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## RIBONUCLEOPROTEIN PARTICLES IN THE AMPHIBIAN OOCYTE NUCLEUS

### Possible Intermediates in Ribosome Synthesis

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#### ABSTRACT

Studies of the sedimentation properties of RNP<sup>1</sup> material from the nucleus of the amphibian oocyte have indicated (1) that there are few, if any, 78S ribosomes in the nucleus, (2) that there are smaller particles sedimenting at 50–55S and 30S, and (3) that the larger of these is the precursor of the 60S subunit of the cytoplasmic ribosomes. Although the nature of the 30S material is not completely clear, it probably includes precursor particles to the 40S ribosomal subunit. Heavy (50–55S) particles are predominant in immature oocytes of *Triturus viridescens*, whereas in immature oocytes of *Amblystoma mexicanum* they are reduced greatly in amount, but are still detectable. Double-labeling studies of RNA and protein reveal that both types of particle incorporate uridine-<sup>3</sup>H, but that the 50–55S material of immature oocytes does not incorporate <sup>14</sup>C-labeled amino acids. However, other evidence exists that favors the RNP nature of this material. Sedimentation analyses after SDS extraction show that 50–55S particles contain 40 and 30S RNA, whereas 30S particles contain 20S RNA. These types of RNA represent at least 80% of all the extractable nuclear RNA. The 50–55S particles are probably heterogeneous, including both particles containing mostly 40S RNA and particles containing only 30S RNA.

Considerable information has now been obtained concerning the nature of the nuclear precursors

<sup>1</sup> The following abbreviations will be used in this paper: RNP, ribonucleoprotein; RNA, ribonucleic acid; RNase, ribonuclease; DNA, deoxyribonucleic acid; SDS, sodium dodecyl sulfate; MgAc and NaAc, magnesium and sodium acetate; PVS, polyvinyl sulfate; MS 222, tricaine methane sulfonate; DOC, sodium desoxycholate; TCA, trichloroacetic acid; PPO, 2,5-diphenyl-oxazole; dimethyl POPOP, 1,4-bis-(2-(4-methyl-5-phenyl-oxazolyl)-benzene; RPH, reconstituted protein hydrolysate.

to cytoplasmic ribosomal RNA in eukaryotes. It is not yet entirely clear when this precursor RNA becomes combined with protein between the time of its synthesis and the time of its exit from nucleus to cytoplasm.

In the amphibian oocyte, radioautographic evidence indicated very early that radioactive precursors to RNA first entered the nucleus, and later passed to the cytoplasm (1). That most of the newly synthesized nuclear RNA, unlike that in many other systems, becomes incorporated into new ribosomes was demonstrated conclusively in

*Xenopus laevis* (2, 3). During oogenesis, the actual percentage of all RNA synthesis which is ribosomal is at least 90%, and often as much as 97% (3). Thus, the inference is that studies of oocyte nuclear RNA should readily reveal the nature of the precursors to ribosomal RNA.

When the types of RNA synthesized in the salamander oocyte nucleus were investigated (4), they were found to differ in certain ways from the RNA in whole ribosomes (here referred to as 28 and 18S RNA). With short periods (e.g. 2 hr) of incubation in radioactive isotope, the nuclear fraction showing peak radioactivity sedimented at 40S, while with longer periods of incubation (these can be as long as a week) the nuclear fraction showing peak radioactivity sedimented at 30S. This latter fraction probably represents the bulk of the nuclear RNA (4).

Conclusive evidence that molecules such as these are, indeed, the precursors to 28 and 18S RNA in the cytoplasm has been obtained for mammalian cells (5-8). By analogy, it is thought that the 40S nuclear RNA of amphibians breaks down into 30 and 18S RNA. Evidence is available that the latter of these two products rapidly leaves the nucleus (4), as it does in mammalian cells (9, 10). The 30S RNA, however, accumulates and only later becomes converted into 28S RNA. It then enters the cytoplasm. Data are here presented which suggest that 30S RNA, while in the nucleus, is associated with protein. 40S RNA also has protein associated with it, as does a third type of RNA—20S RNA—which may represent a precursor to 18S ribosomal RNA (11).

## MATERIALS AND METHODS

### *Animals and Their Care*

The animals used were female *Triturus viridescens* and *Amblystoma mexicanum*. Animals were kept in aerated tap water, *Triturus* at 4°C, and *Amblystoma* at 20°C. Studies on mature oocytes were chiefly done on *Amblystoma*, but a few were done on *Triturus*. Studies on immature oocytes were all done on *Triturus*. Animals were anesthetized with 0.2% MS 222 (Sandoz Inc., Hanover, New Jersey), prior to removal of pieces of ovary through a small abdominal incision.

### *Incorporation Media and Isotopes*

All incorporations were performed at room temperature. Two different media were used: (1) a sterile amphibian Ringer's solution, supplemented with 2 g/l magnesium, and 100 µg/ml each of penicillin G (Nutritional Biochemicals Corp., Cleveland)

and dihydrostreptomycin (Chas. Pfizer & Co. Inc., New York). This solution is hereinafter referred to as sterile Ringer's. (2) a sterile commercial tissue culture medium, TC-199 (Grand Island Biological Co., Grand Island, New York), also supplemented with penicillin and streptomycin (4).

