CHANGES IN TRANSCRIPTION DURING EARLY EMBRYOGENESIS OF SEA URCHINS

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1. Introduction

It is known that qualitative changes in transcription occur early in embryogenesis which are revealed by analysis of the RNA synthesized in embryos at different developmental stages [1–5]. To get further insight into the peculiarities of transcription in early development we compared RNA synthesized *in vivo* with that obtained in a cell-free system where chromatin isolated from sea urchin embryo cells at corresponding stages was used as a template.

Hybridization experiments indicated that RNA synthesized *in vitro* and embryo RNA at homologous stages display similarities in population characteristics, the degree of hybridizability and the kinetics of binding to DNA. It is concluded that isolated chromatin preparations used as templates in a cell-free system retain their ability to support synthesis of RNA molecules specific for a given developmental stage. Transcription of redundant nucleotide sequences of the DNA prevailing during early developmental stages is shown. The results obtained are interpreted to mean that there is a specific restriction of transcription from the unique or less redundant nucleotide sequences of the DNA at early developmental stages.

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2. Materials and methods

Sea urchin embryos of *Strongylocentrotus droebachiensis* were used in this work. Chromatin was isolated at two developmental stages: hatching blastula (36 hr of development at 7–8°) and late gastrula (72 hr of development) as described earlier [6].

Preparative *in vitro* RNA synthesis was carried out using *E. coli* RNA polymerase isolated according to Chamberlin and Berg [7]. The incubation mixture contained per 1 ml: 40 μmoles Tris buffer (pH 8), 4 μmoles $MgCl_2$, 1 μmole $MnCl_2$, 12 μmoles β-mercaptoethanol, 0.4 μmole each of ATP, GTP, CTP and UTP-^{14}C (21 mCi/mmole), 1–2 mg DNA (or DNA as chromatin) and 100–200 units of RNA polymerase. Incubation was at 30° for 40 min. The RNA synthesized was isolated using the phenol-detergent method.

Total RNA was isolated from embryos essentially as described by Glišin et al. [1]. The procedure of Georgiev and Mant'eva [8] was followed for isolation of nuclear RNA. All the RNA samples were treated with DNAase (Worthington) and pronase (Serva) followed by phenol deproteinization. DNA from the sea urchin sperm was isolated as described earlier [9].

RNA–DNA hybridization was done either in UV DNA gels [10] or on Millipore filters, HAWP, according to Gillespie and Spiegelman [11]. Incubation was at 67° to ensure specificity of hybridization [12]. *Tetrahymena pyriformis* RNA was used in com-

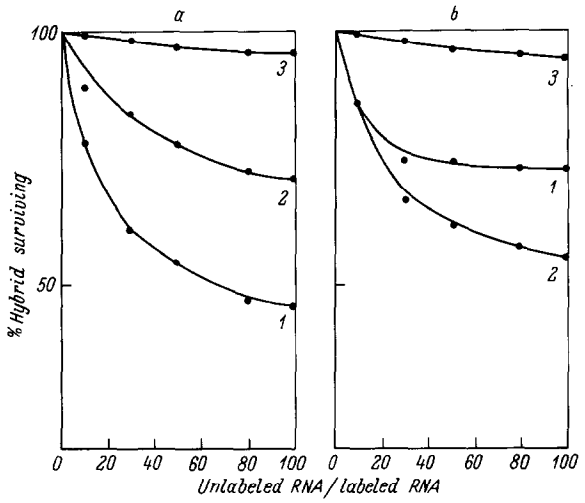

Fig. 1. Competitive hybridization studies of ^{14}C-RNA synthesized in cell-free systems. Embryo chromatin taken at (a) blastula and (b) gastrula stages was used as template. ^{14}C-RNA was incubated at 67° for 18 hr with various amounts of unlabelled nuclear RNA from (1) blastula and (2) gastrula stage embryos and (3) RNA from *Tetrahymena pyriformis*. DNA per filter, 40 μg. ^{14}C-RNA per sample, 0.2 μg (20,000 cpm). Abscissa, ratio of unlabeled to labeled RNA in sample. Ordinate, extent of competitive hybridization (%).

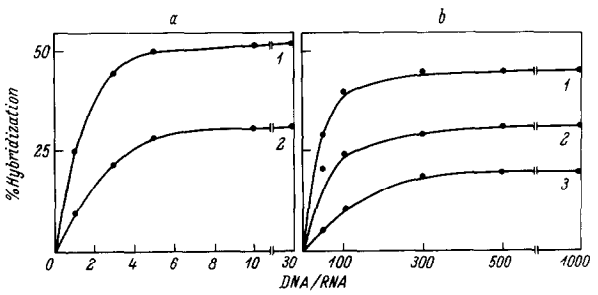

Fig. 2. Hybridization capacity of (a) total ^{3}H-RNA isolated from (1) blastula and (2) gastrula stage embryos and (b) ^{14}C-RNA synthesized *in vitro* where (1) blastula chromatin, (2) gastrula chromatin, or (3) free sea urchin sperm DNA were used as templates. (a) DNA gel per sample, 1–2 mg. Incubation at 67° for 36 hr. (b) DNA per filter, from 5 to 100 μg. ^{14}C-RNA per sample, 0.1 μg (10,000 cpm). Incubation at 67° for 12 hr. Abscissa, DNA:RNA ratio. Ordinate, labeled RNA hybridized (%).

petitive hybridization experiments to serve as a control for non-specific binding.

