THE EFFECT OF RIBOSOMAL PROTEIN EXCHANGE ON THE ACTIVITY OF XENOPUS LAEVIS RIBOSOMES

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Summary Exposure of Xenopus unfertilized egg ribosomes to an adult liver 105,000 g supernate leads to an extensive exchange between some ribosomal proteins and similar proteins in the soluble fraction to give a hybrid product which retains the physical characteristics of control ribosomes. Such exchange does not affect the efficiency of poly U-primed protein synthesis at high Mg^{2+} concentrations, but stimulates synthesis at least 300% at low Mg^{2+} levels. The stimulatory effect is not eliminated by washing the ribosomes in 0.5 M NH_4Cl. It is suggested that during exchange unfertilized egg ribosomes acquire a protein(s) which facilitates the binding of initiation factors, and that such a protein(s) is absent or present in an inactive form on normal egg ribosomes.

The selective translation of preformed messenger RNAs (mRNAs) is the primary means of controlling protein synthesis in the early development of many embryo types. In these forms, unfertilized eggs synthesize proteins at a low rate, despite the presence of all essential components (1). Two major theories have been advanced to account for the lack of developmentally significant protein synthesis before fertilization. These theories propose either an inactivation of the egg mRNA (2,3), or the inhibition of ribosome function (4,5). Most of the current evidence appears to favour the first alternative (6). However, some recent work (7) indicates the presence of an inhibitory factor on at least some unfertilized egg ribosomes, and other observations suggest a heterogeneity in the functional capacity of the egg ribosomes (8,9). Whatever the precise relationship between ribosome heterogeneity and the control of maternal mRNA translation, it is clear that accessory and structural proteins of ribosomes can play a rôle in regulating activity (10,11). The observation of Dice and Schimke (12) that ribosomal proteins are found in the soluble fraction of homogenates, and that exchange is possible between ribosomal proteins in the soluble and particulate compartments in vitro, suggested an approach to the status of unfertilized egg ribosomes. We have tested the effects on function of exchange of ribosomal proteins between sub-cellular fractions of unfertilized eggs and those of liver, a tissue highly active in protein synthesis.

MATERIALS AND METHODS

Xenopus laevis females were induced to ovulate by the procedure of Gurdon

(13). Unfertilized eggs were collected and dejellied by treatment with 0.22 M sodium thioglycolate (pH 8.1) for 5 min. The eggs were then washed, and homogenized in two volumes of buffer (0.25 M sucrose, 0.05 M Tris, pH 7.6, 0.1 M KCl, 0.01 M $MgSO_4$, 0.0001 M EDTA and 0.003 M 2-mercaptoethanol). The homogenate was centrifuged at 20,000 g for 10 min and the supernatant fraction (S_{20}) was retained. Ribosomes and the soluble fraction were prepared from the S_{20} and from Xenopus liver by standard procedures (14). The method for the assay of poly U-directed protein synthesis has been reported (15). The details of other methods used are given in the table and figure legends.

RESULTS

Our first priority was to establish the reality and extent of protein exchange between ribosomal and supernatant fractions. For this purpose, ^{3}H-labelled liver ribosomes were incubated with unlabelled egg supernate and unlabelled egg ribosomes were combined with ^{3}H-labelled liver soluble fraction. Saturation levels of components, at which maximal exchanged occurred, were established in preliminary experiments and used for further work. When labelled liver ribosomes (sp.act., 4 x 10^4 dpm/A_{260nm} unit) were exchanged with unlabelled egg supernate, a 66% decrease in their specific activity resulted (Table 1, line 2). The reverse experiment, combining unlabelled egg ribosomes with labelled liver supernate, gave ribosomes which had acquired 74% of the specific activity of control liver ribosomes (Table 1, line 4). It was apparent, on inspection of the 260/280 nm ratios of the preparations, that some extraneous proteins were present with exchanged ribosomes. This type of contamination, if substantial, could alter ribosome specific activity without necessarily implying exchange of proteins. Gel electrophoretic analysis showed that the contamination was not extensive (Fig. 1 C). Specifically, a major and two minor supernatant components appeared to be selectively adsorbed onto the ribosomes as a result of exchange. The adsorbed supernatant proteins apparently were not contributing substantially to acquired radioactivity, the bulk of which was associated with ribosomal proteins. Washing the exchanged ribosomes in 0.1 M KCl resulted in the loss of some label, although the specific activity was still 65% of controls (Table 1, line 5), while the 260/280 nm ratio was comparable to that of the original ribosome preparation. A wash in 0.5 M salt (see Fig. 1 D) removed all obvious supernatant proteins, as well as reducing the intensity of some bands present in control ribosomes, without a drastic effect on the distribution of label which was acquired during exchange. A 1.0 M KCl wash, which removes all loosely associated proteins, e.g., transfer and initiation factors, produced a pattern on electrophoretic analysis no different from Fig. 1 D, and reduced the specific activity of the ribosomes to 53% of controls (Table 1, line