The total volume of incorporation medium was always 1 ml. The RNA precursor was uridine-5-<sup>3</sup>H (20 or 21 c/mmole; Schwarz BioResearch, Inc., Orangeburg, New York), and the protein precursor was a reconstituted protein hydrolysate-<sup>14</sup>C (RPH-<sup>14</sup>C; Schwarz BioResearch). Amounts of isotopes per experiment are given in the figure legends.

### *Isolation of Nuclei*

**BUFFERS USED:** High magnesium (HM) buffer, 0.05 M Tris, pH 7.8 with 10<sup>-3</sup> M MgAc. Tris-EDTA (TE) buffer, 0.01 M Tris, pH 7.8, with 10<sup>-4</sup> M EDTA. Acetate buffer, 0.1 M NaAc, pH 5.0. All buffers contained 4 µg/ml PVS for the purpose of RNase inhibition.

The giant nuclei of amphibian oocytes are readily isolated manually (12). The procedure used here is essentially that described by Gall (13). It is perhaps the mildest isolation procedure available for use with nuclei. All buffers were used at 4°C. Isolations were performed in cold HM buffer, and nuclei were washed in this buffer, or in TE or acetate buffer, depending upon the nature of the experiment. Before layering on a gradient, nuclei were disrupted by gentle sucking into and out of a sterile syringe. In magnesium concentrations above 10<sup>-4</sup> M, nuclei were lysed with DOC (up to 5%). Enzyme digestion studies were done on disrupted nuclei. Trypsin, boiled RNase, or preincubated pronase were added, and digestion was allowed to proceed for 5-60 min. Enzyme treatments were done at 0°C, because particulate nuclear material tended to degrade at higher temperatures.

### *Cesium Chloride Centrifugation*

The procedure was essentially that described by Perry and Kelly (14), except that all solutions were buffered with 0.05 M phosphate and contained 10<sup>-3</sup> M magnesium. Nuclear material was fixed for 24 hr in an equal volume of 4% buffered formaldehyde at 0°C, after treatment with DNase (RNase-free) for 15 min. Centrifugation was carried out with preformed gradients ranging in density from 1.60 to 1.32, which were spun for 12 hr in the SW39 rotor at 34,000 rpm.

### *Extraction of RNA*

This was carried out, in some cases, by a cold phenol-SDS procedure, described by Brown and Littna (15). In other cases, a simple SDS treatment was used on isolated nuclei or nuclear particles. After washing of nuclei in TE or acetate buffer, the preparation was made 0.5% with SDS. This renders

the nuclei soluble. The resulting solution was layered directly on a gradient and analyzed for radioactivity.

Prior to the extraction of their RNA, nuclear particles were sedimented on a sucrose gradient, from which fractions were collected in cooled vials, each containing 20  $\mu$ l of Macaloid<sup>2</sup> suspension (16). Aliquots (20  $\mu$ l) were collected from each fraction, and their radioactivity was measured in Bray's solution. Fractions containing the particle peaks were then pooled, and subjected to SDS extraction. Extracted RNA was precipitated with 0.1 M NaCl-ethanol (15). Carrier RNA, also used as a velocity marker, was prepared from whole ovaries or ovarian ribosomes, and was added to nuclear preparations prior to ethanol precipitation. In Fig. 4, the amount of 4S RNA is abnormally high in proportion to the amounts of 28 and 18S RNA in this carrier; this was found to be due to the inclusion of a magnesium precipitation step in the extraction procedure (15). This precipitation selectively removes 28 and 18S material as well as phosphoprotein, as observed by Brown and Littna (18). In *Amblystoma*, one magnesium precipitation reduces the amount of 28 and 18S RNA by half, without affecting the 4S region. A relatively high 4S region has also been reported in ovarian tissue of *Xenopus* (2).

#### Sucrose Density Gradient Analysis

Gradients, 4.8 ml in volume, were either 10–30% or 15–40% sucrose. Solutions were made up in 0.01 M NaAc, pH 5, with  $10^{-4}$  M EDTA for runs with extracted RNA, and in HM or TE buffers for nuclear particle analyses. Centrifugation was carried out in the SW39 rotor of the Spinco L-2 ultracentrifuge at 1–4°C. For optical density measurements, fractions were diluted with 0.5 ml of distilled water. Otherwise, fractions were counted directly after addition of 10 ml of Bray's solution (17) or, after precipitation with cold 5% TCA, in toluene-POPOP (4g PPO; 50 mg dimethyl-POPOP per liter toluene) in an Ansitron liquid scintillation counter. Counts shown in figures are uncorrected.

## RESULTS

### *Uptake of Label*

The amphibian oocyte incorporates radioactivity cumulatively over very long periods of time. Measurements of the uptake of precursors into acid-insoluble nuclear RNA and protein showed that their specific activity continues to increase for at least 24 hr, when the oocytes are incubated in sterile Ringer's. All incorporation experiments were therefore, done for periods of 12–24 hr,

since it was desirable to obtain maximal uptake of radioactivity. When commercial culture medium is used, oocytes will incorporate for several days. At the end of this time, many nuclei contain lampbrush chromosomes the morphology of which is normal (4).

