CYTOPLASMIC TRANSFER OF RNA COMPLEMENTARY TO REPETITIOUS DNA SEQUENCES DURING EARLY SEA URCHIN EMBRYOGENESIS

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1. Introduction

It has shown earlier that the RNA population in sea urchin embryos at early developmental stages is characterized by a high content of molecules complementary to the repetitious nucleotide sequences of DNA [1]. Similar results have been reported for loach [2] and amphibian [3] embryos.

Although the genome of most eukaryotes contains repetitious nucleotide sequences [4], the functional role of these DNA sequences has not been established. It has been suggested that these nucleotide DNA sequences serve as regulatory genes [5] or as regulator parts of operons [6]. Another alternative assumes the existence of multiple redundant structural genes with messenger RNA synthesized from them [7,8]. These views are not mutually exclusive and there are experimental data in favour of both views [8–10].

An attempt has been made to elucidate the functional role of RNA molecules complementary to repetitious DNA nucleotide sequences in the early embryogenesis. The method of molecular hybridization, in DNA excess, was used in the analysis of RNA isolated from the nucleus and cytoplasm of sea urchin embyos.

The results obtained are interpreted to mean that the RNA's synthesized in embryo cells on repetitious DNA regions are transferred to the cytoplasm. Furthermore, it was found that qualitative selection of the RNA molecules takes place during the transport to the cytoplasm.

2. Materials and methods

The embryos of *Strongylocentrotus droebachiensis* were cultured as described earlier [11]. The embryos were harvested at two developmental stages: hatching blastula (36 hr of development at 7–8°) and the late gastrula (72 hr development at 7–8°).

Nuclear and cytoplasmic RNA were isolated from the same batches of embryos. Washed embryos were centrifuged and suspended in 10 vol of buffer (0.25 M sucrose, 0.005 M $MgCl_2$, 0.05 M KCl, 0.025 M Tris-HCl, pH 7.6). Disruption of cells was achieved by passage through a hypodermic needle and the homogenate centrifuged for 10 min at 2000 *g*. The pellet was used for isolation of nuclei and cytoplasmic RNP-particles were isolated from the supernatant fluid.

The nuclear pellet was suspended in 10 vol of buffer solution (1 M sucrose, 0.01 M $MgCl_2$, 0.01 M Tris-HCl, pH 7.5, 2% Triton X-100) with a glass homogenizer and centrifuged for 30 min at 5000 *g*. The supernatant solution was discarded. The pellet was suspended in 3 vol of 1 M sucrose, 5 ml of suspension were placed on 20 ml 1.9 M buffered sucrose and purified nuclei were collected by centrifugation for 60 min at 45000 *g*.

Supernatant fluid after separation of nuclei was centrifuged for 10 min at 10000 *g* and the postmitochondrial supernatant used for isolation of cytoplasmic RNP-particles. Triton X-100 was added to final conc. of 2% and cytoplasmic RNP-particles were iso-

lated according to Mg^{2+} precipitation procedure of Takanami [12] as described for isolation of polyribosomes [13].

The pellets of nuclei and cytoplasmic RNP-particles were suspended in a buffer solution (0.1 M NaCl, 0.001 M EDTA, 0.5% sodium dodecyl sulphate (SDS), 0.01 M Tris-HCl pH 7.5) and homogenized in a glass homogenizer. RNA was isolated by phenol deproteinization from nuclei (total nuclear RNA) and cytoplasmic RNP-particles containing polyribosomes (crude polyribosomal RNA).

The procedure of thermal phenol fractionation [14] was followed for isolation of total cytoplasmic RNA and DNA-like nuclear RNA (fraction extracted by phenol –0.14 M NaCl at 65°).

Labeled RNA was isolated from sea urchin embryos incubated for 2 hr in millipore filtered sea water (pore size of the filters is 0.4 μm) with 10 μCi/ml of [^{3}H]uridine (5 Ci/mM). The RNA preparations were repeatedly precipitated with 2.5 M NaCl and ethanol and were treated with DNAase 1 (Worthington), and Pronase (Serva) followed by phenol deproteinization. DNA from sea urchin sperm was isolated by the phenol-detergent method [7]. All the operations were carried out at 4°.

RNA–DNA hybridization was carried out in DNA gels cross-linked by ultraviolet irradiation [15] in 2 × SSC at 67° (SSC is 0.15 M NaCl, 0.015 M sodium citrate). Rat liver RNA and *Tetrahymena pyriformis* RNA were used in experiments to serve as a control for non-specific RNA binding.

