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42S p48 – the most abundant protein in previtellogenic *Xenopus* oocytes – resembles elongation factor 1α structurally and functionally

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We have undertaken an immunological and biochemical analysis of the most abundant soluble protein of previtellogenic *Xenopus* oocytes, 42S p48. We show that this protein shares immunological cross-reactivity with elongation factor 1α (EF-1α). Direct assays of both 42S fractions and purified 42S p48 show that this cross-reactivity is of functional significance since 42S p48, like EF-1α, can transfer charged amino acids to ribosomes. We further demonstrate that 42S p48 is degraded soon after the onset of vitellogenesis, while the EF-1α concentration remains essentially unchanged during this transition. These properties of 42S p48 are discussed with regard to its role in oogenesis.

Key words: 42S p48/elongation factor/*Xenopus laevis*/oogenesis

Introduction

Previtellogenic *Xenopus laevis* oocytes have an unusual protein and RNA content. Roughly 75% of total RNA is 5S RNA and tRNA, while only a small amount of the longer ribosomal RNAs are present (Mairy and Denis, 1971; Ford, 1971). The 5S and tRNA are found in two cytoplasmic ribonucleoprotein (RNP) particles, the 7S and 42S RNP particles (Denis and Le Maire, 1983; Mattaj *et al.*, 1983). The 42S particle consists of tRNA, 5S RNA and two proteins, 42S p48 and 42S p43 of 48 000 and 43 000 daltons, respectively. 42S p48 binds to tRNA and 42S p43 to 5S RNA (Picard *et al.*, 1980). The tRNA in the 42S particle is largely aminoacylated (Denis and Le Maire, 1983). The 7S RNP is composed of one molecule of 5S RNA and one molecule of a 40 000 dalton protein (Picard and Wegnez, 1979), which has been shown to be transcription factor IIIA (TFIIIA) (Pelham and Brown, 1980. Honda and Roeder, 1980). TFIIIA is required for transcription of 5S RNA, and acts by binding to the internal control region of the 5S genes (Pelham and Brown, 1980; Engelke *et al.*, 1980; Sakonju *et al.*, 1981). In contrast to the 7S particle, no information on possible functions of the 42S particle proteins has been available.

Nevertheless, if only because of their abundance, the 42S proteins remain of interest. It has been estimated that 42S p48 accounts for 15–20% and 42S p43 for 8–10% of total soluble protein of stage I (previtellogenic) *X. laevis* oocytes (Mattaj *et al.*, 1983; Denis and Le Maire, 1983). TFIIIA is approximately as abundant as 42S p43 in these immature oocytes.

In the present study we show that 42S p48 shares immunological cross-reactivity with elongation factor 1α (EF-1α), which is responsible for transferring aminoacyl-tRNA to ribosomes during translocation (reviewed in Clark, 1980). Further, 42S particle fractions, like EF-1α, can transfer charged aminoacyl-tRNA to ribosomes. This activity co-purifies with 42S p48. 42S p48 is no longer detectable immunologically in stage 3 (Dumont, 1972) oocytes, i.e. after the start of vitellogenesis. It therefore appears that 42S p48 is a stage-specific elongation factor.

Results

42S p48 and EF-1α show antigenic cross-reactivity

With TFIIIA as a model, we wished to investigate the question of whether the 42S particle proteins were also funtionally interesting. Since 42S p48 is found bound to charged aminoacyl-tRNAs (Denis and Le Maire, 1983), it seemed possible that it would be related to EF-1α, which transfers aminoacyl-tRNAs onto ribosomes engaged in protein synthesis (Clark, 1980). Accordingly, an antibody raised against purified *Artemia salina* EF-1α (the pure EF-1α was a gift of W.Möller, Leiden), which had previously been shown to cross-react with vertebrate EF-1α (Coppard *et al.*, 1982) was used to probe extracts of a mixture of oocytes of stages 1 to 3 (Dumont, 1972), which were either unfractionated or had been separated into 42S and pooled fractions of 7S and lighter (1–7S) by centrifugation through a sucrose