### *The Nuclear Particles*

There are many reports of ribosomes in the nuclei of various cells—reports both of a morphological (19) and of a biochemical nature (20, 21). The original object of this work was, therefore, to attempt to verify these observations. Uridine-<sup>3</sup>H appears in the 28 and 18S RNA extracted from the ribosome fraction after a few hours of incorporation. However, the radioactivity patterns from preparations of nuclei have shown no detectable 28 and 18S material at any time, even after a week's exposure to isotope. The nuclear RNA extracted is 40 and 30S RNA (4, 22). This finding has two implications: first, that ribosomal RNA known to be synthesized in the nucleus is not assembled into 78S ribosomes there, and second, that new (and, therefore, radioactive) ribosomes, completed in the cytoplasm, do not re-enter the nucleus to a significant extent.

When the sedimentation profiles of nuclear RNP material were studied, no radioactivity peaks were observed at 78S. However, there was material, first demonstrated in *Amblystoma*, that sedimented between 20 and 50S. On further investigation, what appear to be three types of nuclear particle were found. These types will now be described. Reference will be made to "immature" and "mature" oocytes: "immature" refers to those oocytes (about 0.5–1.2 mm in *Triturus*) in which the nucleoli are still predominantly at the periphery of the nucleus; "mature" refers to oocytes, of both *Triturus* and *Amblystoma* in which the lampbrush chromosomes are withdrawn into the center of the nucleus, and in which few, if any, nucleoli remain at the nuclear membrane. The sedimentation pattern of nuclear particles is apparently of the mature type only if these cytological properties obtain in the isolated nuclei. Selection of supposedly mature oocytes by size alone does not yield consistent results, apparently because some nuclei from the largest oocytes of any one batch still show peripheral nucleoli.

In gradient centrifugation of nuclei isolated from immature oocytes of *Triturus* after incorporation of labeled uridine, the radioactive ma-

<sup>2</sup> American Transul Co., Baroid Div., Houston, Tex.

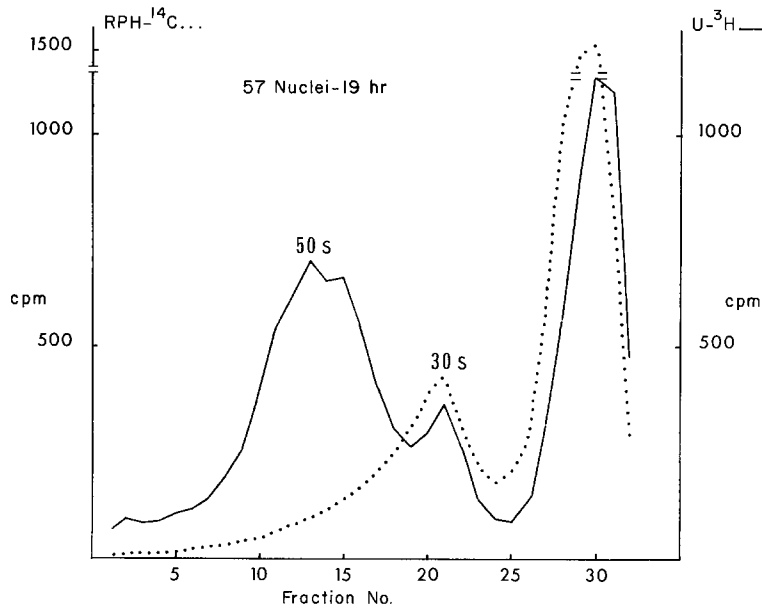


FIGURE 1 The sedimentation profile of a nuclear extract from *Triturus* immature oocytes. Number of nuclei and time of incorporation are shown.  $100 \mu\text{c}$   $\text{U-}^3\text{H}$  and  $10 \mu\text{c}$   $\text{RPH-}^{14}\text{C}$  were used in 1 ml of sterile Ringer's. Nuclei were isolated, washed, and sedimented in HM buffer. Sedimentation was carried out for  $4\frac{1}{2}$  hr at 34,000 rpm and  $5^\circ\text{C}$ . The  $\text{U-}^3\text{H}$  radioactivity at the top of the gradient in this and Fig. 2 is acid soluble, whereas the  $\text{RPH-}^{14}\text{C}$  radioactivity is predominantly acid insoluble.

terial sediments as two nuclear fractions. The mixing of uridine- $^{14}\text{C}$ -labeled nuclei with uridine- $^3\text{H}$ -labeled *E. coli* ribosomal subunits shows that these two fractions have sedimentation coefficients of exactly 50 and 30S. The sedimentation profile of the 50S nuclear fraction shows a peak with a heavy shoulder, sometimes separated into a third peak near 60S. In doubly-labeled immature oocyte nuclei, only the 30S fraction appears labeled with amino acids, whereas both it and the 50S fraction are labeled with uridine- $^3\text{H}$  (Fig. 1). Nuclei from mature oocytes of both *Triturus* and *Amblystoma* show a different sedimentation pattern (Fig. 2), in which most of the 50S material has disappeared and only 30S material remains. Whether the specific activity of the latter is the same as it is in immature oocytes is not known, because nuclear fractions show no measurable optical density. However, it is evident that the level of total RNA synthesis in mature nuclei is greatly reduced (see also reference 3). This reduction has chiefly resulted in the virtual disappearance of 50S material.

