TRANSFER RNA SYNTHESIS IN EARLY EMBRYOGENESIS

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1. Introduction

It has been shown in a number of reports that tRNA in animal and bacterial cells has at least one precursor stage prior to maturation [1–6]. The precursor (pre-tRNA) may be revealed in cells after a very short incubation with label (3–5 min) that suggests a high rate of the pre-tRNA maturation process in a normal cell.

Recently we have demonstrated that tRNA synthesis occurs in loach (*Misgurnus fossilis* L.) embryos at the early cleavage stage [7] in the absence of rRNA and DNA-like RNA synthesis [8, 9]. In this paper some characteristics of early tRNA synthesis in loach embryos are described. The main finding is the low rate of maturation process of pre-tRNA. The incorporation was found to occur mostly in the pre-tRNA molecules in embryos at the early blastula stage (5 hr of development) after incubation with labeled uridine for 1–2 hr. The mature tRNA synthesis appears in embryos at the mid blastula stage (6.5 hr of development).

To elucidate the factors controlling the activation of the tRNA maturation process, experiments were performed with blastoderms (embryos devoid of yolk) isolated at early and mid blastula stage of development and cultivated *in vitro.*

The results obtained indicate that pre-tRNA maturation process is activated in the blastoderm at the mid blastula – 6.5 hr – stage. In order for the activation to be manifasted at this stage the embryos must be kept intact at the earlier stage.

2. Materials and methods

2.1. *Materials*

Mature eggs of loach (*Misgurnus fossilis* L.) were obtained 40 hr after injecting the females with 200 i.u. Choriogonin (Gedeon Richter, Hungary). Eggs were fertilized, left to develop in tap water at 21°C and staged as described previously [10].

2.2. *Introduction of the label*

Since egg membranes are not permeable to usual precursors – nucleotides and nucleosides [8] RNA synthesis was studied in isolated embryos, viz. blastoderms [11]. Blastoderms isolated at desired stages were incubated in 6 vol of Holtfreter's solution containing 0.05 M Tris-HCl buffer, pH 7.8 with [^{3}H] or [^{14}C]methyl-methionine. Sodium formate (2×10^{-5} M) adenosine (2×10^{-5} M) and guanosine (2×10^{-5} M) were added to the incubation medium when [^{14}C]-methylmethionine was used.

2.3. *Preparation and salt-fractionation of RNA*

The nucleic acids were extracted with cold phenol [12]. Blastoderms were homogenized in the ice-cold 0.14 M NaCl solution containing 0.01 M $MgCl_2$ and 2–10 μg/ml polyvinyl sulphate and deproteinized with water-saturated phenol three times. Before the second deproteinization the aqueous phase was made 1% in respect to sodium dodecyl sulphate. Nucleic acids were precipitated from the aqueous phase with ethanol after addition of potassium acetate. The nucleic acid precipitates were dissolved in a small volume of cold water and made 1.5 M with respect to NaCl. The precipitate of "salt-insoluble" RNA (si RNA) formed overnight (–10°C) contained rRNA's and heterogen-

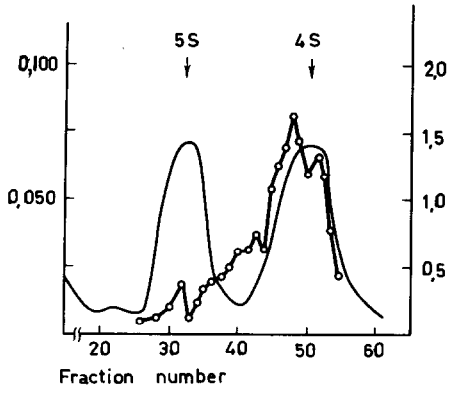


Fig. 1. The low molecular weight RNA of salt soluble fraction synthesized at early blastula stage (5 hr of development). 0.3 ml 5 hr-blastoderms were incubated for 1 hr in 1.2 ml solution A (0.05 M Tris-HCl buffer, pH 7.8, containing 0.14 mM NaCl, 1.5 μM KCl, 6 μM $NaHCO_3$, 4.2 μM $CaCl_2$ and antibiotics – 100 i.u./ml penicillin, 50 i.u./ml streptomycin) with [^{3}H]uridine (12 μCi/ml). RNA was extracted by phenol procedure and ss RNA preparation was obtained as described under Materials and methods. The preparation was mixed with 17 o.e. of unlabeled ss RNA from blastoderms and analysed by gel-filtration on Sephadex G-100 column. (——) O.D.; (o—o—o) radioactivity.

eous RNA including mRNA [13]. DNA, 5 s rRNA and tRNA as "salt-soluble" fraction (ssNA) remained in the supernatant [14].

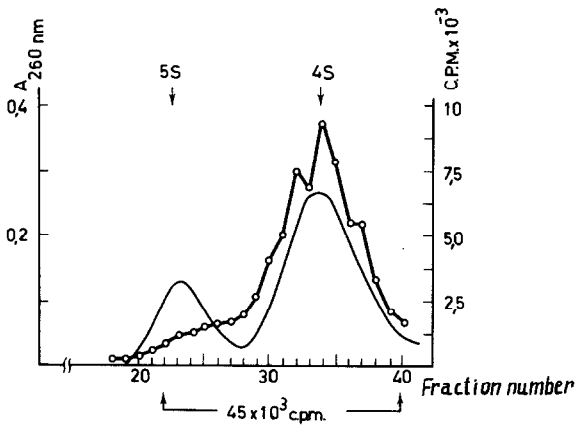


Fig. 2. The low molecular weight RNA of salt soluble fraction synthesized at mid blastula stage (6.5 hr of development) 1 ml 6.5 hr-blastoderms were incubated in 1 ml of solution A with [^{14}C]uridine (2 μCi/ml) for 1 hr. ss RNA was obtained and analysed as in fig. 1. (——) O.D., (o—o—o) radioactivity.