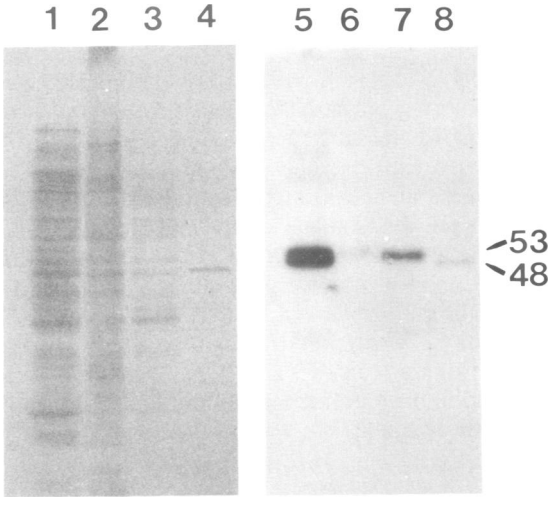

Fig. 1. Anti-EF-1α antibodies recognize two protein bands in extracts of pooled stage 1 to 3 oocytes. After electrophoresis on 15% polyacrylamide-SDS gels, proteins were transferred to nitrocellulose and stained lightly with amido-black (**lanes 1–4**) or with anti-EF-1α followed by [^{125}I]protein A and autoradiography (**lanes 5–8**). Lanes 1 and 5: total extract of stage 1 to 3 oocytes. **Lanes 2** and **6**: total extract of *Xenopus* kidney culture cells. **Lanes 3** and **7**: 1 to 7S fraction of oocyte extracts. **Lanes 4** and **8**: 42S fraction of oocyte extract. The size of bands was determined by comparison with molecular size markers (BioRad). The positions of 42S p48 (48) and EF-1α (53) are indicated. The most abundant protein in the 42S fractions is 42S p48 (see also Figure 3). Although 42S p48 is the most abundant protein in stage 1 oocytes, its abundance in the pooled stage 1 to 3 oocytes is such that it is not easily identifiable in total protein (**lane 1**).

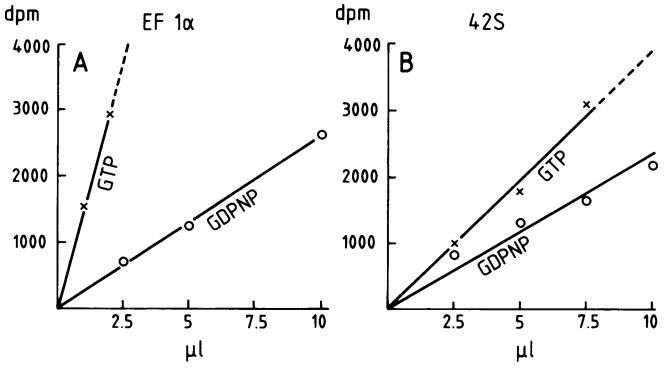

Fig. 2. The 42S fraction has EF-1α activity. Crude *A. salina* EF-1α fractions and *X. laevis* oocyte 42S fractions were assayed for EF-1α activity either with GTP(+) or, to prevent EF-1α recycling, GDPNP(○). (**A**) Assay of crude *A. salina* EF-1α (protein concentration 14 mg/ml). (**B**) Assay of *X. laevis* oocyte 42S fraction (protein concentration 6.4 mg/ml). The assay measures the binding of [^{14}C]phenylalanyl-tRNA^{Phe} to ribosomes. At saturation binding equals 4000 d.p.m. Dashed lines represent extrapolations of values which reached 4000 d.p.m.

Table I.