To avoid confusion, it should be stated that RNP material previously reported by this author

for *Amblystoma* oocyte nuclei (22, 23) corresponds with that just described as sedimenting at 30S. Previous estimates had averaged nearer 40S. This value now appears to be too high—as confirmed by experiments involving mixing with markers. The sedimentation profiles of nuclear extracts from mature oocytes of *Amblystoma* and *Triturus* as obtained on sucrose gradients are practically identical, and are as shown in Fig. 2. Investigation of immature oocytes (and hence 50S material) in *Amblystoma* was not possible, owing to the sudden outbreak of disease in the colony.

For the purpose of determining whether both 50S and 30S particles are RNP complexes, enzyme studies were performed, followed by some preliminary buoyant density analyses on nuclear material of immature oocytes. Treatment with  $10 \gamma/\text{ml}$  of preincubated (to remove RNase) pronase demonstrated a definite shift in the sedimentation coefficient to a lighter value, as compared with the controls. This shift was not due to degradation of RNA. Cesium chloride sedimentation (Fig. 3) revealed densities in the range expected for RNP complexes for both 50S (and

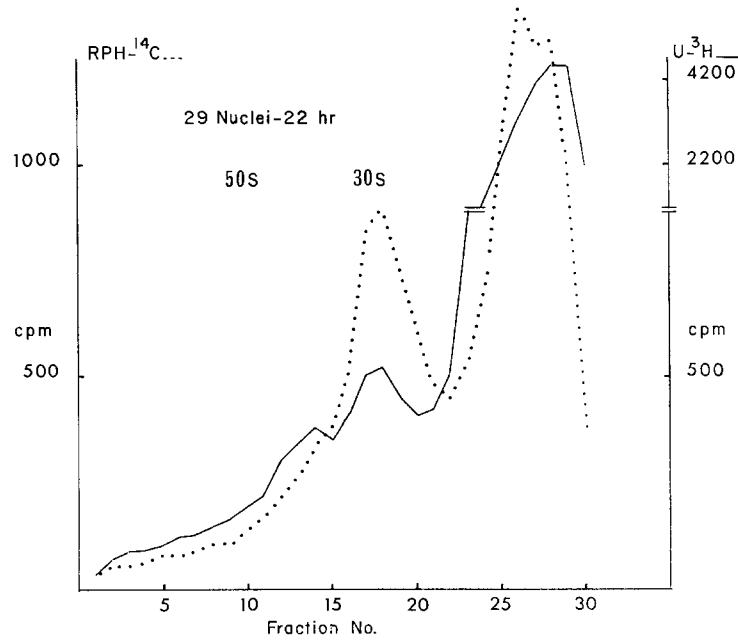


FIGURE 2 The sedimentation profile of a nuclear extract from mature oocytes of *Triturus*. Other details were as given in Fig. 1.

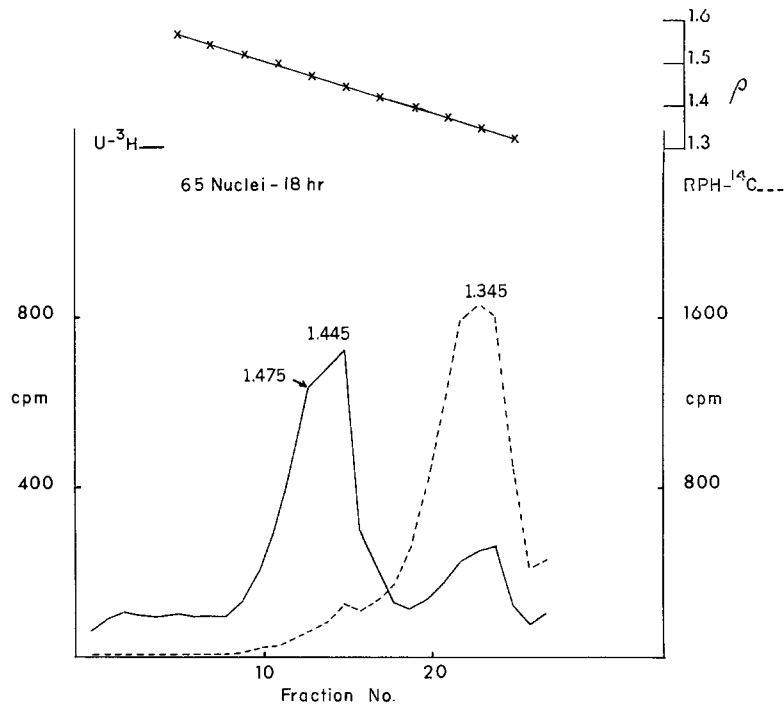


FIGURE 3 The radioactivity profile of a nuclear extract from immature oocytes of *Triturus* after sedimentation in a preformed cesium chloride gradient. Details of the procedure are given in Methods. Density of fractions was determined from measurements of refractive index at 25° made on a blank gradient. Isotopes as in Fig. 1. Each fraction from the experimental gradient was treated with cold 5% TCA, filtered, dried, and counted in toluene-POPOP.

