, U.S.A.; GTP (sodium salt) from Sigma Chemical Co., St Louis, Mo., U.S.A.; poly U (Control no. 46859) from Miles Chemical Corp., Elkhart, Ind., U.S.A.; ^{14}C -labelled amino acids from The Radiochemical Centre, Amersham, Bucks., U.K.

Determination of protein and RNA. The amount of protein in the pH5 enzyme fraction and the concentration of ribosomes in 0.25 M-sucrose, 0.25 M-KCl, 1 mM-MgCl₂, 0.05 M-tris-HCl buffer, pH7.6, were measured by means of a nomograph based on the extinction coefficients of enolase and nucleic acid (California Corp. for Biochemical Research, Los Angeles, Calif., U.S.A.).

Standard cell-free system. The procedure of Schweet,

Lamfrom & Allen (1958) as modified by Arnstein, Cox & Hunt (1964) was followed. The incubation mixture (0.5 ml) used for testing the incorporation of labelled amino acids into protein had the following composition: 50 mM-KCl, 5 mM-MgCl₂, 10 mM-glutathione, 25 mM-tris-HCl buffer, pH7.6 at 25°C, 0.05 ml of an amino acid mixture containing ^{14}C -labelled phenylalanine (specific radioactivity 50 $\mu\text{Ci}/\mu\text{g}$) and 19 unlabelled amino acids (0.1 μmol of each), ribosomes (0.25 mg), pH5 enzymes (1–4 mg) and an ATP-generating system consisting of 2.5 μmol of sodium phosphoenolpyruvate, 0.125 μmol of ATP, 0.03 μmol of GTP and 25 μg of pyruvate kinase. The incubation was carried out in air at 37°C for 1 h.

Isolation of labelled protein from the incubation mixture. After incubation, 0.2 ml of M-NaOH containing 25 μmol of unlabelled amino acid/ml was added to the reaction mixture (0.5 ml), which was then either kept at 37°C for 5 min and then at about 20°C for 15 min, or kept at about 20°C for 1 h. After the addition of 10% (w/v) trichloroacetic acid (2.5 ml) the mixture was kept at 4°C overnight, the protein was filtered on to a 3 cm diam. Oxoid membrane and washed with cold 5% (w/v) trichloroacetic acid (2 $\times$ 5 ml), hot 5% (w/v) trichloroacetic acid (2 $\times$ 5 ml) and cold water (2.5 ml).

Radioactivity measurements. An automatic ultra-thin end-window counter (model C-110B; Nuclear-Chicago Corp., Des Plaines, Ill., U.S.A.) was used. The background was low (about 1.5 c.p.m.). The counting efficiency for ^{14}C was 15% and the radioactivity of samples was counted for at least 4000 counts.

Isolation of reticulocyte ribosomes and the pH5 enzyme fraction. Reticulocytes, ribosomes and pH5 enzyme fractions were isolated by the standard method of Arnstein *et al.* (1964).

Preparation of *X. laevis* ovary ribosomes. The ovary from a mature animal was removed into ice-cold homogenizing medium (0.25 M-sucrose, 35 mM-tris-HCl buffer, pH7.6, 50 mM-KCl, 1.5 mM-MgCl₂) and weighed. The tissue was homogenized in 2.5 vol. of medium with five passes of a loose-fitting (radial clearance approx. 100 μm) Teflon plunger rotating at 1500 rev./min. The homogenate was filtered through a Buchner funnel, which removed the

connective tissue, and then centrifuged for $500\,000g_{\max.}$ -min in a Sorval 8×40 angle rotor. The supernatant was carefully removed with a syringe from beneath the thick layer of fat and centrifuged for $13.2 \times 10^6g_{\max.}$ -min in either an MSE 8×50 rotor or a Spinco 40 rotor. The supernatant was removed with a syringe and used for preparation of the pH5 enzyme fraction. The tube was dried and the pellet rinsed with homogenizing medium before being resuspended in homogenizing medium to give the ribosome suspension. Zonal centrifugation revealed that the ribosome fraction was made up mainly of single ribosomes with less than 10% of subparticles.