Fraction	Specific activity (pmol aatRNA Phe/μg)
42S	0.69 ± 0.05
42S − RUTC	0.36 ± 0.03
42S − RUTC + GDP	0.33 ± 0.03
42S + GDP	0.34 ± 0.02

The assays were carried out as described in Materials and methods or with the following modifications: 42S − RUTC, assay in the absence of Ribosome, poly(U), tRNA complex; 42S + GDP, assay in the absence of GTP but in the presence of 2 mM GDP. The assay volume was maintained at 50 μl.

density gradient (Mattaj *et al.*, 1983). The extracts were transferred from denaturing polyacrylamide gels to nitrocellulose and probed with anti-EF-1α antiserum. The antibody stains two bands in unfractionated extracts, one of 48 000 and one of 53 000 (Figure 1, lanes 1 and 5). Only the upper of the two is present in extracts of an *X. laevis* kidney cell culture line (Figure 1, lanes 2 and 6). Vertebrate EF-1α has a mol. wt of 53 000 (Slobin and Möller, 1979), and we therefore assume the upper band is EF-1α. Fractionation of the oocyte extract into 1 to 7S and 42S fractions results in the separation of EF-1α, which is found in the light fraction (Figure 1, lanes 3 and 7), from the 48 000 species, which is found in the 42S fraction (Figure 1, lanes 4 and 8) comigrating with 42S p48.

EF-1α activity co-purifies from the 42S fraction with 42S p48

In order to determine whether the antigenic cross-reactivity had functional significance, the 42S fraction was tested for EF-1α activity. The assay involves measuring the ability of the protein under test to transfer [^{14}C]phenylalanine-tRNA^{Phe} to ribosomes loaded with poly(U) (Merrick, 1979; Slobin and Möller, 1979). The activity of partially purified *A. salina* EF-1α (Figure 2A) and of the 42S fraction (Figure 2B) are shown. The 42S fraction clearly does have activity although it contains no detectable (53 kd) EF-1α (Figure 1, lane 8).

EF-1α can recycle in the presence of GTP but not the nonhydrolyzable analogue GDPNP. The difference in activity measured under these two conditions (Figure 2A) is thus a measure of EF-1α recycling. Comparing Figures 2A and B it is clear that the 42S fraction does not show such a large difference in activity when assayed with GDPNP instead of GTP, and thus presumably does not recycle as efficiently as 53 kd EF-1α.

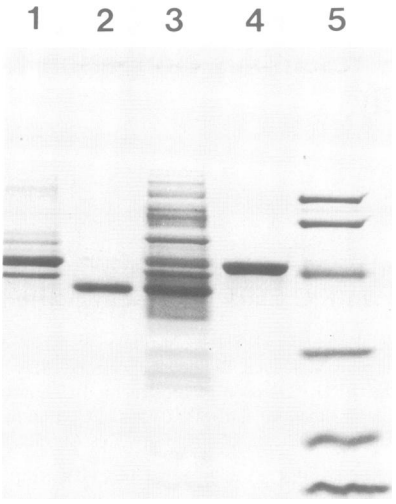

Fig. 3. Purification of 42S p48. Extracts of stage 1 and 2 oocytes were fractionated as described in the text, then, after electrophoresis on a 12.5% polyacrylamide-SDS gel, were stained with Coomassie Blue (42S p48 stains poorly with silver stain). **Lane 1**: 42S fraction. **Lane 2**: 7S fraction. **Lane 3**: <7S fraction. **Lane 4**: purified 42S p48. **Lane 5**: Molecular size markers (BioRad). From the top; phosphorylase *b*, 92 500; BSA, 66 000; ovalbumin, 45 000; carbonic anhydrase, 31 000; soybean trypsin inhibitor, 21 500; lysozyme, 14 400 daltons. Note that the <7S fraction is contaminated with 7S RNP.

Table II.

Fraction	Specific activity (pmol [^{14}C]phenylalanyl tRNA^{Phe} incorporated per μg protein)
<7S	1.19 ± 0.1
7S	0
42S	1.66 ± 0.2
42S p48	0.94 ± 0.1

The assays were carried out as described in the legend to Table I.

