SOME PROPERTIES OF THE 24S PARTICLE ISOLATED FROM THE CYTOPLASM OF SEA URCHIN EGGS

Sabera Ruzdijic, Georgi Milchev[+], Nevenka Bajkovic and Vladimir Glisin, Laboratory of Molecular Biology, Institute of Biological and Medical Sciences in Montenegro, Kotor[*] and Center for Multidisciplinary Studies, University of Belgrade, Belgrade, Yugoslavia

Received May 10, 1973

Summary

Discrete independent protein-RNA particles with a sedimentation constant of about 24S have been isolated from the cytoplasm of unfertilized sea urchin eggs or developing embryos. They contain about 8% of the total protein of the egg/embryo. The particles are 3-4% RNA by weight. Therefore, these particles bind the amount of RNA equal to about 4% of the total RNA of the egg/embryo. On the basis of labeling kinetics and sedimentation properties we tentatively conclude that this RNA represents the nonpolyribosome-bound RNA of the cytoplasm.

Discrete entities with a sedimentation constant of about 24S can be isolated from the 100,000x g(S_{100} fraction) supernatant of developing sea urchin embryos. These particles are obtained when the S_{100} fraction is centrifuged for 2-4 hours at 275,000 x g(1,2,3,4). It has recently been shown by immunological studies (5,6) that these 24S particles isolated from embryos of different stages of development are similar and yet distinct from the yolk granules. They contain about 8% of the total protein of the unfertilized egg/embryo.

We have investigated and confirmed the existence of these particles in two Adriatic sea urchins, Arbacia lixula and Paracentrotus lividus. We have also found that a very large fraction (about 70% or more) of the nonpolyribosome-bound newly synthesized RNA found in the cytoplasm can be recovered in the form of these particles. Sedimentation analysis showed that the RNA

+ Permanent Address: Biochemistry Laboratories, Bulgarian Academy of Sciences, 13 Sofia, Bulgaria

* Mailing Address: P.O.Box 69, Kotor, Yugoslavia

complexed to 24S particles does not correspond to the ribosomal, 4S or 5S RNAs. The amount of RNA in these particles constitutes only 3-4% of the total particle weight. Nevertheless, because of the high concentration of these particles in the embryo, the RNA found here makes up about 3-4% of the total RNA of the sea urchin egg of embryo.

Materials and Methods

Ripe eggs of Paracentrotus lividus and Arbacia lixula were obtained respectively by excision of the ovaries and by electrical stimulation. Freely sedimenting eggs were placed in filtered sea water supplemented with antibiotics(7). The eggs were fertilized with 3-4 drops of concentrated sperm. Only those cultures were used in which more than 95% of eggs were fertilized. At different stages of development ^{3}H-guanosine, ^{3}H-uridine, ^{3}H-amino acid mixture, ^{14}C-amino acid mixture or ^{14}C-lysine (New England Nuclear) were added to a final concentration of 1 C/ml. Following incubation (usually for hours) the embryos were sedimented by hand centrifugation through a mixture of one part of 0.88M sucrose and one part of sea water. The eggs/embryos were then homogenized with a Dounce (size B) homogenizer in three volumes of the triethanolamine buffer (0.05M triethanolamine, 0.005M $MgCl_2$, 0.24M KCl and 0.25M sucrose (Merck), pH 7.8).

The homogenate was placed over a 1 ml cushion of 0.5M sucrose and centrifuged for 15 minutes at 15000 x g. The postmitochondrial supernatant was then placed over a 2 ml cushion of 0.5M sucrose and further centrifiged for 60 minutes at 225,000 x g in a 50 rotor in a Beckman L2 preparative ultracentrifuge. The resulting postmicrosomal supernatant was finally centrifuged in the same rotor over the same type of cushion for an additional four hours at 275,000 x g. The pellet (the crude 24S particles) were then analyzed for thier sedimentation properties, RNA and protein content and kinetics of the RNA and protein radioactive label.