3. Results and discussion

Comparison of the RNA population synthesized *in vitro* on chromatin isolated from embryos at blastula and gastrula stages with that synthesized in embryos at the same stages was made by competitive hybridization. As seen from fig. 1a, RNA from blastula embryos inhibits binding of ^{14}C-RNA synthesized *in vitro* on blastula chromatin as template to a greater extent than RNA taken from gastrula embryos. On the other hand, the gastrula stage RNA competes more effectively with ^{14}C-RNA synthesized *in vitro* on gastrula chromatin (fig. 1b). Thus, homologous RNA displays higher competitive efficiency than RNA derived from embryos at other developmental stages. This supports the assumption that the population of RNA molecules synthesized *in vitro* resembles that of RNA derived from embryos at corresponding developmental stages.

Investigations of the kinetics of DNA renaturation revealed a significant proportion of redundant nucleotide sequences in the genome of eukaryotes [13]. The efficiency of binding RNA molecules to DNA as well as hybridization kinetics can reflect the relative content of the RNA molecules synthesized on redundant nucleotide sequences of DNA [9, 14, 15].

As seen from fig. 2a, 50% of blastula RNA reacts with DNA, while gastrula stage RNA hybridizes to a lesser extent (30%). High hybridizability of RNA isolated from cytoplasmic particles of sea urchin embryos [16] and fish embryos RNA [15] was observed earlier.

Similar hybridization behaviour was observed for RNA synthesized *in vitro* on blastula and gastrula chromatin (fig. 2b). However, as seen from the graphs of fig. 2, DNA/RNA ratios corresponding to complete reaction of hybridizable RNA molecules under given conditions are different for RNA samples obtained *in vitro* and from embryo cells. This may be due to the presence of a high proportion of unlabeled ribosomal RNA in preparations of total RNA from embryos.

Further, as seen from experiments on hybridization kinetics, RNA synthesized *in vitro* is similar to

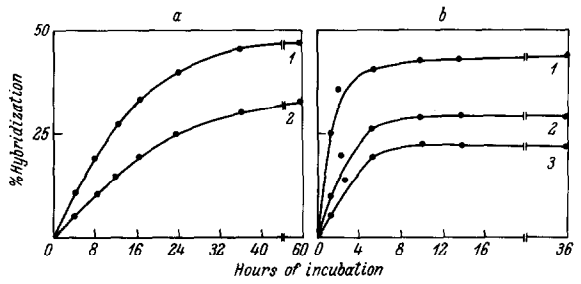

Fig. 3. Hybridization kinetics of (a) total ^{3}H-RNA isolated from (1) blastula and (2) gastrula stage embryos and (b) ^{14}C-RNA synthesized in a cell-free system where (1) blastula chromatin, (2) gastrula chromatin, or (3) free sea urchin sperm RNA were used as templates. (a) DNA gel per sample, 1 mg. ^{3}H-RNA per sample, 200 μg. Incubation at 67°. (b) DNA per filter, 20 μg. ^{14}C-RNA per sample, 0.1 μg. Incubation at 67°. Abscissa, incubation time (hr). Ordinate, labeled RNA hybridized (%).

embryo RNA (fig. 3). In both cases blastula stage RNA hybridizes at a higher rate than gastrula RNA.

High hybridizability and a higher rate of binding of blastula RNA than gastrula stage RNA testify to a higher relative content of RNA classes transcribed from redundant DNA nucleotide sequences in the former. Thus, RNA synthesized *in vitro* on chromatin resembles the corresponding natural RNA not only in the distribution of molecular species but also in relative content of RNA molecules copied from redundant nucleotide sequences of DNA.

However, as seen from fig. 3b, RNA synthesized *in vitro* forms hybrids with DNA at a higher rate than corresponding embryonic RNA (fig. 3a). This evidently reflects the higher homogeneity of the RNA synthesized *in vitro*.

The data available show that RNA synthesized in a cell-free system, on chromatin of differentiated cells as a template, displays lower hybridizability as compared to that of RNA transcribed from free DNA [14]. In our case the situation is different. RNA synthesized *in vitro* on embryo chromatin has characteristically a considerably higher hybridization efficiency (fig. 2b) and a higher rate of hybrid formation (fig. 3b) than RNA transcribed from free DNA.

There is evidence in favour of enhancement of chromatin template activity of embryo cells in the course of development, that is, progressively greater amounts of nucleotide sequences are involved in transcription [17–19]. Therefore it can be assumed that at early developmental stages there occurs a restriction of transcription from unique and less redundant sequences of DNA. This restriction is eventually reduced in the course of embryogenesis.

Earlier investigations carried out in this laboratory disclosed changes in the fractional content of histone in the course of development [6]. Other authors have also shown changes in the protein components of chromatin to occur during embryogenesis [17, 19]. It is possible that the characteristics of transcription at early developmental stages are affected by the chromatin proteins of embryo cells.

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