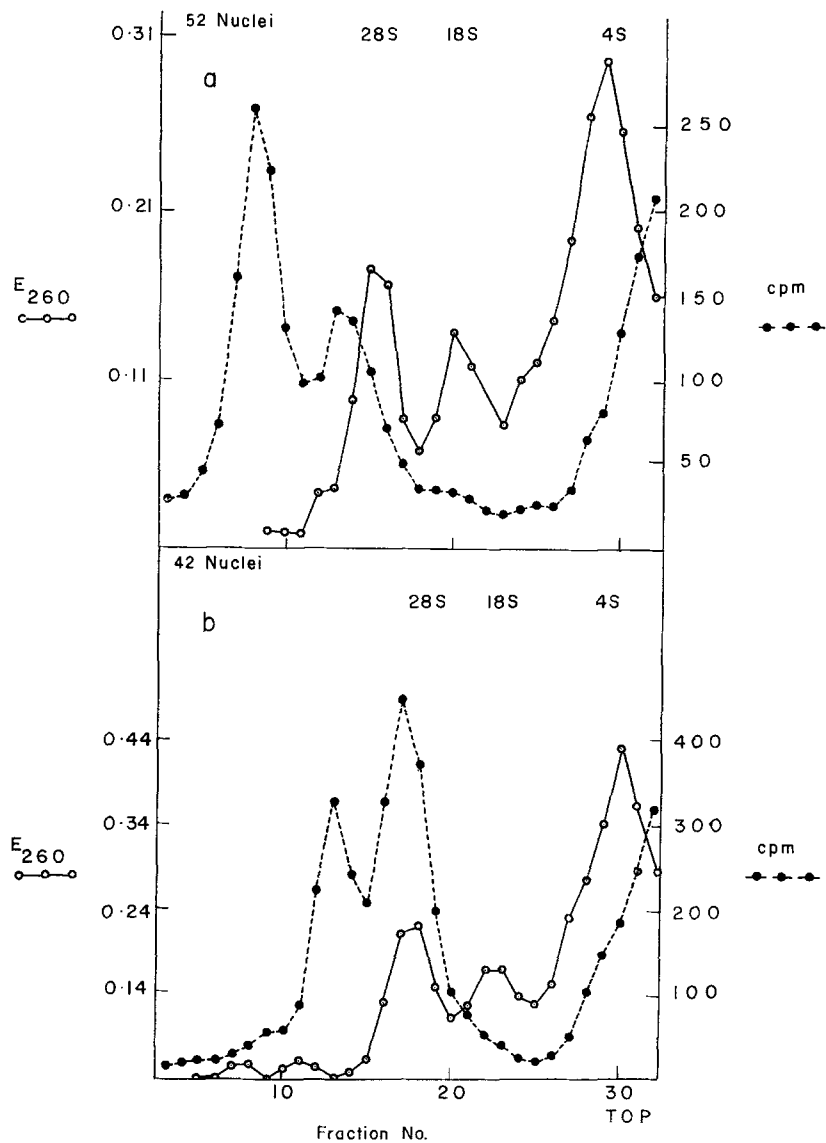


FIGURE 4 Typical sedimentation profiles of total nuclear RNA from mature oocytes of *Amblystoma*. Profiles from *Triturus* were in all cases similar. Incorporation was for 28 (Fig. 4 a) and 44 (Fig. 4 b) hr, with  $100 \mu\text{c U-}^3\text{H}$  in TC-199. Nuclei were washed in acetate buffer after isolation and lysed with 5% SDS (final concentration, 0.5%). Extracts were layered directly onto sucrose gradients (see Methods).  
 Note: in this and all subsequent figures, the 28S and 18S positions were obtained from cold carrier RNA run in the same tube with the radioactive nuclear RNA.

heavier material, arrow in Fig. 3) and 30S materials. The two fractions are distinguished by the fact that, in nuclear material from immature oocytes, only the 30S fraction is doubly labeled (Fig. 1). The density of the latter is unexpectedly low, indicating a very high proportion of protein.

The results have, however, been confirmed several times with different nuclear preparations.

More extensive enzyme studies upon the 30S particle (22) in nuclei from mature oocytes of *Amblystoma* showed that this particle is sensitive to both RNase and trypsin. Sedimentation pro-

