Poly(A)-containing RNA in early embryogenesis of sea urchins

I.V. Prokopenko and V.V. Sova*

Kamchatka Branch, Institute of Marine Biology, Far Eastern Research Center, Academy of Sciences of the USSR, Petropavlovsk-Kamchatsky, USSR

Received 16 January 1984

The content, biosynthesis and template activity of poly(A)$^+$ RNA in the early stages of sea urchin development have been studied. The amount of poly(A)$^+$ RNA reaches a maximum at the middle blastula stage in polyribosomes and at the 8-blastomere stage in the cytoplasm. Poly(A)$^+$ RNA synthesis becomes noticeable at the 64-blastomere stage and the spectrum of newly synthesized molecules is different from that at the middle blastula stage. The products of translation in vitro of poly(A)$^+$ RNA at all the stages studied show insignificant differences and contain a major group of polypeptides of molecular mass 10–20 kDa.

Sea urchin embryogenesis *Poly(A)$^+$ RNA* *In vitro translation*

1. INTRODUCTION

Among the many sets of poly(A)$^+$ RNA functioning at the early stages of sea urchin embryo development, tubulin mRNA [1,2] and actin mRNA [3] have been identified. A comprehensive study has been made of the mRNA in histones which in sea urchins do not possess the poly(A) fragment [4,5]. A systematic study of poly(A)$^+$ RNA at early stages of sea urchin development has not been performed.

We have shown previously that the translation product of polyribosomal poly(A)$^+$ RNA isolated at the middle blastula stage from sea urchin embryos (*Strongylocentrotus intermedius*) reacts with antibodies against glycoproteins of the embryo cell plasma membrane [6], the synthesis of which is maximal at the middle blastula stage [7]. Apparently there is a significant amount of templates in the fraction of poly(A)$^+$ RNA coding for the synthesis of embryo-specific glycopolypeptides of the embryo cell plasma membrane. It remains unclear whether these polypeptides are synthesized on the accumulated maternal mRNA or on the newly transcribed templates.

* To whom correspondence should be addressed

This study is devoted to elucidating the role of poly(A)$^+$ RNA in the synthesis of a group of embryo-specific polypeptides of the embryo cell plasma membrane, the study of which was made in parallel.

2. MATERIALS AND METHODS

2.1. *Sea urchins*

St. intermedius, St. nudus and *Scaphechinus mirabelis* collected during active spawning in Posiet Bay (Sea of Japan) were used here. Fertilization and development of the embryos were done as in [8].

2.2. *Polyribosome preparation*

The postmitochondrial supernatant, polyribosomes and polyribosome RNA were prepared as in [9].

2.3. *Preparation of cytoplasmic RNA*

Triton X-100 and EDTA were added to the postmitochondrial supernatant to concentrations of 2% and 0.015 M, respectively, in a 2.5-fold volume of ethanol. Such a mixture can be stored at −20°C for a prolonged period. The pellet obtained by centrifugation of the mixture was

Published by Elsevier Science Publishers B V
00145793/84/$3 00

dissolved in a buffer containing 0.1 M Tris–HCl (pH 8), 0.1 M NaCl, 0.001 M EDTA and 0.5% SDS. The mixture with an addition of proteinase K (0.1 mg/ml, Merck) was incubated for 20 min at room temperature. Deproteinization and reprecipitation of RNA were done as in [9].

2.4. *Poly(A)$^+$ RNA preparation*

Poly(A)$^+$ RNA was prepared by two cycles of oligo(dT)–cellulose chromatography (PL Biochemicals) as in [10].

2.5. *Sedimentation analysis of poly(A)$^+$ RNA*

RNA (100–200 μg) was centrifuged for 3.5 h at 49000 rpm (Beckman rotor SW-50.1) in a linear sucrose gradient (5–20%) prepared on a buffer containing 0.01 M Tris–HCl (pH 7.5), 0.1 M NaCl and 0.005 M EDTA.

2.6. *Cell-free protein synthesis*

The RNA template activity was tested in a wheat germ cell-free protein-synthesizing system prepared as in [9]. Ten μCi [^{3}H]leucine or 0.5 μCi [^{14}C]leucine (Amersham, spec. act. 160 and 0.33 Ci/mmol, respectively) per 100 μl sample were used in the experiments.

