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Isolation of a 7S particle from *Xenopus laevis* oocytes: A 5S RNA–protein complex

(oogenesis/ribonucleoprotein particles/ribosome unfolding)

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ABSTRACT **Previtellogenic oocytes of *Xenopus laevis* contain a free 5S RNA–protein complex sedimenting at 7 S. This particle consists of one molecule of 5S RNA and one 45,000-dalton protein. The protein of the 7S particle and the protein that is released in association with 5S RNA when the ribosome is treated with EDTA are unrelated. Because the 5S RNA accumulated by small oocytes in storage particles is incorporated into the ribosome later in oogenesis, we conclude that 5S RNA is successively associated with two proteins during the life span of the oocyte.**

Previtellogenic oocytes of *Xenopus laevis* are known to contain very large amounts of tRNA and of 5S RNA (1–3). These RNA species make up approximately 80% of the oocyte RNA content. Only a very small proportion of 5S RNA is associated with the ribosomes in these cells (3–5). About half the 5S RNA is associated with tRNA in 42S ribonucleoprotein particles (3, 4). It has been suggested that the remainder of the 5S RNA is free in the cell sap (4). Here we show that this is not the case. All 5S RNA that is not included in the 42S particles is associated with a protein within 7S particles.

The search for proteins that interact with 5S RNA in the eukaryotic ribosome has led to the discovery of a 5S RNA–protein complex (6–11). This complex is released from the 60S ribosomal subunit by an EDTA treatment (6, 7, 9–11) or a formamide treatment (8). It consists of one molecule of 5S RNA and one protein whose molecular weight has been reported as 32,000–45,000 by various authors (6–11). We show that the protein of the 7S particle from immature oocytes is different from the protein that is released in association with 5S RNA when the ribosomes of mature oocytes are treated with EDTA.

MATERIALS AND METHODS

Preparation and Fractionation of Homogenates from Immature Ovaries. Homogenates from immature ovaries of *X. laevis* (4–6 cm) were prepared according to Denis and Mairy (4). After a rapid disruption of the ovaries in a Potter homogenizer in 50 mM Tris·HCl, pH 7.6/25 mM KCl/5 mM $MgCl_2$, the homogenate was centrifuged at 10,000 × *g* for 15 min. The supernatant was then layered on top of 15–30% sucrose gradients made in the same buffer and centrifuged in a Beckman SW 27.1 or SW 41 rotor. All these operations were carried out at 0–4°C.

Purification of the 7S Particle. We purified the 7S particles by preparative electrophoresis. A 12 × 2.5 cm tube, closed at the bottom with nylon tissue, was filled with 30 ml of 7.2% acrylamide/0.18% bisacrylamide/0.1% sodium persulfate/0.1% *N,N,N′,N′*-tetramethylethylenediamine/20 mM sodium acetate/40 mM Tris, pH 8.4. This tube was adjusted in a cell at a distance of 1 mm above a dialysis membrane and a solution of 20 mM sodium acetate/40 mM Tris, pH 8.4, was continuously passed through the elution chamber (20 ml/hr). A current of 30 mA was applied for 12 hr.

Determination of the Physical Properties of the 7S Particle. The sedimentation coefficient of the 7S particle was measured at 20°C in a Beckman An-H titanium rotor by standard procedures. The buoyant density in metrizamide was determined by centrifuging 0.5-ml samples through preformed gradients (20–50%) in 50 mM Tris·HCl, pH 7.6/25 mM KCl/0 or 5 mM $MgCl_2$. The samples were spun at 45,000 rpm for 48 hr in a Beckman SW 50.1 rotor. RNA and protein were localized in the gradient fractions after removal of metrizamide by three precipitations with 66% (vol/vol) ethanol.