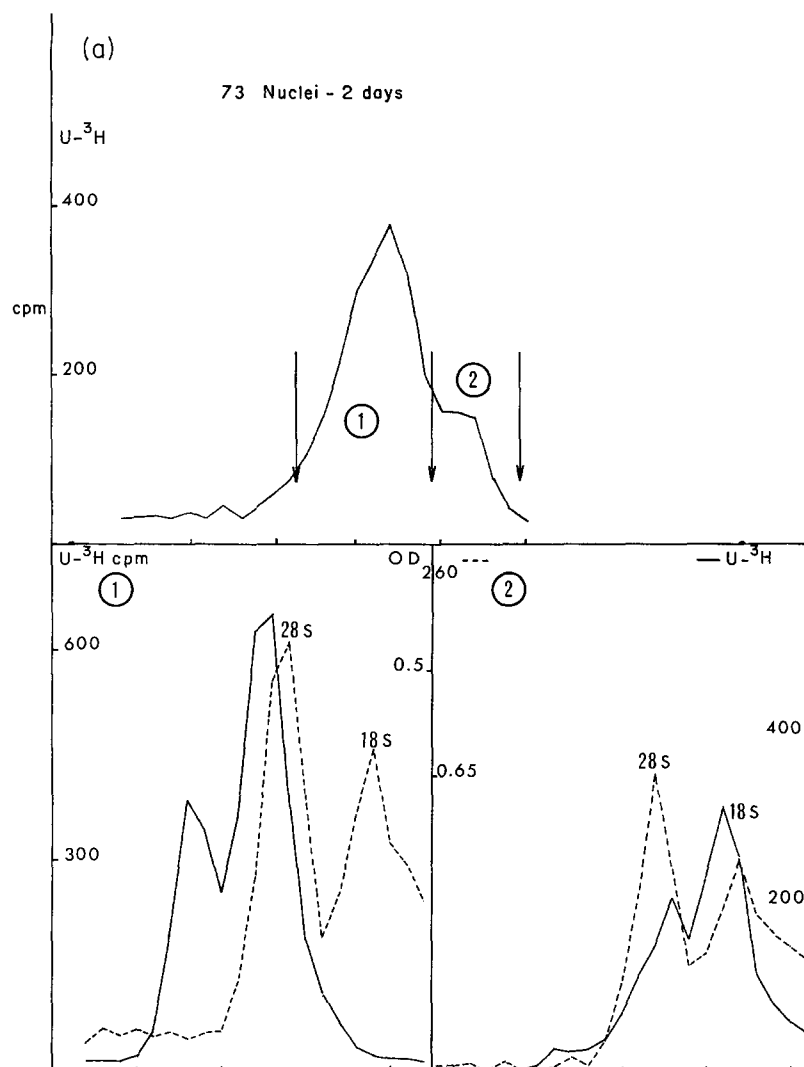


FIGURE 5 Analysis of particle RNA from the nuclei of immature oocytes of *Triturus*. Incorporation was for 2 days in TC-199 + 150  $\mu$ c U-<sup>3</sup>H. Graph (a) illustrates the particle gradient prepared as described in the legend to Fig. 1. Fractions were pooled as shown and separately extracted with SDS (see Methods). Graphs 1 and 2 show the resulting RNA profiles. ---, O.D.<sub>260</sub> of the carrier RNA. There is no measurable OD for nuclear material.

files of treated particles show that some degradation occurs within the first 5 min of exposure to either enzyme (at 10  $\gamma$ /ml). This degradation gives rise in each case to RNP material that sediments between 20 and 25S, and which is not further degraded after an hour in RNase.

The effect of magnesium ions upon the structure of nuclear particles was also investigated. The influence of these ions on ribosomal integrity is well known (24-26). Removal of magnesium ions

from a preparation of particles obtained from mature oocyte nuclei, by washing and sedimenting their contents in 10<sup>-4</sup> or 10<sup>-3</sup> M EDTA, does not significantly alter the sedimentation properties of the particles.

Analysis of the RNA of these particles was of key importance in understanding their function, particularly since the size of the nucleotide pool in oocytes does not allow the use of pulse-chase experiments. Macaloid was used as an RNase

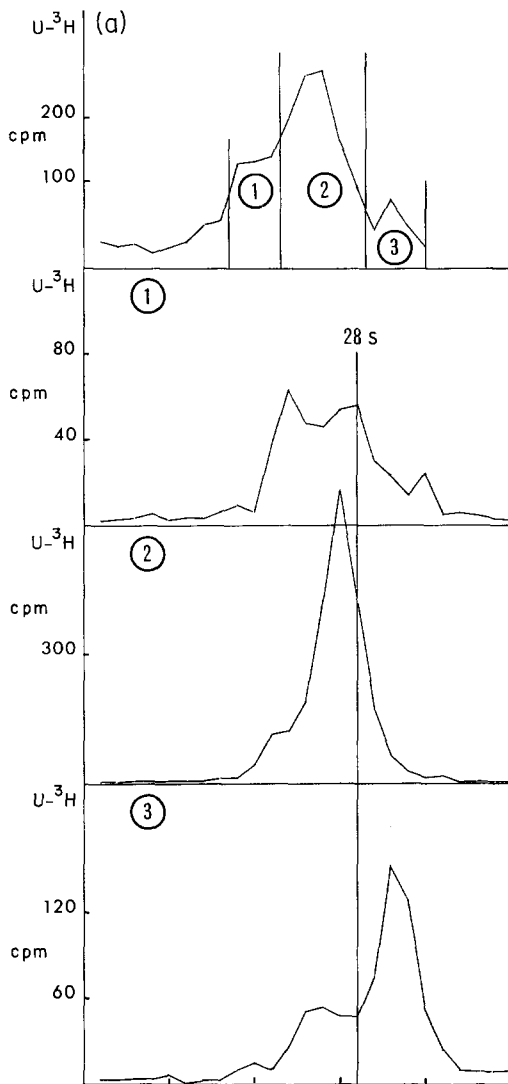


FIGURE 6 More extensive analysis of particle RNA from *Triturus* immature oocyte nuclei. Details were as in the legend for Fig. 5. The 28S marker was cold oocyte ribosomal RNA.

inhibitor, and it proved effective in maintaining the integrity of the very small amount of particle RNA during its isolation. Salamander oocyte nuclei contain chiefly 40 and 30S RNA (4). Representative sedimentation profiles of nuclear RNA extracted with SDS are shown in Fig. 4. A third peak around 20S is sometimes visible. Cold phenol-SDS extraction gives similar results. After phenol extraction, 80% or more of all nuclear RNA sediments at 40 or 30S. Radioactivity at the

top of the gradient in Fig. 4 is in soluble nucleotides.