2.7. *Analysis of cell-free synthesis products*

After incubation the fractions were dialyzed against 0.1% SDS, 0.5% 2-mercaptoethanol and lyophilized. The samples were dissolved in buffer comprising 0.625 M Tris–HCl (pH 6.8), 2% SDS, 0.002 M EDTA, 5% 2-mercaptoethanol, 10% glycerine. Electrophoresis was performed as in [11] in a 10–18% polyacrylamide gel gradient. Autofluorography was performed as in [12]. A set of proteins (Pharmacia) was used as markers: phosphorylase *b* (94000), albumin (67000), ovalbumin (43000), trypsin inhibitor (20000), α-lactalbumin (14000).

2.8. *Preparation of [^{3}H]poly(A)$^+$ RNA*

[^{3}H]Uridine (2 μCi/ml, Isotop, USSR) was added to the suspension of developing embryos an hour before culmination. After development, the embryos were washed with seawater and a 10-fold volume of the lysis buffer containing 1.5% SDS, 0.05 M EDTA (pH 7.8) and 0.1 M NaCl was added. Incubation was done by mixing until lysis was completed. Two vols of ethanol were added to the lysate. Storage was at −20°C.

Total RNA was extracted from the precipitate after centrifugation of the ethanol mixture as described in section 2.3, clarified from RNA by precipitation with 2 M LiCl [13] and poly(A)$^+$ RNA obtained as detailed in section 2.4.

3. RESULTS AND DISCUSSION

3.1. *Content of poly(A)$^+$ RNA at early stages of sea urchin embryo development*

Poly(A)$^+$ RNA was prepared from polyribosomes and the cytoplasmic fraction (postmitochondrial supernatant) of sea urchin embryos (*St. intermedius*) at early stages of development: unfertilized egg, 8 blastomeres, 64 blastomeres, middle blastula and gastrula. The poly(A)$^+$ RNA content was determined by the amount of RNA bound to oligo(dT)–cellulose. Table 1 shows data on the quantitative content of poly(A)$^+$ RNA at defined stages of embryo development. The fraction of poly(A)$^+$ RNA sharply increases at the middle blastula stage for polyribosomal RNA. For cytoplasmic RNA the maximum content of poly(A)$^+$ RNA was observed at the 8-blastomere stage which apparently indicates the occurrence of cytoplasmic polyadenylation in the first hours after fertilization as reported in [14]. The different content of poly(A)$^+$ RNA in polyribosomes and in the cytoplasmic fraction can be explained by the large amount of translating histone templates at the first stages of development [15] devoid of the poly(A) fragment; however in the cytoplasm there is a large store of maternal poly(A)$^+$ RNA which is not translated. At the middle blastula stage the amount of histone mRNA on polyribosomes decreases and that of the active translatable poly(A)$^+$ RNA increases.

3.2. *Translation activity of poly(A)$^+$ RNA*

Poly(A)$^+$ RNA-directed protein synthesis from polyribosomes and the cytoplasmic fraction increases linearly with the addition of increasing amounts (up to 4–5 μg) of RNA to the cell-free protein-synthesizing system (not shown, see [9]).

All the RNA obtained at different stages of *St. intermedius* development was tested for template activity in the wheat germ cell-free system (table 1). The maximum activity in vitro is displayed by both the polyribosomal and cytoplasmic poly(A)$^+$ RNA

Table 1

Determination of poly(A)$^+$ RNA template activity

Sea urchin species	Stages of embryo development	Polyribosomal poly(A)$^+$ RNA		Cytoplasmic poly(A)$^+$ RNA	
		Poly(A)$^+$ RNA fraction of the total RNA (%)	Radio-activity (cpm^{-1})[a]	Poly(A)$^+$ RNA fraction of the total RNA (%)	Radio-activity (cpm^{-1})[b]
–	–	without RNA	2500	without RNA	20000
St. intermedius	egg	0.1	10350	0.13	186000
St. intermedius	8 blastomeres	0.2	12500	1.2	420000
St. intermedius	64 blastomeres	0.35	15300	1.0	450000
St. intermedius	middle blastula	0.8	30200	0.7	520000
St. nudus	middle blastula	–	–	0.6	450000
Sc. mirabelis	middle blastula	–	–	0.4	560000
St. intermedius	gastrula	0.5	11000	0.24	312000

Poly(A)$^+$ RNA was prepared from total polyribosomal or cytoplasmic RNA by chromatography on oligo(dT)–cellulose; the amount of bound material was estimated and poly(A)$^+$ RNA translated into the wheat germ cell-free system. The RNA template activity was measured by the radioactivity in the trichloroacetic acid precipitate. The translation mixture sample (100 μg) contained: [a] 4 μg poly(A)$^+$ RNA, 0.5 μCi [^{14}C]leucine; [b] 5 μg poly(A)$^+$ RNA, 10 μCi [^{3}H]leucine

obtained at the middle blastula stage. For comparison, poly(A)$^+$ RNA from the cytoplasmic fraction of *St. nudus* and *Sc. mirabelis* embryos at the middle blastula stage was translated into the cell-free system (table 1). These RNAs also display a high template activity.