Purification of the 5S RNA–Protein Complex from Ribosomes. Ovaries from mature animals were homogenized in 5 vol of 50 mM Tris·HCl, pH 7.6/35 mM KCl/5 mM $MgCl_2$/0.5% sodium deoxycholate/250 mM sucrose at 0°C. The homogenate was filtered through three layers of cheesecloth and centrifuged at 20,000 × *g* for 15 min, and the resulting supernatant was spun at 45,000 rpm for 90 min in a Beckman 60 Ti rotor. The pellet of ribosomes was resuspended in the homogenization buffer and centrifuged again at the same speed. The ribosomes were then solubilized in 10 vol of 50 mM Tris·HCl, pH 7.6/500 mM KCl/5 mM $MgCl_2$/250 mM sucrose and layered on top of 15–30% sucrose gradients made in the same buffer. The separation of 40S and 60S subunits was achieved by a 15-hr centrifugation at 20,000 rpm in an SW 27.1 rotor. The 60S particles were recovered by centrifugation, dissolved in a small volume of 50 mM Tris·HCl, pH 7.6/25 mM KCl, and treated with EDTA (1 μmol per 10 A_{260} units). The sample was layered onto a 15–30% sucrose gradient made in 50 mM Tris·HCl, pH 7.6/25 mM KCl/1.5 mM $MgCl_2$ and centrifuged at 39,000 rpm for 24 hr in an SW 41 rotor. The 5S RNA–protein complex released from the 60S particles was recovered as an UV-absorbing peak at midheight of the gradient. We routinely obtain 1 mg of the complex from 80–100 g of ovarian tissue.

Extraction and Analysis of RNA. RNA was extracted from the particles by the dodecyl sulfate/cold phenol method and analyzed by electrophoresis in 7.4% polyacrylamide gels (12).

Extraction and Analysis of Proteins. The proteins were extracted from the particles in 66% (vol/vol) acetic acid/33 mM $MgCl_2$ (13). The RNA precipitate was removed by a low-speed centrifugation. The supernatant was dialyzed against 5% acetic acid and lyophilized. We used the method of Lowry *et al.* (14) to determine the concentration of proteins, with bovine serum albumin as a standard.

Analytical electrophoreses were carried out using dodecyl sulfate/urea/polyacrylamide gels (15). The proteins (10–40 μg)

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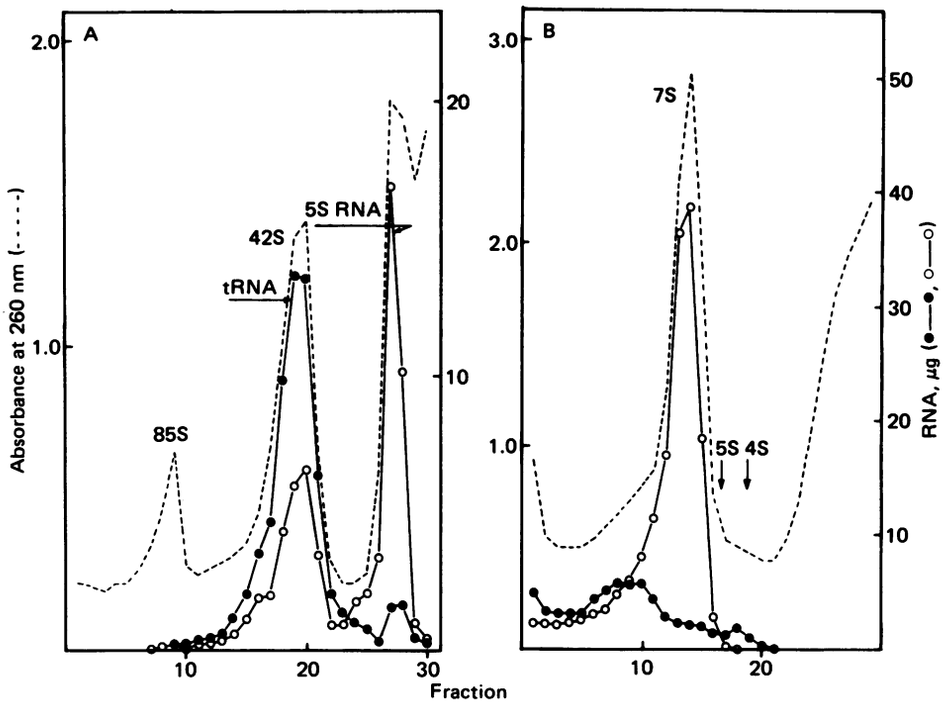