In Figs. 5-7 the results of particle RNA analyses after SDS extraction are shown—for immature nuclei (Figs. 5 and 6), and mature nuclei (Fig. 7). In each figure, profile (a) represents the radioactivity of the particle preparation. Fractions from separate regions of these gradients were pooled as shown, and their RNA was extracted and run on an acetate-EDTA gradient. In immature oocytes of *Triturus*, nuclear 40 and 30S RNA are present in the 50S particles (Fig. 5). Further analysis indicated that 40S RNA is not equally distributed through the whole 45-60S region, since it can be extracted only from RNP fractions which are heavier than 50S (Fig. 6). Some 30S RNA is always recovered with 40S RNA, however (Fig. 6, 1). 40S RNA is virtually absent from the material in the major 50S peak (Fig. 6, 2). Thus, the nuclear RNP material that sediments between 45 and 60S probably includes two classes of particles—one containing 30S RNA alone, and one containing 40S RNA together with some 30S RNA.

RNA from 30S nuclear particles sediments at about 20S (Fig. 5 and Fig. 6, 3). Such RNA has not been previously reported in amphibians. It is not regularly distinguishable in total nuclear RNA extractions. For mature oocyte nuclei (Fig. 7) the main RNA fractions are similar to those found for immature oocyte nuclei (Fig. 5), but the sedimentation profiles are not so sharp, and the total amount of incorporation into all types of RNA is not so great. This is manifested most strikingly by a decrease in the proportion of 40 and 30S RNA to 20S RNA, and is a reflection of the reduction in the amount of 50S particles (Fig. 2). Whether the broadening of RNA profiles represents the appearance of heterogeneous nuclear RNA which was previously masked by the huge amounts of ribosomal RNA is a question worthy of consideration.

## DISCUSSION

Students of the amphibian oocyte are unusually fortunate in having available large nuclei which can be isolated readily from cytoplasm without damage (12, 13, 27). The availability of these nuclei has led to an appreciable increase in our knowledge of nuclear structure and function (3, 4, 13, 27-30). The data reported here will be interpreted in the light of this knowledge and of that gained from other systems.



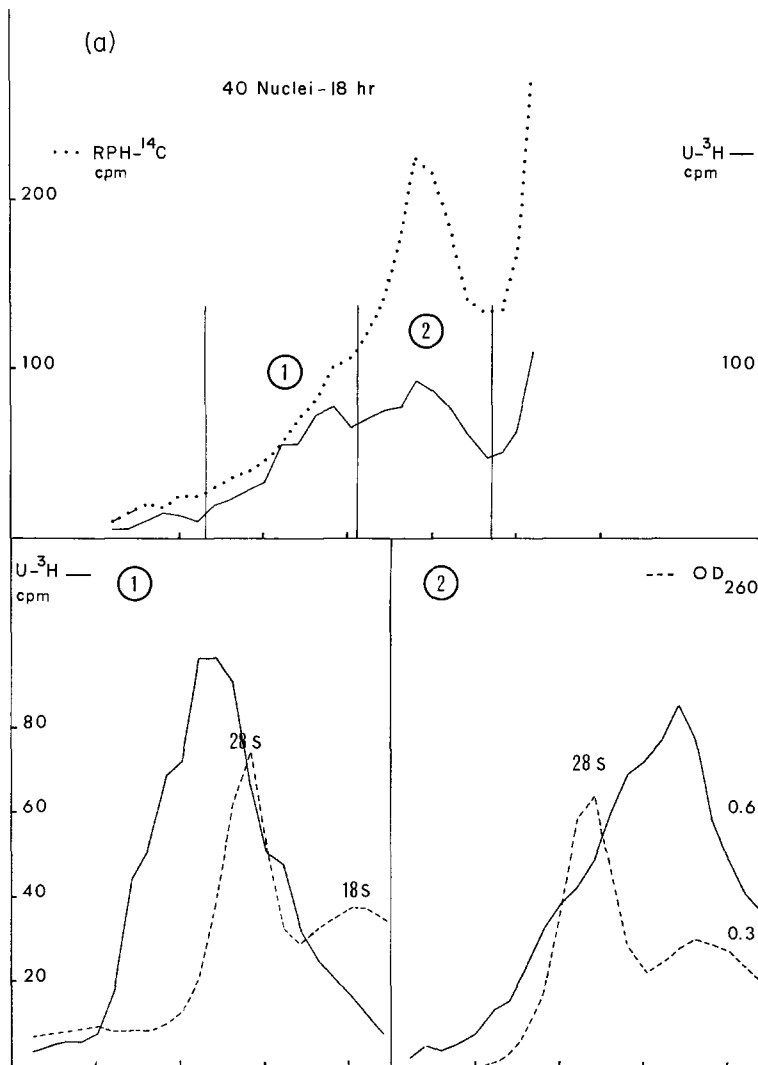


FIGURE 7 Analysis of particle RNA from mature oocyte nuclei of *Triturus*. Incorporation was for 18 hr in sterile Ringer's + 100  $\mu$  U- $^3$ H and 10  $\mu$  RPH- $^{14}$ C. Other details are given in the legend for Fig. 5.