TABLE 1. Exchange of ribosomal proteins between Xenopus egg and liver subcellular preparations.

Composition of exchange mixtures*		Post-exchange treatment**	Specific activity of ribosomes (dpm/A_{260nm} unit)
Ribosomes	Soluble fraction		
1. Liver (^3H)	-	None	40,000
2. Liver (^3H)	Egg (unlabelled)	None	13,600
3. Egg (unlabelled)	-	None	0
4. Egg (unlabelled)	Liver (^3H)	None	29,650
5. Egg (unlabelled)	Liver (^3H)	0.1 M KCl	26,200
6. Egg (unlabelled)	Liver (^3H)	1.0 M KCl	21,500
7. Egg (unlabelled)	Liver (^3H)	2.0 M LiCl	9,840

*8 A_{260nm} units of egg or ^3H-labelled liver ribosomes were combined with 15 mg of ^3H-labelled liver 105,000 g supernatant protein (approx. 750,000 dpm) or 15 mg of egg 105,000 g supernatant protein and incubated in TKM buffer (0.5 M Tris, pH 7.6, 0.025 M KCl, 0.01 M $MgSO_4$) for 20 min at 4°C followed by 5 min at 23°C. After incubation the mixtures were diluted to 7 ml with TKM and centrifuged for 2 h at 105,000 g. The supernatant fraction was discarded and the pellets were resuspended in TKM and cleared by low speed centrifugation.

**Exchanged ribosomes, in TKM buffer, were layered over 4 ml of a solution containing 0.25 M sucrose, 0.05 M Tris, pH 7.6, 0.005 M $MgSO_4$ and the indicated amount of KCl or LiCl, and then centrifuged at 37,000 rev/min for 4.5 h in the Spinco SW-50 rotor. Pelleted material was resuspended in TKM for the determination of ribosomal specific activity.

6). Of interest is the effect of treating exchanged ribosomes with 2.0 M LiCl/ 5 mM $MgSO_4$; approximately 1/3 of the total protein is removed by this treatment, but a substantial amount of label is still found associated with the ribosomes, the specific activity is 24% of controls (Table 1, line 7). We interpret the results as follows: On exposure to the supernatant fraction, some proteins on the ribosomes remain unaffected; but others, including some of the "core", as well as the "split" proteins, undergo exchange with similar proteins in the supernate, to give a hybrid product which retains the absorbance characteristics and electrophoretic pattern of unexchanged ribosomes.

The effect of ribosomal protein exchange on poly U-directed protein synthesis is shown in Fig. 2 and Table 2. Firstly, as indicated in Table 2, no discernible effect on activity at optimum Mg^{2+} concentration of 6.65 mM is seen with either exchanged egg or liver ribosomes. However, there is a substantial effect on activity at a low, sub-optimal Mg^{2+} concentration. With products of the exchange reaction between liver ribosomes and egg supernate, there was a drastic inhibition of incorporation at low Mg^{2+} levels. In the reverse exchange, using egg ribosomes and liver supernate, there was an increase in protein synthetic