FIG. 1. Sucrose density centrifugation of homogenates of *X. laevis* immature ovaries. The ovaries of two females were homogenized in 1 ml of buffer. The homogenate was layered on top of two 15–30% sucrose gradients and centrifuged at 39,000 rpm for 210 min (*A*) or 15 hr (*B*) in an SW 41 rotor. The gradients were divided into 0.4-ml fractions. RNA was extracted from each fraction and analyzed by polyacrylamide gel electrophoresis [5S RNA (○), tRNA (●)]. The arrows in *B* indicate the positions of pure 5S RNA and tRNA centrifuged in parallel.

were dissolved in 50 μl of 1% sodium dodecyl sulfate/7 M urea/2 mM 2-mercaptoethanol/50 mM sodium phosphate, pH 7.2, and heated at 100°C for 10 min. Bromophenol blue (5 μl, 0.05%) and glycerol (10 μl) were added and the sample was layered on 7.7% polyacrylamide gels made in 1% sodium dodecyl sulfate/7 M urea/50 mM sodium phosphate, pH 7.2. A current of 8 mA/tube was applied for 7–8 hr. The electrophoresis buffer was 1% sodium dodecyl sulfate/50 mM sodium phosphate, pH 7.2. The following standards were used for the molecular weight determinations: bovine serum albumin (68,000), aldolase (40,000), carbonic anhydrase (29,000), and lyzozyme (14,300). The gels were stained with 0.05% Coomassie brilliant blue and destained by shaking in 10% acetic acid/20% methanol (vol/vol).

Amino Acid Analyses. Proteins were hydrolyzed at 110°C under reduced pressure for 24 hr in 5.7 M twice-distilled HCl containing 0.5% 2-mercaptoethanol. Cysteic acid was determined according to Spencer and Wold (16). Analyses were performed with a Beckman 120 C analyzer, using the two-column system. The recorder was equipped with a 4–5 mV range card. For determination of tryptophan we used the fluorometric method of Pajot (17).

RESULTS

Evidence for a 5S RNA–Protein Complex in Immature Ovaries of *X. laevis*. Three peaks of UV-absorbing material are obtained when homogenates of immature ovaries of *X. laevis* are centrifuged through sucrose density gradients (3, 4). A small peak of ribosomes (85S) is visible along with two large peaks sedimenting at the middle (42S) and the top of the gradients. Fig. 1*A* shows the distribution of 5S RNA and tRNA in such a gradient. As previously shown by Ford (3) and by Denis and Mairy (4), the 42S particles contain more than 90% of the tRNA and approximately 40% of the 5S RNA of immature ovaries. More than 50% of 5S RNA is present in the surface peak, the amount present in ribosomes being negligible (Fig. 1*A*). It was previously assumed that 5S RNA not associated with the 42S particles or with ribosomes is free in the cell sap (4). However, when ovary homogenates are centrifuged for a long time, the top peak is found to sediment, not at 5 S, but at 7 S (Fig. 1*B*). If an aliquot of the material present in this peak is submitted to electrophoresis, a band of UV-absorbing material is obtained that migrates more slowly than free 5S RNA (Fig. 2*A*). This band stains with Coomassie blue as well as with pyronine Y. When sodium deoxycholate is added to the extract, a significant part of the material migrates to the 5S RNA position, and the slow-moving band is strongly reduced (Fig. 2*B*). When 1% sodium dodecyl sulfate or 7 M urea is added to the extract, all the UV-absorbing material migrates to the 5S RNA position (experiments not shown).