First to be considered is the question of the existence of 78S ribosomes in the nucleus. Morphological evidence for the existence of particulate nuclear components has been available for some time (19). In amphibian oocytes, particulate components are most conspicuous in and around the nucleolus (28, 29). Since the nucleolus is functionally involved in the synthesis of ribosomes (31), an obvious conclusion would be that the granular elements associated with it represent completed ribosomes. An additional justification for such a conclusion is that the oocyte is a non-

dividing cell that produces large numbers of ribosomes. The conservation of these ribosomes throughout oogenesis (3) might involve their accumulation in the nucleus, at least during the stages of maximum synthesis.

Considerable evidence is now available that strongly suggests that whole ribosomes are neither assembled nor stored in oocyte nuclei. Data are also available concerning the RNA in HeLa cell nuclei (10) and the bulk RNA and protein of starfish oocyte nucleoli (32). Amphibian oocyte nuclei, layered directly on a gradient under condi-

tions that permit preservation of cytoplasmic ribosomes, do not contain radioactive 78S material (22). The extracted RNA of such nuclei does not include 18S material (Fig. 4, and reference 4). This is also true of nucleolar RNA from HeLa cells (10). 28S RNA, if present, is associated with heavier material in these oocyte nuclei, and sediments nearer 30S (Fig. 4, and reference 4). Again, this is found in HeLa cell nuclei (10, 33). Collectively, these results support the concept that new 78S ribosomes are neither completed in the nucleus nor returned to it after assembly in the cytoplasm. Large quantities of ribosomes are almost certainly not lost from the nuclei during their isolation, for several reasons. First, it has been shown (34) that nuclear sap is not lost in the first 2-3 min following isolation; in these experiments the nuclei were isolated at a rate greater than one per minute. Second, the use of magnesium-containing buffers ensures precipitation, and hence fixation, of the nuclear contents. Finally, electron micrographs of isolated nuclei show no evidence of structural abnormalities such as might result from loss of their contents (28).

The major part of this paper has been concerned with the analysis of some nuclear components which are not identical with ribosomes or their subunits, but which are probably their precursors. It has been shown, in the oocyte nuclei of two species of amphibia, that material containing both RNA and protein and having sedimentation coefficients of 50 and 30S is demonstrable after incubation of oocytes in radioactive precursors. Measurements of the proportion of the nuclear acid-insoluble uridine-<sup>3</sup>H radioactivity incorporated into this RNP material have shown that 80% or more of the radioactive nuclear RNA is present as RNP. Since over 90% of all nuclear RNA synthesis is ribosomal, this result means that the RNA in the nuclear particles is largely ribosomal RNA.

Analysis of particle RNA indicates the presence of three types of nuclear particle. The heaviest, containing 40S RNA and perhaps also 30S RNA, sediments between 55 and 60S (Fig. 6, 1). The predominant particle sediments at 50S and contains almost exclusively 30S RNA (Fig. 6, 2). The third sediments at 30S and contains 20S RNA (Figs. 5, 2; 6, 3; 7, 2). That 40 and 30S RNA are ribosomal precursors in this system is suggested by the fact that almost all the RNA made in the nucleus ultimately becomes incorporated into

cytoplasmic ribosomes as 28 and 18S RNA. Their precursor nature is also suggested by analogy with findings in mammalian cells (33, 35, 36). Hybridization-competition data on nuclear RNA and DNA from *Triturus*<sup>3</sup> have also demonstrated the ribosomal nature of nuclear RNA. The nature of 20S RNA, on the other hand, has not been elucidated directly. It has been reported also in *Chironomus tentans* (11) and in HeLa cell nucleoli (37), and may be a precursor to 18S ribosomal RNA. In HeLa cells, 20S RNA is present only in small amounts compared with 30 and 40S RNA. The larger proportion of it reported in these experiments with immature oocyte nuclei indicates some degree of accumulation of ribosome precursors in the nucleus. Such accumulation is, to some extent, predictable from the massive enrichment and activity of the ribosome-synthesizing machinery in oocytes (2, 3).

RNP particles containing 40S RNA have been previously reported in L-cell nuclei (38), and particles containing 30 and 40S RNA have been found in HeLa cell nucleoli (39). In both systems, particles containing 40S RNA were themselves heavier than 60S. It is not possible to say whether this situation is basically different from that reported in this paper, because the conditions of sedimentation and the ionic properties of the buffers were different. Thus, the heaviest particles reported here, containing all the 40S RNA and some 30S RNA, may be found to be equivalent to the 80S RNP particles reported in HeLa cell nucleoli (39), which also contain 40 & 30S RNA. The 50S particle reported here seems closely similar to that found in HeLa cell nucleoli (39).

Although the preliminary nature of these results leaves much that is far from clear, it is postulated that the oocyte nucleus contains RNP complexes between protein and the RNA precursors to cytoplasmic ribosomal RNA. The biochemical analysis of such complexes should provide information concerning the initial steps in the attachment of ribosomal protein to ribosomal RNA.

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<sup>3</sup> Gall, J. G. Unpublished observations.

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