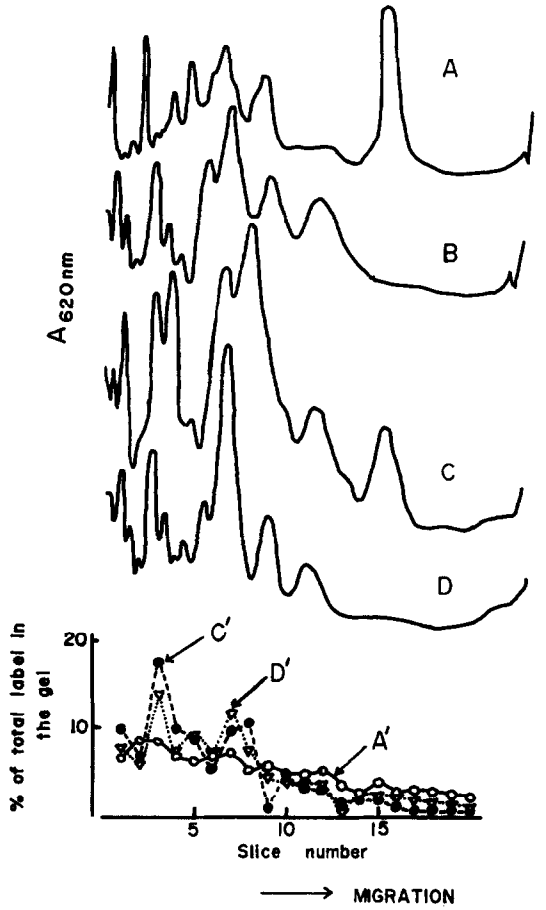

Figure 1. Electrophoretic analysis of Xenopus soluble and ribosomal proteins. Samples were run on SDS-polyacrylamide gels by the procedure of Bickle and Traut (16), at 2.5 ma per tube for 11 h.
A-D : scans of amido black stained gels pm the model 240 Gilford recording spectrophotometer at 620 nm using the Gilford linear transport gel scanner.
A'-D' : radioactivity distribution in gel slices, solubilized in H_2O_2 and counted in 10 ml of Aquasol (New England Nuclear Corp.).

A, A' : Xenopus liver 105,000 g supernatant protein. B : Normal Xenopus egg ribosomes. C, C' : Unlabelled egg ribosomes exchanged with labelled liver supernate. D, D' : as in C, but the exchanged ribosomes were washed in 0.5 M NH_4Cl.

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activity at low Mg^{2+}. In a double label experiment (Fig. 2), in which the degree of exchange was determined at the same time and on the same samples as incorporation efficiency, there was a stimulation of incorporation proportional to the degree of exchange. The maximal amount of stimulation varied slightly but was always above 300%. To check if the effect was due to binding of some low molecular weight component, the exchanges were carried out with dialysed

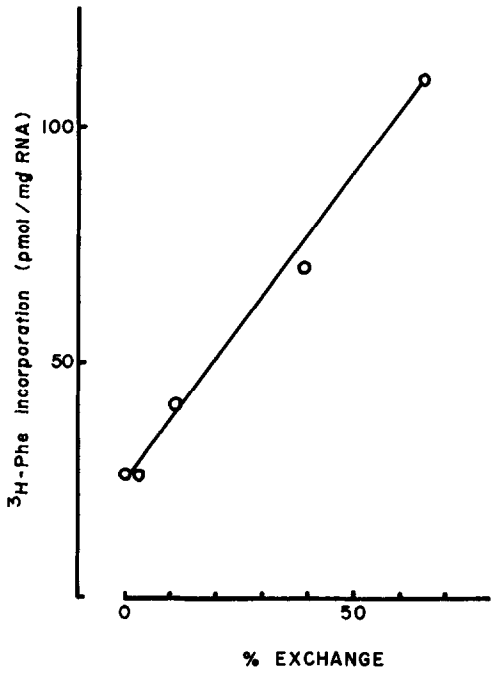

Figure 2. Correlation between the extent of ribosomal protein exchange, using egg ribosomes and liver supernatant fraction, and the efficiency of poly U-directed protein synthesis at low (3.45 mM) Mg^{2+} concentration.

The exchanges were carried out using ^{14}C-labelled liver supernate. The percent exchange was calculated from ^{14}C counts on the same samples as used to determine the ^{3}H-phenylalanine incorporation. Other conditions as in Table 2.

supernatant fractions; stimulation of activity was still observed. To eliminate the possibility that some liver ribosomal subunits were being introduced during exchange, and that these subunits were really responsible for the increased ribosome efficiency at low Mg^{2+}, the dialysed supernate was centrifuged for 4.5 h at 105,000 g before use in exchanges; the effect was still observed, if anything somewhat more clearly. Whatever component in the supernate is responsible, it is unstable, since repeated freezing and thawing destroys its capacity to produce the increase in incorporation efficiency.

Of particular interest is the effect of washing exchanged ribosomes with 0.5 M salt; the stimulatory capacity is reduced, but not eliminated, remaining at least double that for control, unwashed ribosomes. Similarly, the inhibitory effect of egg supernatant proteins on liver ribosomes is not removed by high salt washing.