Further experiments showed that the activity measurement shown in Figure 2B was exaggerated since 42S particles bound some [^{14}C]phenylalanyl-tRNA^{Phe} even in the absence of the ribosome-poly(U)-tRNA complex (Table I). Additionally assay of the 42S particles in the presence of GDP, which inhibits EF-1α, showed that roughly 50% of the 42S particle activity was GDP resistant. This most probably indicates that, in addition to having EF-1α-like activity, the 42S particles have spare binding capacity for charged amino acids, and we are currently investigating the properties of this binding further. Nevertheless, roughly 50% of the activity measured in Figure 2B and in other similar experiments is dependent on the presence of ribosomes, poly(U), and GTP, and is inhibited by GDP, and thus is similar to EF-1α activity.

To show that 42S p48 was indeed responsible for the EF-1α activity, it was necessary to devise a method which resulted in its purification away from other 42S fraction components, the major of which is 42S p43. To this end, the following purification was devised. Extract from stage 1 and 2 oocytes was frac-

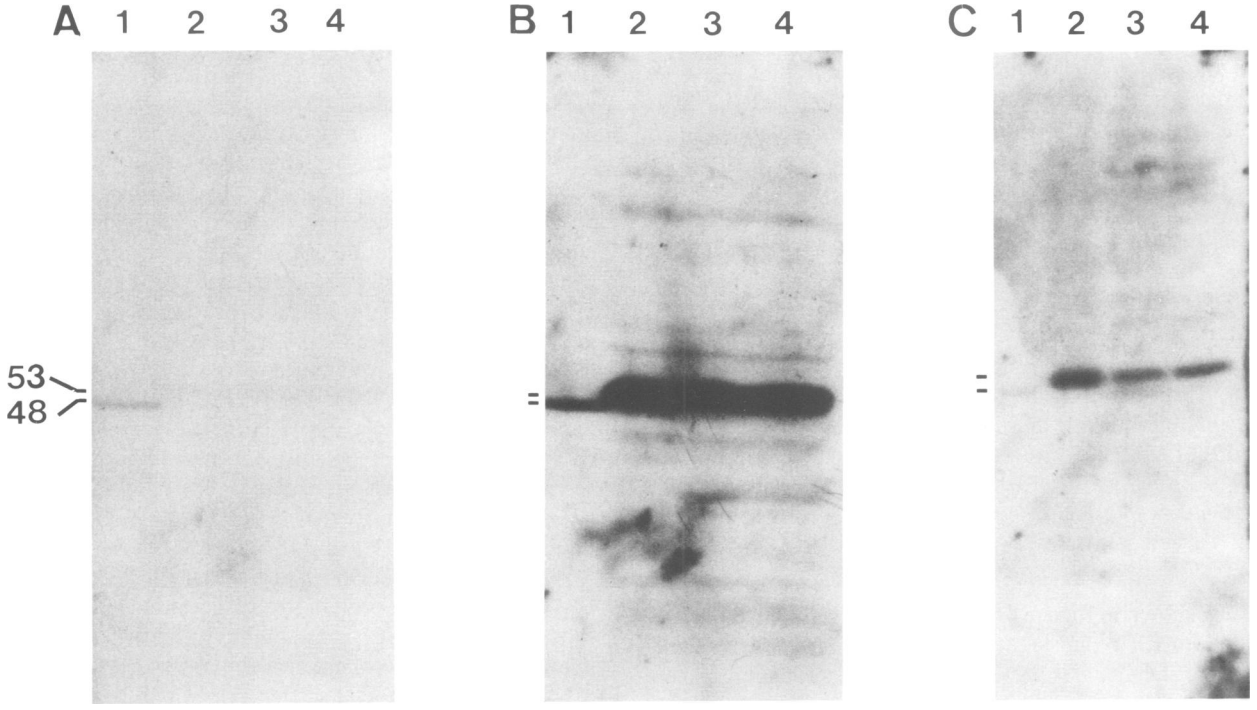

Fig. 4. Accumulation of 42S p48 and EF-1α during oogenesis. Oocytes were separated into pools of stages 1 and 2 (**lanes 1**), 3 (**lanes 2**), 4 (**lanes 3**) and 5 (**lanes 4**) (Dumont, 1972). After electrophoresis of either 5 (**panels A, B**) or one (**panel C**) oocyte equivalent, the proteins were transferred to nitrocellulose and decorated with anti-42S p48 (**panel A**, exposure time 7 days) followed by anti-EF-1α (**panel B**, exposure time 20 h). **Panel C** was decorated with anti-EF-1α (exposure time 44 h). The positions of 42S p48 (48) and EF-1α (53) are indicated.

tionated on an S-200 Sephacryl column into 42S, 7S, <7S, and other fractions. The protein compositions of the major fractions are shown in Figure 3, lanes 1−3, note that the <7S fraction is contaminated with 7S RNP.