Typically 0.2 ml of the resuspended crude 24S pellet was layered onto linear 25 ml 15-30% sucrose gradient in the appro-

priate buffer and centrifuged for 12 hours at 24,000 rev/min. in a Spinco SW 25 rotor at 4°. Following centrifugation, 1 ml fractions were collected starting from the bottom of the tube. The absorbance was read at 235, 260 and 280 nm and aliquots were taken for a determination of radioactivity.

Radioactivity was measured in toluene-based fluid using a Beckman 150-LS scintillation counter.

The sedimentation coefficient was measured by the sedimentation velocity method in a Beckman Model E analytical ultracentrifuge. The sedimentation analysis was for each sample performed a 3 different concentrations of the particles and the S value was extrapolated to zero concentration.

The RNA was extracted from the 24S particles by the hot phenol-SDS method(8).

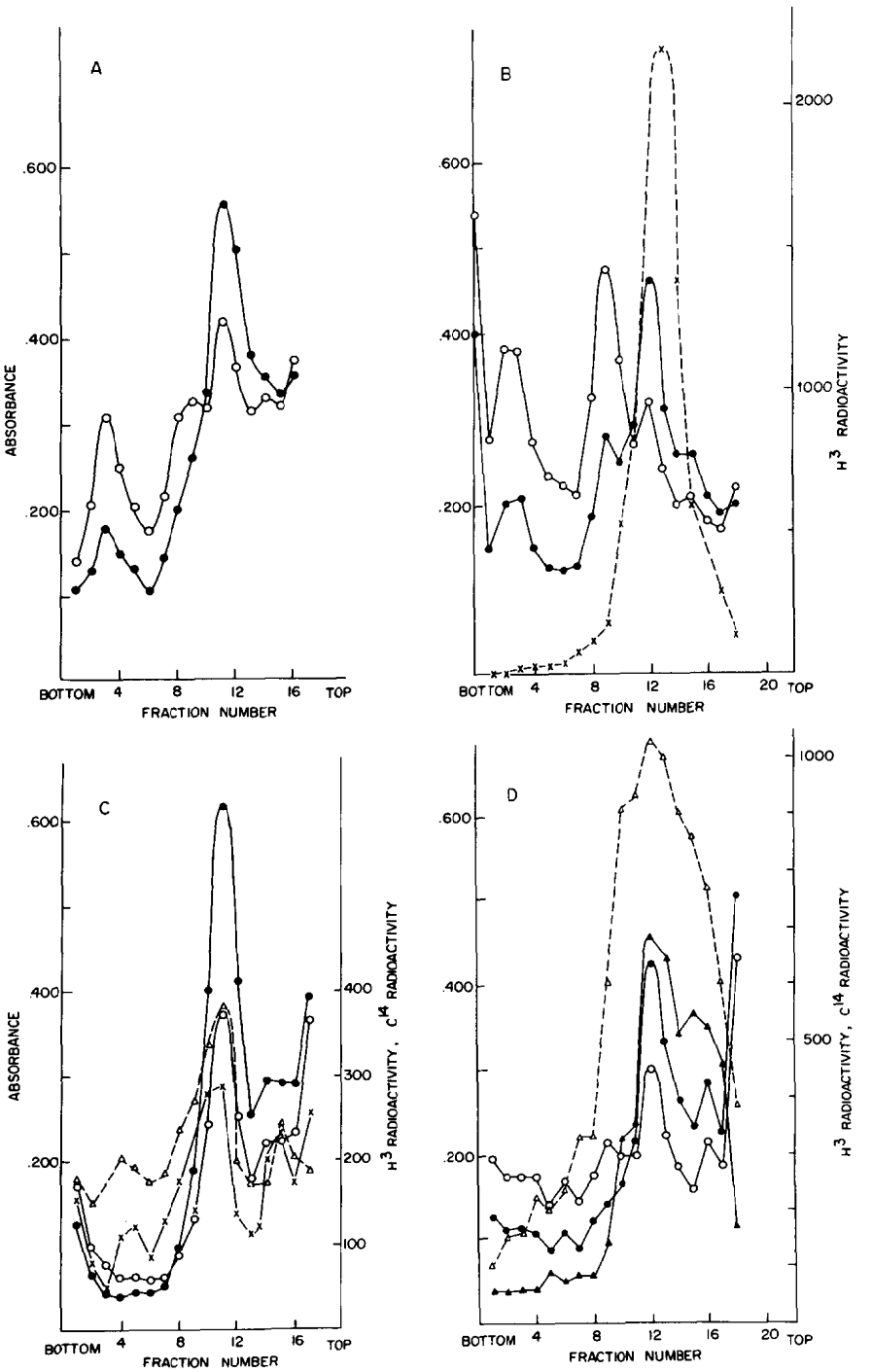
Results and Discussion

Figures 1a, 1b, 1c and 1d represent the sedimentation profiles and the distribution of radioactive labels(RNA and protein precursors) of the crude 24S particles in 15-30% sucrose gradients. The crude particles are usually contaminated with ribosomes or ribosomes or ribosomal subunits. The extent of this contamination depends on the stage of development of embryos from which the 24S particles have been isolated. The largest contamination was found in the unfertilized egg. As the embryos develop the amount of polyribosomes increases (9), and hence the contamination by these subunits becomes gradually smaller (at blastula or pluteus stage the 24S particles are almost completely free of the contaminants). The yield of 24S particles does not vary significantly from stage to stage (including the unfertilized egg) and these particles always contain about 8% of the total protein of the egg. Since in the earlier stages of sea urchin development significant synthesis of either ribosomal RNA or ribosomal protein does not take place (10), the incorporated RNA and protein precursors are confined to the 24S peak (fractions: 8-14) of the crude 24S particles only.