DISCUSSION

It seems reasonably clear that the stimulatory or inhibitory effects of ex-

TABLE 2. Poly U-directed protein synthetic capacity of control and exchanged *Xenopus* ribosomes.

Ribosomes*	Supernate*	Wash	Incorporation (pmol/mg RNA)** at 3.45 mM	Incorporation (pmol/mg RNA)** at 6.65 mM Mg^{2+}
Egg	-	None	36	2134
Egg	-	0.5 M NH_4Cl	26	2037
Egg	Normal liver	None	110	2109
Egg	Normal liver	0.5 M NH_4Cl	65	2037
Egg	Dialysed liver	None	142	2320
Egg	Dialysed liver	0.5 M NH_4Cl	92	2564
Egg	Centrifuged liver	None	154	nd
Egg	Centrifuged liver	0.5 M NH_4Cl	85	nd
Liver	-	None	400	2293
Liver	-	0.5 M NH_4Cl	309	2236
Liver	Dialysed egg	None	132	2560
Liver	Dialysed egg	0.5 M NH_4Cl	114	2422

*The sources of ribosomes and supernatant fractions used in the exchange experiments. Normal supernate : undialysed. Dialysed supernate : dialysed against TKM buffer for 24 h at 4°C. Centrifuge supernate : centrifuged at 105,000 g for 4.5 h after dialysis, to remove remaining ribosomal subunits.

**Figures are means of duplicate determinations, corrected for endogenous incorporation, which for egg ribosomes ranged between 28 and 32 pmol/mg RNA and for liver ribosomes between 92 and 233 pmol/mg RNA. The incubation mixtures contained, in a volume of 0.25 ml, 5 µg of ribosomal RNA in ribosomes, 400 µg of ovary 105,000 g supernatant fraction protein, 125 µg of poly U, 125 µg of ^{3}H-phenylalanine tRNA. Ionic conditions were, 25 mM Tris, pH 7.6, 60 mM KCl, 11.25 mM 2-mercaptoethanol, and $MgSO_4$ as indicated. Other conditions as in MATERIALS AND METHODS and Table 1. nd : not determined.

changes are due to the proteins newly acquired by the ribosomes. The characteristics of the stimulatory effect are very similar to those observed on supplying ribosomes with initiation factors (17). The effect is certainly not due to the acquisition of transfer factors from the supernate, since the assays are run at saturating amounts of such factors, and the incorporation level at optimum Mg^{2+} is not affected (the larger amounts of incorporation should be more strongly affected by a shortage of transfer factors). It is possible that initiation factors are involved in the stimulatory effect, since a high salt wash, which removes such factors from ribosomes, partially reduces the increased translational efficiency. However, this cannot be the only component which

is involved, since a high salt wash does not totally eliminate the effect, although it does remove all initiation factors (unpublished observations).

Possible, but in our view less likely, interpretations of the results include, 1) Removal or addition of an inhibitory factor as a result of exchange (7,18). However, washing control or exchanged ribosomes in high salt does not result in a stimulation of activity, so a loosely associated inhibitor (7) appears unlikely. 2) The introduction of a protein which helps bind poly U to ribosomes (19); again, such a protein would have to be tightly associated after exchange. In our view, the most likely interpretation is as follows: The exchange reaction introduces onto the ribosomes a protein(s) which is not removable by a high salt wash and which is involved or facilitates the binding of initiation factors. In the presence of an active form of this protein(s), initiation factors can bind and the ribosomes function more efficiently at low Mg^{2+} concentrations. Washing in high salt removes the bound initiation factors, but leaves the protein(s) which facilitates their binding, so that when the washed ribosomes are tested for activity at low Mg^{2+} in the presence of ovary supernatant fraction, they bind initiation factors which are present in this supernate (Garrison and Kaulenas, unpublished), and are able to demonstrate the increased efficiency of polyphenylalanine synthesis at low Mg^{2+} concentrations. The simplest interpretation for the inhibitory effect of egg supernate on liver ribosomes would be that the former possesses non-functional or inactive initiation factor-binding protein(s), which is exchanged for the active proteins found on control liver ribosomes.

It appears from these data that the functional capacity of ribosomes is at least partly related to the presence of specific ribosomal proteins. The results may mean that egg ribosomes are not all totally capable of initiation with natural mRNAs, but may have to be "activated" by some as yet undefined means.

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