The 42S peak was then further fractionated in the following steps. First the 42S particles were disrupted with 1 M KCl. Passage over an ultragel HA column resulted in the removal of tRNA and 5S RNA. The 42S p48 and 42S p43 present in the flow through were concentrated by vacuum dialysis, then dialysed into 0.2 M KCl. Under these conditions, 42S p43 precipitates and can be removed by centrifugation leaving electrophoretically pure 42S p48 (Figure 3, lane 4). The purification is described in detail in Materials and methods.

Measurement of EF-1α activity showed that, while activity was found in both the 42S and <7S fractions, there was no detectable EF-1α activity in the 7S fraction, indicating that a good separation of the activities in the <7S and 42S fractions had been achieved. Purified 42S p48 was shown to have activity (Table II). 42S p48 activity had to be measured at low protein concentration (below 16 μg/ml). At higher concentrations specific activity decreased. This was not due to non-specific inhibition since the addition of purified p48 protein to 42S fractions did not result in inhibition (data not shown). A possible explanation of this observation is that pure 42S p48 aggregates at high concentration. Indeed, it was possible to observe 42S p48 precipitating on lowering of the salt concentration. This lack of solubility is also likely to be the explanation for the decrease in specific activity seen when 42S p48 is compared with the 42S fraction (Table II).

42S p48 and EF-1α accumulate at different times during oogenesis

Previous results (Dixon and Ford, 1982; Mattaj *et al.*, 1983) indicated that the maximal concentration of 42S p48 was found

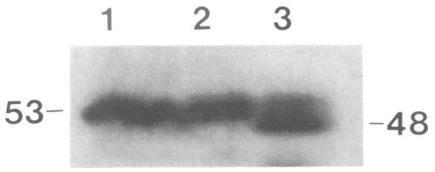

Fig. 5. EF-1α is present in stage 1 and 2 oocytes. Extracts of *Xenopus* kidney culture cells (**lane 1**), and stage 5 (**lane 2**) or stage 1 and 2 (**lane 3**) oocytes containing 50 μg of protein were transferred to nitrocellulose after polyacrylamide-SDS gel electrophoresis, incubated sequentially with anti-EF-1α and [^{125}I]protein A, then visualized by autoradiography. The positions of 42S p48 (48) and EF-1α (53) are indicated.