Preparation of pH5 enzyme fraction. The pH5 enzyme fractions were prepared as described by Arnstein *et al.* (1964).

RESULTS

Mg^{2+} optimum and the response to added poly U. Fig. 1 summarizes the results of two experiments

Fig. 1. Effect of $MgCl_2$ concentration and of the amount of poly U on the biosynthesis of polyphenylalanine by ribosomes from ovaries of *X. laevis*. Each incubation mixture (final volume 0.5 ml) contained ribosomes (0.25 mg), pH5 enzymes (approx. 1 mg), L-[^{14}C]phenylalanine (0.125 μCi , sp. radioactivity 50 $\mu Ci/\mu mol$), tris-HCl buffer, pH 7.6 at $20^\circ C$ (12.5 μmol), KCl (25 μmol), phosphoenolpyruvate (2.5 μmol), ATP (0.125 μmol), GTP (0.03 μmol), pyruvate kinase (50 μg) and various concentrations of $MgCl_2$ and poly U as indicated. There was no incorporation when ribosomes or the energy system were omitted. Samples were incubated in air at $37^\circ C$ for 1 h. Δ , First experiment; $\circ$ and $\square$, second experiment. The concentrations of $MgCl_2$ were as follows: 1, 5 mM and 30–85 mM; 2, 7 mM; 3, 10 mM (first experiment); 4, 10 mM (second experiment); 5, 12 mM; 6, 15 mM.

Fig. 2. Effects of temperature on *X. laevis* and rabbit reticulocyte cell-free systems. The composition of each incubation mixture is given in the legend of Fig. 1, but the amounts of $MgCl_2$ (6 μmol) and poly U (105 μg) were the same throughout. Each assay was incubated for 30 min at the appropriate temperature. Δ , ∇ and $\blacktriangle$, Independent experiments with ribosomes and enzymes from rabbit reticulocytes; $\circ$ and $\bullet$, independent experiments with both ribosomes and enzymes from ovaries of *X. laevis*. The values shown by $\square$ were obtained by multiplying the radioactivity of those shown by $\circ$ and those shown by $\bullet$ at $37^\circ C$ and so facilitate comparison of the two experiments at other temperatures.

in which the incorporation of phenylalanine into acid-insoluble protein by *X. laevis* ovary ribosomes was assayed at different concentrations of poly U and Mg^{2+} . The results were consistent in the two experiments. They show that, at optimum concentrations of Mg^{2+} , 0.25mg of ribosomes is saturated by 70 μg of a particular batch of poly U. At saturating concentrations of poly U the incorporation of phenylalanine is 60-fold greater at 12mM- Mg^{2+} than at 5mM- Mg^{2+} . Above 12mM-magnesium chloride there is an inhibition of polyphenylalanine synthesis and in a solution of 25mM-magnesium chloride, or higher concentrations, there was no poly U-directed synthesis.

Fig. 3. Effect of KCl on the cell-free system. The composition of each incubation mixture was as described in the legend of Fig. 1; O, 10mM- $MgCl_2$; Δ , 15mM- $MgCl_2$. The amount of poly U was 8.8 μg /0.5ml.

Temperature-dependence of the system. Fig. 2 shows the results of experiments comparing the temperature-sensitivity of the *X. laevis* ovary and rabbit reticulocyte poly U-stimulated systems. The results show clearly that both the systems are most efficient at 37°C. It appears that the optimum is broader for *X. laevis* ovary ribosomes than for rabbit reticulocyte ribosomes.

Effect of potassium chloride concentration. An increase in the concentration of potassium chloride beyond 0.03M did not increase the amount of acid-insoluble polyphenylalanine that was synthesized. An increase in the concentration of magnesium chloride from 10mM to 15mM did not compensate for the inhibitory effect of increasing the concentration of potassium chloride (Fig. 3).

pH optimum. The incubation mixture was buffered at a particular pH within the range 7–8.2 (measured at about 20°C) with tris-hydrochloric acid buffer. The pH optimum was about 7.4–7.6 and the synthesis of acid-insoluble polyphenylalanine was decreased to 75% at pH 7.2 and to about 50% at pH 8.