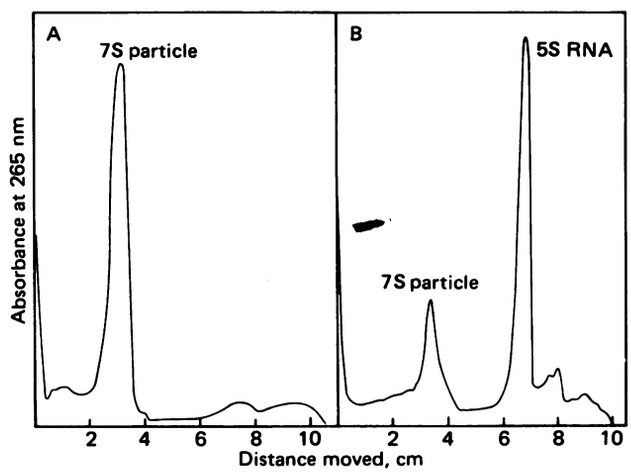

FIG. 2. Analytical gel electrophoresis of the material present in the 7S peak of a sucrose gradient (Fig. 1*B*). In *A*, 50 μl of the material was layered on a 7.6% polyacrylamide gel and submitted to electrophoresis (150 min, 90 V). In *B*, the sample was treated with 5 μl of 0.5% sodium deoxycholate.

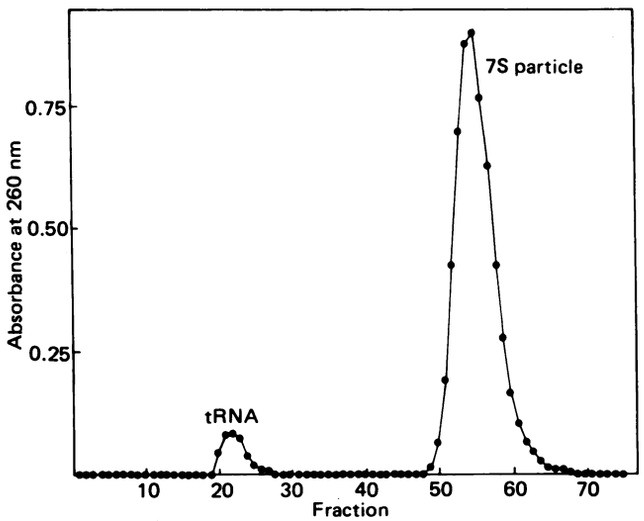

FIG. 3. Purification of the 7S particles by preparative electrophoresis. The 7S peaks from three sucrose gradients were pooled, concentrated to 1.5 ml, and layered on a 2.5 × 7 cm preparative gel (fractions: 2 ml).

We conclude from these results that homogenates of immature ovaries contain a 5S RNA–protein complex sedimenting at 7 S. This complex can be completely disrupted by sodium dodecyl sulfate and urea. It will be referred to as the 7S particle in the next part of the report.

Purification of the 7S Particles. A preparative electrophoresis system was used for purification of the 7S particles. The surface peaks from three or four gradients were pooled, concentrated, and layered on a preparative gel. Two peaks of UV-absorbing material were recovered at the bottom of the gel (Fig. 3). The larger peak corresponds to the 7S particles and the smaller one to free tRNA that is present in the top fractions of the sucrose density gradients (Fig. 1). The 7S particles were precipitated with 2 vol of ethanol for subsequent analysis.

Composition and Physical Properties of the 7S Particles. No trace other than that of 5S RNA was ever observed when the RNA extracted from 7S particles was analyzed on polyacrylamide gels. A single protein species was also detected in our analyses (Fig. 4, lane C). Its molecular weight was estimated to be 45,000. The molar ratio of 5S RNA to the protein in the 7S particles was determined to be 0.99 (average of three measurements). We conclude that 7S particles consist of the association of one molecule of 5S RNA with one molecule of protein.

The physical properties of the 7S particles are summarized in Table 1. The sedimentation coefficient was found to be dependent on the concentration. The buoyant density in metrizamide, in the absence of Mg^{2+} (1.222 g cm^{-3}), is half way between the densities of 5S RNA (1.169 g cm^{-3}) and protein (1.275 g cm^{-3}). This confirms the 5S RNA/protein molar ratio because these molecules have approximately the same molecular weight (40,000 and 45,000, respectively). As previously observed for pure RNA and ribosomes, the 7S particles have a higher buoyant density in metrizamide in the presence of Mg^{2+} than in its absence (18).