in stage 1 oocytes. We used antibodies against 42S p48 (whose characterization is described by Mattaj *et al.*, 1983) and EF-1α to determine the relative levels of the two proteins at different stages of oogenesis. Oocytes were staged (Dumont, 1972) and divided into four pools. Stages 1 and 2 were taken together and stages 3,4 and 5 separately. Extracts of the pools were made and the equivalent of five oocytes of each pool were loaded onto the SDS−polyacrylamide gel. After electrophoresis, the proteins were transferred to nitrocellulose where they could be immunodecorated. A single band, 42S p48, was visualized in the stage 1 and 2 pool by binding to anti-42S p48 antibodies followed by ^{125}I-labelled protein A and autoradiography (Figure 4A, lane 1). This experiment was incubated with very dilute antibody such that detection of autoradiographic signals required 7 days exposure. This procedure was chosen so that the same blot could then be restained with EF-1α antibody (Figure 4B). After overnight exposure, 42S p48 is obviously labelled more strongly (Figure 4B, lane 1). In later stages, no 42S p48 is visible, but 53 kd EF-1α is strongly stained (Figure 4C, lanes 2−4). Several conclusions can be drawn from these experiments. 42S p48 is

detectable only in stage 1 and 2 oocytes. EF-1α is not detectable in this experiment in stage 1 and 2 oocytes, but is easily detectable in later stages. The quantity and concentration of EF-1α is maximal in stage 3 oocytes. This is clearer in panel 4C where the results of a separate experiment in which extract from a single oocyte equivalent from each of the pools was stained with anti-EF-1α. Other antisera used later to stain the same blot as controls (anti-nucleoplasmin, anti-eIF.4A, data not shown) showed patterns of accumulation which were correlated with oocyte growth, i.e. these proteins were present in largest amount in stage 5 oocytes.

Comparison of Figures 4A and B show that the anti-EF-1α from *Artemia* stains *Xenopus* EF-1α more strongly than it does 42S p48, while the anti-42S p48 antibody recognized 42S p48 more strongly (although in other experiments with higher concentrations of antibody, cross-reaction between anti-42S p48 antibodies and *Xenopus* EF-1α has been observed). These results indicate that *Xenopus* and *Artemia* EF-1α are more closely related than EF-1α and 42S p48.

EF-1α is present in previtellogenic oocytes

The results shown in Figure 4 raised the possibility that 42S p48 substituted for EF-1α in stage 1 and 2 oocytes. In order to determine whether EF-1α was present in these previtellogenic stages, we next analysed equal quantities of protein from a pool of stage 1 and 2 oocytes, stage 5 oocytes, and from a *Xenopus* kidney cell culture line. These extracts were probed with anti-EF-1α in the experiment shown in Figure 5. The result clearly shows that EF-1α is present in all stages tested, and confirms the lack of 42S p48 in stage 5 oocytes and culture cells.

Discussion

We have shown that 42S particle fractions as well as a <7S fraction from previtellogenic *Xenopus* oocytes can transfer charged $tRNA^{Phe}$ to ribosomes loaded with poly(U). The activity in the <7S fraction is due to the presence of 53 kd EF-1α, the standard eukaryotic EF-1α (Slobin and Möller, 1979). The activity in 42S particles was co-purified to electrophoretic homogeneity with 42S p48, the larger of the two 42S particle proteins, and − at very early oocyte stages − the most abundant soluble protein. Why should oocytes need a large amount of EF-1α-like activity? The RNA in previtellogenic oocytes is roughly 75% tRNA plus 5S RNA (Mairy and Denis, 1971; Ford, 1971). These RNAs are thought to be synthesized and stored for use when the amplified (Brown and Dawid, 1968; Evans and Birnstiel, 1968; Gall, 1968) rRNA genes of the oocyte begin to be heavily transcribed at the onset of vitellogenesis. The tRNA in previtellogenic oocytes is largely aminoacylated (Denis and Le Maire, 1983). Aminoacylation of tRNA is a prerequisite for protein synthesis. Aminoacylated tRNA can undergo non-enzymatic deacylation and EF-Tu, the prokaryotic equivalent of EF-1α, prevents this breakdown (Pingoud and Urbanke, 1980). Since the oocyte synthesizes such a large quantity of aminoacyl tRNA, it would be logical to store it as a complex to prevent breakdown. In addition, it could be that large amounts of free tRNAs are toxic, and have to be 'neutralized' by protein. Previtellogenic oocytes are relatively deficient in ribosomes and other components of the protein synthetic machinery. The fact that 42S p48 has EF-1α-like activity would allow the stored aminoacylated tRNA to be transferred to ribosomes when protein synthesis in the oocyte increases. Although this rationalizes the oberved properties of 42S p48, it is not clear why the oocyte does not simply make a large quantity of EF-1α to perform this function. It may be that in addition to storing aminoacyl tRNAs and later transferring them to ribosomes 42S p48 has other biochemical functions (it should be remembered that TFIIIA, which stores 5S RNA in oocytes and presumably transfers it to nascent ribosomes either directly or indirectly, is also a transcription factor). It may also be that 42S p48, or the gene encoding it, has properties making it suitable for the accumulation of large amounts of a single product. Alternatively, extremely high concentrations of EF-1α may have a detrimental effect on the cell.