When the RNA is extracted from these crude particles with phenol and sedimentation analysis carried out (figures 2a and 2b) the labeled RNA originally bound to the 24S particles sediments with a sedimentation coefficient of about 9-12S. At later stages of development (fig.2b) a pronounced optical density peak is observed as well. This peak (fraction 13, fig.2b) is not derived from any of the ribosomal RNAs, since under the experimental conditions used, these RNAs have not yet incorporated the label. Therefore, the RNA associated with the 24S particle should have unique labeling properties as compared to ribosomal RNAs.

Since the 24S particles are found in the same large amount in different developmental stages (about 8% of the total protein of the embryo), they must play an important role in the metabolism of the embryo. The constant amount of 24S particles from the unfertilized egg to the investigated stages of development(pluteus) and its anabolic activity, as determined by radioactive amino acid incorporation (fig. 1b, 1c and 1d) clearly excludes the possibility that these particles are derived from the yolk.

The similarity of the sedimentation coefficient of the 24S particles to the reported for RNA polymerase of eukaryotics (11) and the fact that surpluses of RNA polymerase sufficient to support transcription for about 4×10^5 somatic cells has been reported in frog eggs (12) suggested the possibility that the 24S particles may be RNA polymerase. However, no RNA polymerase activity was found associated with the 24S particle. Obviously, a negative result does not necessarily exclude the possibility that these particles are molecules or part of the molecules of an RNA polymerase. Inhibitors attached to or factors missing from them could yield a negative answer. However, the amount of these particles per se urchin embryo is extremely high for an enzyme. Also, since these particles have been found in the cytoplasm of the unfertilized eggs they are most probably distinct from the RNA polymerase enzyme. Therefore, we tentatively propose that these <u>independent protein-RNA complexes</u> represent cytoplasmic entities in which stable, maternal mRNA is stored prior


A
B
C
D
ABSORBANCE
.600
.400
.200
BOTTOM
4
8
12
16
20
TOP
FRACTION NUMBER
2000
1000
H^3 RADIOACTIVITY
400
300
200
100
500
C^{14} RADIOACTIVITY