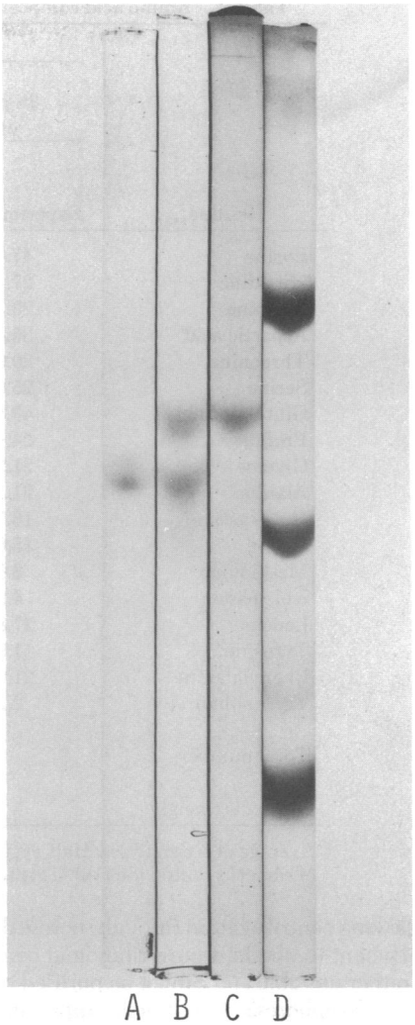

FIG. 4. Dodecyl sulfate/urea gel electrophoresis of the protein extracted from the 7S particle (lane C) and from the ribosomal 5S RNA–protein complex (lane A). The two proteins were mixed in lane B. Bovine serum albumin (68,000 daltons), carbonic anhydrase (29,000 daltons), and lyzozyme (14,300 daltons) markers are shown in lane D.

Comparison between the 7S Particle and the 5S RNA–Protein Complex that Is Released from Ribosomes by Treatment with EDTA. When the 60S subunit of the mammalian ribosome is treated with EDTA, 5S RNA is released in association with one or two proteins (6, 7, 9–11). The major protein corresponds to L_3 (11). The minor one is probably a degradation product of L_3, because it is not visible in two-dimensional electrophoreses of proteins extracted directly from ribosomes (11). The ribosome of *X. laevis* behaves in the same way. We have compared the 7S particle from previtellogenic oocytes with the 5S RNA–protein complex of ribosomal origin.

Table 1. Physical properties of the 7S particles from *X. laevis* ovaries

Property	5S RNA	Protein	7S particle
Molecular weight	40,000	45,000	85,000
Sedimentation coefficient s_0	—	—	7.53 ± 0.15
Buoyant density in 25 mM KCl, g cm^{-3}	1.169 ± 0.004	1.275 ± 0.006	1.222 ± 0.005
Buoyant density in 25 mM KCl/5 mM $MgCl_2$, g cm^{-3}	1.184 ± 0.004	1.278 ± 0.006	1.259 ± 0.006

Table 2. Amino acid composition of the protein associated with 5S RNA in the 7S particles

	Residues/molecule			
	7S particle of immature ovaries of *X. laevis*		5S RNA–protein complex from ribosomes of mature ovaries of *X. laevis*	
Residue	Experimental*	Probable integral value	Experimental*	Probable integral value
Lysine	47.0	47	32.6	33
Histidine	27.4	27	8.1	8
Arginine	23.3	23	19.3	19
Aspartic acid	36.4	36	36.8	37
Threonine	20.9	21	17.0	17
Serine	25.0	25	26.0	26
Glutamic acid	43.8	44	41.1	41
Proline	24.4	24	13.9	14
Glycine	21.9	22	38.0	38
Alanine	21.1	21	31.4	31
Half-cystine	16.7	17	—	—
Valine	15.0	15	23.4	23
Methionine	3.0	3	5.1	5
Isoleucine	4.9	5	15.8	16
Leucine	27.8	28	23.6	24
Tyrosine	11.9	12	10.4	10
Phenylalanine	21.0	21	10.9	11
Tryptophan	2.2	2	—	—
Total number		393		353
Molecular weight		45,300		38,300

* Average of six analyses. Half-cystine was determined as cysteic acid after hydrolysis in the presence of dimethyl sulfoxide (16). Tryptophan was determined by fluorescence measurements (17).