The translation products of all the RNAs studied were analysed by polyacrylamide gel electrophoresis in the presence of SDS with subsequent autofluorography (fig.1). At all presented stages of *St. intermedius* embryo development predominant synthesis of polypeptides of 10–20 kDa is observed. The results obtained on analysis of translation products of polyribosomal poly(A)$^+$ RNA and RNA isolated from sea urchin embryos of various species at the middle blastula stage are, in general, analogous to the data shown in fig.1 and so are not presented.

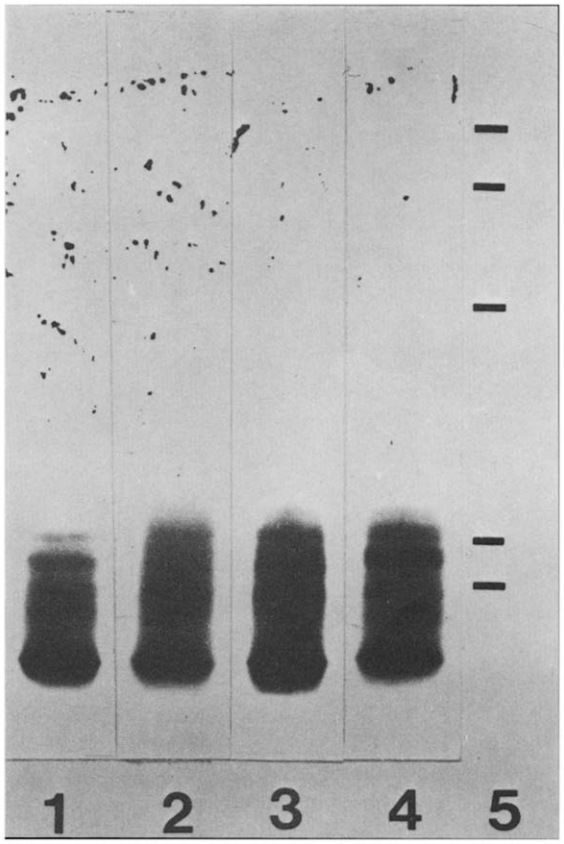

Fig.1. SDS-polyacrylamide gel electrophoresis autofluorogram of sea urchin (*St. intermedius*) embryo poly(A)$^+$ RNA translation products in the wheat germ cell-free system: 1, egg; 2, 8 blastomere; 3, middle blastula; 4, gastrula; 5, a set of proteins was used as markers (see section 2.7). Control (without RNA) is not shown as no activity in the given sample was observed.

Table 2

Incorporation of [^{3}H]uridine in the RNA of sea urchin embryos (*Strongylocentrotus intermedius*)

Stage of development	RNA fraction	Radioactivity ($cpm^{-1} \cdot \mu g^{-1}$)
64 blastomeres	total	300
	poly(A)$^-$	250
	poly(A)$^+$	3500
Middle blastula	total	800
	poly(A)$^-$	550
	poly(A)$^+$	7000

[^{3}H]Uridine was introduced in vivo as indicated in section 2.8, RNA chromatographed on oligo(dT)–cellulose, aliquots taken from different fractions and radioactivity measured in a scintillator (toluene: Triton X-100 = 30:1)