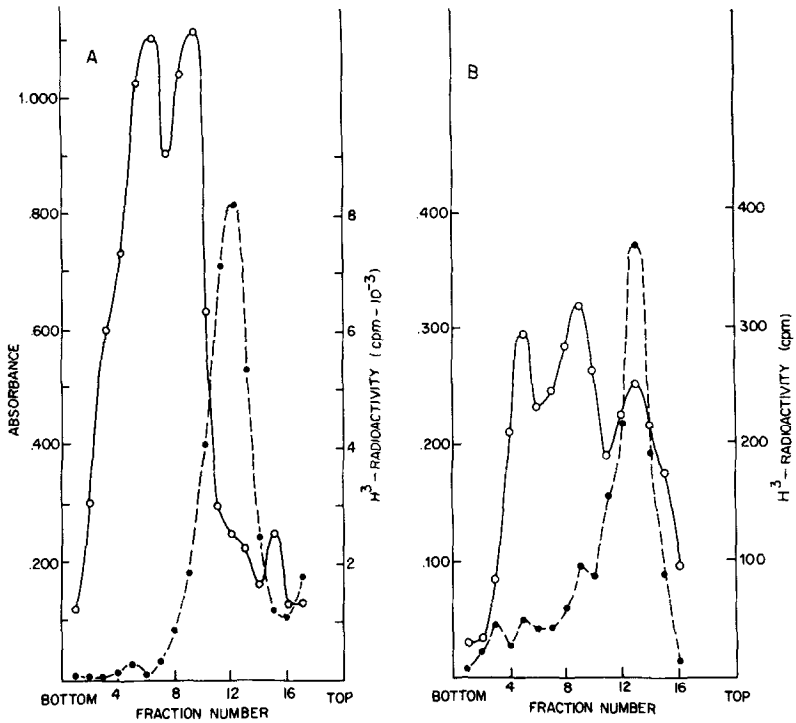


Figure 2. Sedimentation profiles on 4-20% linear sucrose gradients of RNA isolated from crude 24S particles. A. 8-cell stage; B. blastula.
o------o O.D. at 260 nm; ●------● ^{3}H-guanosine

to its activation. They may also represent one of the obligatory steps in the general biochemical processes of the transport (or storage) of the newly synthesized mRNA from the nuclei to the polyribosomes. The biological role of these particles is obscure presently, and further work is required to elucidate their biological function.

Whatever biological role these particles may play, the most

Figure 1. Sedimentation profiles of crude 24S particles on 15-30% linear sucrose gradients.
A. unfertilized egg; B. 8-cell stage; C. blastula; D. gastrula;
o------o$_3$ O.D. at 260 nm; o------o$_{14}$ O.D. at 280 nm;
x------x ^{3}H-guanosine; ▲------▲ ^{14}C-lysine;
▲------▲ ^{3}H-uridine

immediate use of them would be as a tool for isolation of large quantities of nonpolyribosome-bound RNA of the cytoplasm, free of other RNAs.

Acknowledgments

We are indebted to Dr. Ana Savic for useful discussions and critical evaluation of our work. This work was supported by grant No. 02-020-1 from the National Institutes of Health, U.S. Department of Health, Education and Welfare (PL-480 program).

REFERENCES

1. Infante, A. A. and Nemer, M., J. Mol. Biol., 32, 543 (1968).
2. Kane, R. E., J. Cell Biol., 32, 243 (1967).
3. Malkin, L. I., Mangan, J. and Gross, P. R., Dev. Biol., 12, 520 (1965).
4. Stephens, R. E., J. Cell Biol., 32, 255 (1967).
5. Kondo, H., Exptl. Cell Res., 72, 519 (1972).
6. Kondo, H. and Koshira, H., Exptl. Cell Res., 75, 385 (1972).
7. Glisin, V. R. and Glisin, M. V., Proc. Natl. Acad. Sci., 52, 1548 (1964).
8. Penman, S., J. Mol. Biol., 17, 117 (1966).
9. Ruzdijic, S and Glisin, V. R., Biochim. Biophys. Acta, 269, 441 (1972).
10. Bajkovic-Moskov, N. and Glisin, V. R., unpublished observation.
11. Seifert, H. K., Lepetit Coloquia on Biology and Medicine, RNA-polymerase and Transcription, p. 233, Wiley Interscience, New York, 1970.
12. Wassarman, P. M., Hollinger, T. G. and Smith, L. D., Nature New Biol., 240, 208 (1972).