One single long centrifugation through sucrose density gradients is sufficient to obtain a pure ribosomal complex. This complex is rather unstable and cannot be purified by means of preparative electrophoresis. It sediments approximately 0.5 S slower than the oocyte particle. When treated with sodium dodecyl sulfate, the ribosomal complex releases 5S RNA and a 38,000-dalton protein (Fig. 4, lane A), which is thus 7000 daltons lighter than the protein of the oocyte particle.

Table 2 shows the amino acid compositions of the proteins from both particles. It shows that the 45,000-dalton protein of the oocyte particle cannot be a precursor of the 38,000-dalton ribosomal protein because four amino acids (glycine, alanine, valine, and isoleucine) are more abundant in the smaller protein than in the larger one. Four other amino acids (lysine, histidine, proline, and phenylalanine) are markedly more abundant in the protein of oocyte particles than in the protein of ribosomal origin.

DISCUSSION

We have purified from immature ovaries of *X. laevis* a 7S particle that is composed of one molecule of 5S RNA and one molecule of a 45,000-dalton protein. This particle is disrupted by urea and ionic detergents. This disruption demonstrates that RNA and protein interact by noncovalent bonds.

All 5S RNA of immature oocytes is present in either 7S or 42S particles. No trace of free 5S RNA can be detected. In the 42S particles, 5S RNA is associated with tRNA and two proteins (45,000 and 54,000 daltons). The mathematics of these four molecules will be discussed elsewhere (H. Denis and B. Picard). The finding to be emphasized here is the presence in both particles of a 45,000-dalton protein. We have not yet made the demonstration that these proteins are identical, but it is likely that they are. Both proteins have exactly the same molecular weight and show a characteristic fluorescence when stained with Coomassie blue. Purification of the 45,000-dalton protein from the 42S particles and immunological methods should answer this question. Though the two constituents of the 7S particles are probably present in the 42S particles, the physiological relationship between the particles remains unclear. The lower percentage of di- and triphosphorylated 5S RNA molecules in the 7S particles than in the 42S particles (5) argues against a precursor relationship between the two particles. The 7S particle does not seem to be a subunit of the 42S particle because we were unable to release 7S particles from the 42S particles by increasing the salt concentration or treating them with EDTA (H. Denis and M. Wegnez, unpublished observations).

The function of the 7S and 42S particles is yet not clear. The simplest interpretation is to attribute to them a storage function. RNA would be more protected against endogenous ribonucleases in nucleoprotein particles than in a free form (5). The particles remain present in the oocytes during the whole previtellogenic period and progressively disappear when oocytes enter vitellogenesis. At the end of the oogenetic process, 5S RNA is found exclusively in ribosomes. At least part of 5S RNA stored in small oocytes is taken up by the ribosomes in large oocytes (19). Our data show that the protein of the 7S particle has a significantly higher molecular weight than the protein that is associated with 5S RNA in the ribosome (Fig. 4). The amino acid analyses exclude the possibility that the ribosomal protein can be derived by cleavage from the constitutive protein of the 7S particle (Table 2). It follows that the same molecules of 5S RNA are successively associated with two different proteins during the life span of the oocyte. The transition probably takes place at the beginning of the vitellogenic period. We do not know whether the 7S particle protein is conserved or destroyed in vitellogenic oocytes.

The 5S RNA–protein complex isolated from ribosomes is rather unstable. We were unable to purify that complex by preparative electrophoresis. In contrast, the 7S particle from immature ovaries is stable. It is difficult to explain the instability of the ribosomal complex. It either is due to a naturally weak interaction between 5S RNA and the protein or more probably arises from the EDTA treatment. As for the high stability of the 7S particles from immature ovaries, it may be remembered that these particles are stored for months in the oocytes.

The 42S particles are probably present in the previtellogenic oocytes of all amphibians and teleosts. They were always found, when searched for, in species belonging to these two vertebrate classes (3, 4, 12, 20). We have purified and characterized 7S particles from the immature ovaries of *Tinca tinca*, a freshwater European fish (unpublished observations). We have also found 7S particles in another amphibian species, *Pleurodeles waltlii*, and in three teleost species: *Cyprinus carpio*, *Phoximus phoximus*, and *Scorpaena porcus*. It is thus likely that 7S particles exist in the immature oocytes of all amphibian and teleost species.

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