3. Results and discussion

To establish if the RNA synthesized on repetitious DNA is transported to the cytoplasm we studied the degree of hybridization of different blastula stage RNA fractions at various DNA/RNA ratios. The hybridization conditions of DNA excess employed in this investigation enable analysis of RNA which is complementary to repetitious DNA sequences [2,7,16]. The efficiency of hybridization under these conditions reflects the proportion of RNA-species which contain sequences complementary to repetitious DNA nucleotide sequences.

As seen in fig. 1 cytoplasmic and crude polyribosomal RNA's isolated from blastula stage embryos

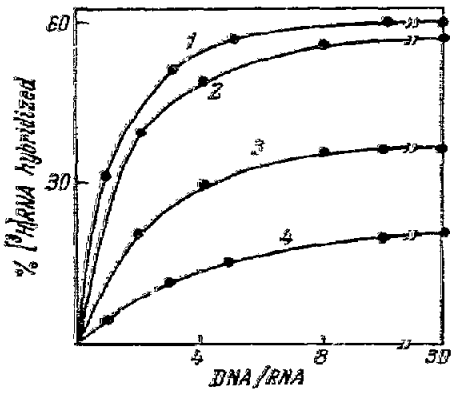

Fig. 1. Hybridization capacity of (1) crude polyribosomal, (2) total cytoplasmic, (3) total nuclear and (4) DNA-like nuclear [^{3}H]RNA isolated from sea urchin hatching blastula stage embryos. [^{3}H]RNA was incubated with DNA gel (0.5–3 mg) at 67° for 36 hr in 2 × SSC.

have a high hybridization capacity with DNA. The maximum degree of hybridization of these RNA samples is about 60%. Fig. 1 also shows that the hybridizability of total nuclear RNA (curve 3) and of DNA-like nuclear RNA (curve 4) is considerably lower than that of cytoplasmic RNA. This is consistent with the assumption that a certain degree of preferential transport of nuclear RNA complementary to repetitious nucleotide DNA sequences to the cytoplasm occurs.

Similar results were obtained in loach embryos when the hybridizability of cytoplasmic and chromo-

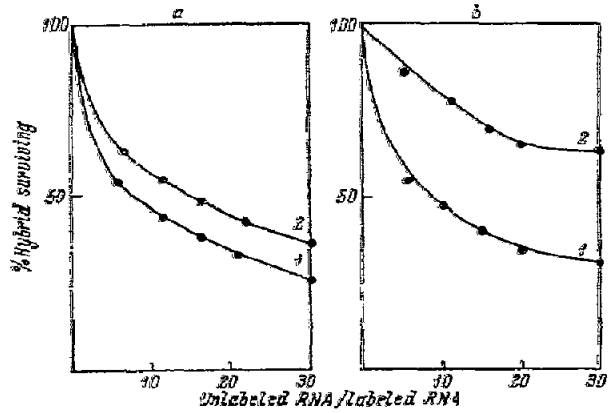

Fig. 2. Competitive hybridization studies of (a) DNA-like nuclear [^{3}H]RNA and (b) cytoplasmic [^{3}H]RNA isolated from hatching blastula stage embryos. [^{3}H]RNA was incubated at 67° in 2 × SSC for 36 hr with various amounts of (a) unlabeled DNA-like nuclear RNA and (b) total cytoplasmic RNA from (1) hatching blastula and (2) late gastrula stage embryos. The concentration of DNA gel per sample was (a) 0.5 mg and (b) 2 mg with a DNA:[^{3}H]RNA ratio of 5.

somal-nucleolar RNA [2] were compared. The high degree of hybridizability was also revealed earlier by an analysis of polyribosomal RNA synthesized in sea urchin embryos at the blastula stage [17].

Changes in the RNA populations present during early embryogenesis of sea urchins occur [1,18,19]. It is important to know if these changes are similar for both cytoplasmic and nuclear RNA. We have attempted to answer this question by making use of competitive hybridization.

Labeled preparations of DNA-like nuclear and cytoplasmic RNA isolated from blastula embryos and corresponding unlabeled preparations from blastula and gastrula embryos were used in competitive hybridization experiments. The difference between the capacity of unlabeled blastula and gastrula stage RNA preparations to suppress binding of nuclear labeled RNA from blastula stage embryos is rather limited (fig. 2a). This suggests that nuclei of gastrula embryo cells contain most of the RNA species which are present in nuclei of blastula embryo cells. In contrast, competitive hybridization experiments with cytoplasmic RNA (fig. 2b) revealed greater differences in efficiency of competition between unlabeled RNA preparations from embryos at different developmental stages. Therefore, differences in RNA populations synthesized in blastula and gastrula stages would seem to be a reflection of the changes in the cytoplasmic RNA population.