The amount of 42S p48 decreases from very high to undetectable levels on the transition from previtellogenic to vitellogenic oocytes (Dixon and Ford, 1982; Mattaj *et al.*, 1983; this paper). The other protein component of the 42S particle, 42S p43, behaves similarly (Dixon and Ford, 1982). This pattern of accumulation differs from that of TFIIIA, the other extremely abundant protein of previtellogenic oocytes. The quantity of TFIIIA remains constant through oocyte stages 1−5, before falling 20- to 30-fold at stage 6 (Roeder *et al.*, 1981). This abrupt decrease in quantity would also support the view that the role of 42S p48 is in the storage of accumulated aminoacyl-tRNA. Once this store has been utilized in protein synthesis, the EF-1α present is sufficient to support translation and the 42S p48 can therefore be degraded. It will be of interest to clone 42S p48 and compare it with EF-1α in order to determine which regions of the proteins have been conserved, especially since comparison between EF-1α and EF-Tu, its prokaryotic and mitochondrial equivalent, has already been possible (van Hemert *et al.*, 1984). In addition, given the information now available for 42S p48 and TFIIIA, it will be interesting to determine whether 42S p43 also has a specific functional activity.

Materials and methods

Preparation and fractionation of extracts

Oocytes from immature *X. laevis* were homogenized and fractionated by sucrose density gradient centrifugation as described by Picard *et al.* (1980). For column chromatography, ovaries were homogenized in 20 mM Hepes−KOH, pH 7.0, 50 mM NaCl, 2.5 mM $MgCl_2$, 1 mM DTT, 1 mM benzamidine−HCl and 1 mM NaN_3. Following centrifugation at 25 000 *g* for 30 min to remove cell debris, the supernatant was applied to a Sephacryl S-200 column. Fractions containing the 42S particle, the 7S particle and a <7S fraction were collected and, when required for storage, made 50% in glycerol. Without the addition of glycerol the 42S fraction was further fractionated as follows. KCl was added to the 42S fraction to a final concentration of 1 M. 5S and tRNA were removed by passage through an ultragel HA (LKB) column in 10 mM Hepes−KOH pH 7.0, 1 M KCl, 5 mM β-mercaptoethanol, 1 mM benzamidine−HCl and 10% glycerol. The proteins eluted in the flow-through and were concentrated by vacuum dialysis in a colloidion sac (Sartorius). They were dialysed against the same buffer containing 0.2 M KCl. The precipitated 42S p43 was removed by centrifugation at 12 000 *g* for 20 min. The supernatant containing 42S p48, was collected and stored after addition of glycerol to 50%. The KCl concentration was maintained at 0.2 M. The protein concentration in the fractions was determined (Lowry *et al.*, 1951) using bovine serum albumin as a standard.

Antibody staining

After electrophoresis in SDS−polyacrylamide gels (Laemmli, 1970), proteins were transferred to nitrocellulose (Towbin *et al.*, 1979). Following incubation with antibody, reactive proteins were visualized by ^{125}I-labelled protein A (a gift of K.Suda).