Function with rabbit reticulocyte enzymes. *X. laevis* ovary ribosomes were prepared by the standard method. A sample (12mg/ml in 0.025M-potassium chloride, 1mM-magnesium chloride, 0.05M-tris-hydrochloric acid buffer, pH 7.6) was made 0.5M in potassium chloride to remove bound enzymes and the washed ribosomes were recovered by ultracentrifugation and then redissolved (2.5mg/ml) in the original buffer. Washing did not diminish the activity of the ribosomes in the cell-free system. Protein biosynthesis was observed when homologous enzymes were replaced by rabbit reticulocyte pH 5 enzymes (see Table 1). Comparable experiments showed that rabbit reticulocyte ribosomes were active when supplemented with *X. laevis* pH 5 enzymes.

Table 1. *Effect of washing in 0.5M-potassium chloride and the response to rabbit reticulocyte enzymes on biosynthesis of polyphenylalanine by X. laevis ribosomes*

Except where stated each assay contained, in a final volume of 0.5ml: ribosomes (0.25mg), pH 5 enzyme fraction (1.2mg), KCl (25 μmol), $MgCl_2$ (7 μmol), tris-HCl buffer, pH 7.6 (12.5 μmol), glutathione (5 μmol) sodium phosphoenolpyruvate (2.5 μmol), ATP (0.125 μmol), GTP (0.03 μmol), and an amino pyruvate kinase (25 μg). The incubation was carried out in air at 37°C for 1 h.

Ribosomes	Enzymes	Radioactivity of acid-insoluble protein ($\mu Ci/\mu g$)
Standard	Homologous	7.5
	Rabbit reticulocyte	7.86
Washed in 0.5M-KCl	None	0.13
	Homologous	8.9
	Rabbit reticulocyte	3.3
	pH 5 enzyme minus energy system	0.13
None	Homologous	2.2
	Rabbit reticulocytes	0.14

Kinetics of polyphenylalanine synthesis. The conditions given in Table 1 were used. The radioactivity of acid-insoluble protein was $3.75 \mu\text{Ci}/\mu\text{g}$ after 15 min, $6.35 \mu\text{Ci}/\mu\text{g}$ after 30 min and $7.5 \mu\text{Ci}/\mu\text{g}$ after 1 h at 37°C . Thus biosynthesis was largely complete after 30 min. In this and the other experiments, in the absence of poly U, the endogenous incorporation was very low and was scarcely different from the incorporation found in the absence of an added energy system.

DISCUSSION

An optimum concentration of 10–12 mM-magnesium chloride compared favourably with an optimum of about 10 mM-magnesium chloride for the poly U-directed phenylalanine incorporation by reticulocyte ribosomes (Arlinghaus & Schweet, 1962; Arnstein, Cox & Hunt, 1962) and by *Escherichia coli* ribosomes (Nirenberg & Matthaei, 1962). Williamson (1969) found that the optimum amount of magnesium chloride for the overall reaction is a compromise between the optimum for the attachment of poly U to ribosomes and the optimum for the translation of poly U and the synthesis of polyphenylalanine. The observed optimum (10–12 mM-magnesium chloride; Fig. 1) probably represents a compromise between the optima for the two reactions as in the reticulocyte system. The pH optimum and the effect of increasing the concentration of potassium chloride are also in line with the

rabbit reticulocyte and *E. coli* cell-free systems. Unsworth & Cohen (1968) reported that for ribosomes from *Rana catesbiana* tadpole liver the response to poly U was optimum at 25°C and 60 mM-Mg^{2+} , conditions that appear exceptional. Although ribosomes from *X. laevis* ovaries have a low endogenous activity they actively synthesize polyphenylalanine in the presence of poly U. It is inferred that the low endogenous activity arises from a dearth of mRNA.

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