The differences in populations of nuclear and cytoplasmic RNA's were also revealed by a direct comparison of nuclear RNA and RNA from cytoplasmic particles in the competitive hybridization experiments (fig. 3). The capacity of unlabeled nuclear and crude polyribosomal RNA preparations from blastula and gastrula embryos to compete in the hybridization reaction with nuclear [^{3}H]RNA from gastrula embryos was investigated. As seen from fig. 3, nuclear RNA from gastrula stage embryos (curve 1) inhibits binding of labeled nuclear RNA to a greater extent than RNA from cytoplasmic particles of the embryos at the same stage (curve 3). The experiments with blastula stage embryos RNA's as competitors also show significant differences in competitive efficiency between nuclear and cytoplasmic RNA (fig. 3, curves 2 and 4). Thus, the results obtained (figs. 2 and 3) reveal the qualitative differences in RNA populations of nuclear and cytoplasmic RNA. Furthermore, as can be

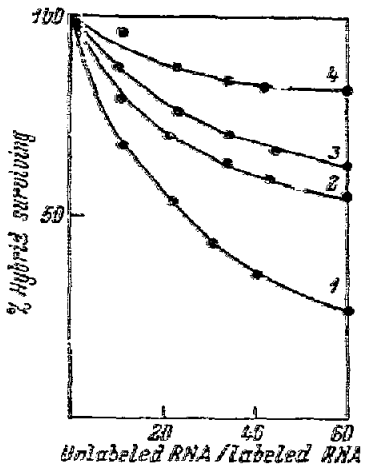

Fig. 3. Competitive hybridization studies of total nuclear [^{3}H]RNA isolated from late gastrula embryos. [^{3}H]RNA was incubated at 67° for 36 hr in 2 × SSC with various amounts of unlabeled total nuclear (1,2) and crude polyribosomal (3,4) RNA isolated from (1,3) late gastrula and (2,4) hatching blastula stage embryos. The DNA gel concentration per sample was 0.5 mg with a DNA:[^{3}H]RNA ratio of 10.

seen from fig. 3, nuclear blastula RNA competes more effectively with the gastrula stage nuclear [^{3}H]RNA than cytoplasmic RNA derived from gastrula stage embryos (curves 2 and 3). This indicates that there is more resemblance between the populations of nuclear RNA from different developmental stages than between nuclear and cytoplasmic RNA of the same stage. Nuclear RNA from blastula and gastrula stage differs insignificantly in competitive efficiency with nuclear blastula [^{3}H]RNA (fig. 2a). Competitive hybridization experiments with labeled nuclear gastrula RNA reveal more differences between RNA populations of nuclear RNA from the two developmental stages (fig. 3, curves 1 and 2), suggesting that some new nuclear RNA species are synthesized during the gastrula stage. At the same time most of the nuclear RNA species synthesized in blastula stage embryos can be found also in the nuclei of gastrula embryos (fig. 2a). However, the low competitive efficiency of cytoplasmic blastula RNA with nuclear gastrula [^{3}H]RNA (fig. 3, curve 4) indicates that most of the nuclear RNA's synthesized on similar repetitious DNA regions in embryos at blastula and gastrula stages are not transported to the cytoplasm.

Thus, during early embryogenesis the RNA species complementary to repetitious DNA regions are prefer-

entially transported to the cytoplasm. Moreover at each stage of development particular kinds of RNA molecules with certain nucleotide sequences are selected for transport to the cytoplasm. We have no direct evidence that these cytoplasmic RNA molecules copied on repetitious DNA sequences take part in protein synthesis. It is known, however, that ribosomal RNA synthesis at the early developmental stages of sea urchin embryos is at a relatively low level in comparison with that of the DNA-like RNA [20]. Therefore it is reasonable to assume that at least part of the RNA molecules synthesized early in embryogenesis on repetitious nucleotide DNA sequences and selectively transferred into the cytoplasm is messenger RNA.

However, a substantial part of the RNA synthesized in embryo cells on repetitious nucleotide DNA sequences seems not to be transferred to the cytoplasm and therefore it cannot be considered as messenger RNA. This part of the RNA is retained in the nuclei and is involved in some other, possibly regulatory, functions. It appears that in embryo cells there is a mechanism which qualitatively selects RNA molecules to be transported to the cytoplasm. The significance of preferential RNA transport to the cytoplasm and stabilization of particular RNA molecules there in early embryogenesis has recently been shown for frog embryos [21].

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