Charging of tRNA

Charging of $tRNA^{Phe}$ from yeast was performed essentially as described by Merrick (1979) with the following modifications. The reaction mixture contained in 700 μl: 42.7 nmol [^{14}C]phenylalanine (504 Ci/mol, Amersham, UK), 12.9 A_{260} $tRNA^{Phe}$ (Boehringer Mannheim, FRG), 100 mM Tris/HCl pH 7.5, 50 mM NH_4Cl, 12 mM $MgCl_2$, 2 mM ATP, 2.4 mM CTP, 2.8 mM β-mercaptoethanol and saturating amounts of partially purified yeast aminoacyl-tRNA synthetase (laboratory stock).

The separation of aminoacyl-tRNA from amino acids and synthetases was carried out on Sephadex G-25 (Pharmacia, Sweden) pre-equilibrated and eluted with 5 mM

sodium acetate (pH 4.8). After ethanol precipitation, the concentration was adjusted to 2 pmol/μl. Counting efficiency was found to be 89.5% in Brays scintillation fluid counted in a liquid scintillation counter (Beckman LS 7500). The specific activity of the phenylalanine was thus found to be 1180 d.p.m. $pmol^{-1}$.

Ribosome-poly(U)-tRNA complex

The salt-washed ribosomes were pre-incubated with poly(U) and tRNA in the presence of a high concentration of Mg^{2+} in order to avoid the characteristic 'lag time' due to the poly(U)-dependent binding of $tRNA^{Phe}$ to the ribosome. The reaction mixture contained in a final volume of 220 μl: 54 mM Tris/HCl pH 7.5, 13 mM Mg $(CH_3CO_2)_2$, 0.1 M NH_4Cl, 37 mM KCl, 0.2 mM DTT, 63 mM sucrose, 2.5 mM β-mercaptoethanol, 0.03 mM EDTA, 875 μg poly(U), 440 μg $tRNA^{Phe}$ and 44 A_{260} units of salt-washed ribosomes. After incubation at 37°C for 15 min, the mixture was stored on ice until use (normally 1–2 min, maximum 5 min).

Binding assay

The assay was based on Slobin and Möller (1979), Merrick (1979) and Fonzi *et al.* (1975) with modification by Cavallius *et al.* (1986). The reaction mixture contained in 50 μl: 60 mM Tris/HCl pH 7.5, 6 mM Mg $(CH_3CO_2)_2$, 20 mM NH_4Cl, 100 mM KCl, 0.7 mM DDT, 13 mM sucrose, 0.5 mM β-mercaptoethanol, 0.6 mM PMSF, 1.1 mM benzamidine, 14% glycerol, 0.06 mM EDTA, 0.01% w/v BSA, 10 pmol [^{14}C]Phe-$tRNA^{Phe}$ (350.5 c.p.m./pmol), 2 A_{260} units ribosome complex and 0.1 mM GDPNP for the stoichiometric assays or 2 mM GTP for the catalytic assays.

In order to avoid hydrolysis of GDPNP to the inhibitor GDP, it was stored in aliquots in liquid nitrogen, and thawed once only just before incubation. After incubation, the reaction was stopped by adding 50 μl of 4% glutaraldehyde to stabilize the EF-1α complex bound to the ribosome and collected immediately on a nitrocellulose filter (Millipore, type HA pore size 0.45 μm), pre-wetted in wash buffer (20 mM Tris/HCl pH 7.5, 5 mM Mg $[CH_3CO_2]_2$ and 100 mM NH_4Cl). The filter was washed with 4.5 ml ice cold wash buffer and dissolved directly in 3 ml Brays scintillation fluid and counted in a liquid scintillation counter (Beckmann LS 7500). Background was routinely determined and subtracted.

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Note added in proof

Recently Le Maire and Denis (*J. Biol. Chem.*, **262**, 654–659, 1987) have published evidence that charged tRNA is transferred from 42S particles to ribosomes both *in vivo* and *in vitro*.