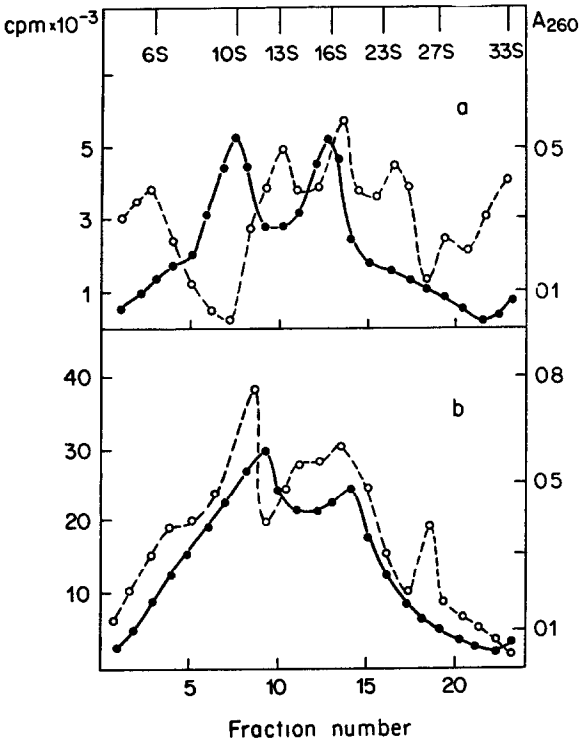

Fig.2. Fractionation of sea urchin (*St. intermedius*) embryo poly(A)$^+$ RNA at the stage of 64 blastomeres (a) and at middle blastula (b) in a linear sucrose gradient (5–20%). Centrifugation conditions are given in section 2.5. (●—●) A_{260}, (○---○) radioactivity.

3.3. *Analysis of newly synthesized poly(A)$^+$ RNA*

We could not observe a noticeable incorporation of the label into poly(A)$^+$ RNA at the 8-blastomere stage. Table 2 shows the distribution of label in the RNA fractions at the 64-blastomere and middle blastula stages. It follows from these data that uridine is actively incorporated into poly(A)$^+$ RNA, especially at the middle blastula stage.

The results of [^{3}H]poly(A)$^+$ RNA centrifugation in the linear sucrose gradient (5–20%) indicate that the absorbance profiles of both RNA preparations have no essential differences. The RNAs under study are presented in two main groups: 8–10 S and 14–16 S RNAs (fig.2). However, the sedimentation analysis of radioactive RNA distribution reveals differences in the character of poly(A)$^+$ RNA synthesis at early stages of embryo development: If 8–10 S RNA at the 64-blastomere stage does not synthesize (fig.2a), the fraction corresponding to 8–10 S poly(A)$^+$ RNA exhibits a significant level of radioactivity at the middle blastula stage (fig.2b).

4. CONCLUSION

From the above data it follows that poly(A)$^+$ RNA in sea urchin early embryogenesis and right up to the middle blastula stage predominantly directs the synthesis of a group of polypeptides of 10–20 kDa among which are a significant number of embryo-specific peptides found within the content of sialoglycoproteins of the embryo cell plasma membrane as shown in [6,7,9].

Poly(A)$^+$ RNA is actively transcribed starting from the 64-blastomere stage but the synthesis of polypeptides of 10–20 kDa apparently proceeds on the maternal 8–10 S poly(A)$^+$ RNAs. The question of transcription of these mRNAs at the middle blastula stage remains open and the answer will be supplied as a result of 8–10 S [^{3}H]poly(A)$^+$ RNA hybridization with recombinant plasmids carrying gene fragments for embryo-specific proteins of sea urchin embryo cell plasma membranes.

REFERENCES

[1] Raff, R.A., Colot, H.V , Selvig, S.E. and Gross, P R. (1972) Nature 235, 211–214.
[2] Hiruma, M.N. and Mano, J. (1974) Exp. Cell Res. 86, 15–24.

[3] Merlino, G.T., Water, R.D., Chamberlain, J.P., Jackson, D.A., Raafat-Gewely, M. and Kleinsmith, L.J. (1980) Proc. Natl. Acad. Sci. USA 77, 765–769.
[4] Kedes, L.H. (1976) Cell 8, 321–331.
[5] Woodland, H R and Wilt, F.H. (1980) Dev. Biol. 75, 199–213.
[6] Sova, V.V., Prokopenko, I.V. and Yurin, V.L. (1979) Dokl. Akad. Nauk SSSR 249, 743–746.
[7] Chuguev, Yu.P., Strongin, A.Ya. and Sova, V.V. (1977) Comp. Biochem. Physiol. 56B, 381–384.
[8] Parsnickaya, A.I. and Sova, V.V. (1976) Marine Biol. USSR 2, 74–77.
[9] Sova, V.V., Prokopenko, I.V., Polyakova, N.E., Goryunova, L.E., Sacharova, N.K. and Yurin, V.L. (1980) Mol. Biol. USSR 14, 1243–1252.
[10] Aviv, H. and Leder, P. (1972) Proc. Natl. Acad Sci. USA 69, 1408–1412.
[11] Laemmli, U.K. (1970) Nature 227, 680–685.
[12] Laskey, R.A. and Mills, A.D. (1975) Eur. J. Biochem. 56, 335–341.
[13] Auffray, C. and Rougeon, F. (1980) Eur. J. Biochem. 107, 303–314.
[14] Wilt, F.H. (1977) Cell 11, 673–681.
[15] Nemer, M., Dubroff, L.M. and Graham, M. (1975) Cell 6, 171–178.