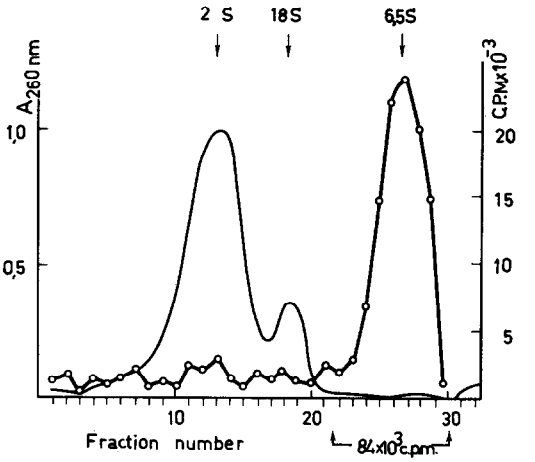


Fig. 3. The low molecular weight RNA of salt insoluble fraction synthesized at mid blastula stage (6.5 hr of development) si RNA preparation was obtained from 6.5 hr-blastoderms in the experiment described in fig. 2. 3 mg si RNA dissolved in 1 ml 0.1 M acetate buffer pH 5, containing 0.01 M EDTA was layered on a 5–20% sucrose gradient prepared in the same buffer. After 14 hr centrifugation at 2400 rpm in the SW-25 rotor of the Spinco L ultracentrifuge at 4°C the bottom of the tubes was pierced and fractions were collected. The RNA content in the fractions was determined by measuring UV-absorption (at 260 nm), radioactivity – after precipitation of RNA with 5% TCA as described in Materials and methods. (——) O.D.; (o—o—o) radioactivity.

2.4. *Analysis of RNA*

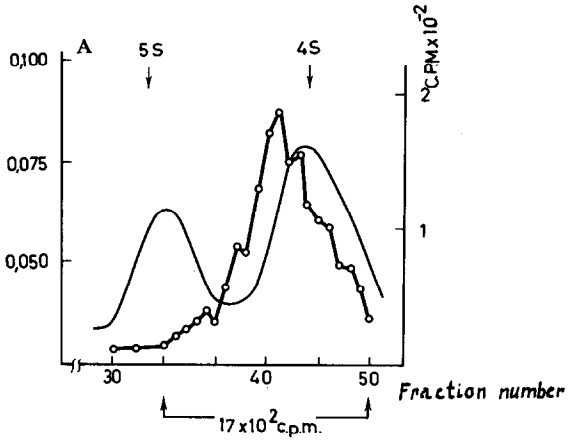
ss NA preparations were analyzed by gel-filtration on a Sephadex G-100 column [15]; the procedure was modified as described earlier [14]. si RNA preparations were analysed by sucrose gradient centrifugation.

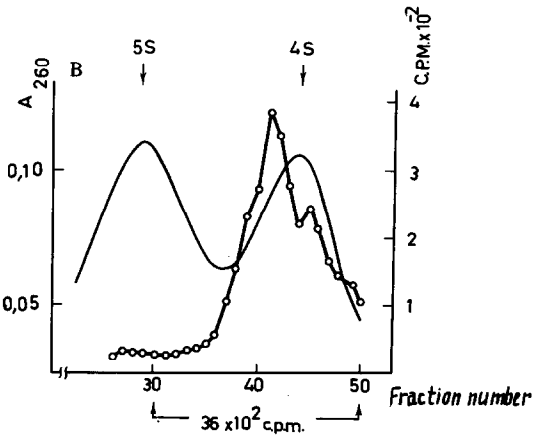
2.5. *Radioactivity measurements*

RNA was precipitated with cold 5% trichloroacetic acid (TCA). The precipitates were collected on membrane filters and washed with 5% TCA and ethanol. The radioactivity was counted in a liquid scintillation spectrometer (SL-40, France) with a toluene scintillator.

3. Results and discussion

The tRNA synthesis at the early blastula (5 hr, synchronic cleavage stage) and at the mid blastula (6.5 hr, period of desynchronization of the cell cleavage) was studied in isolated blastoderms. After in-





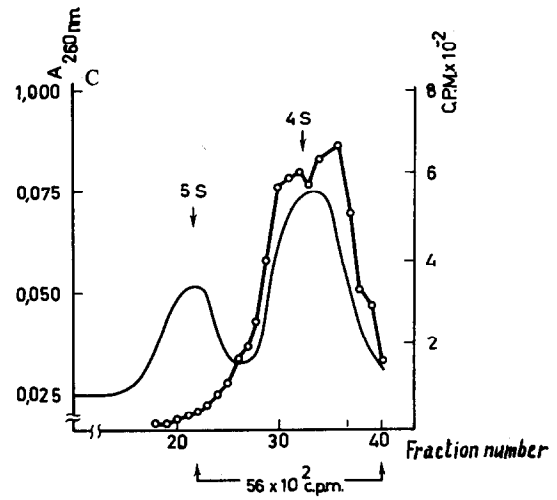


Fig. 4. The comparison of ss RNA synthesis in 5 hr-blastoderms (A), cult. 5 hr-blastoderms (B) and 6.5 hr-blastoderms (C). 5 hr-, cult. 5 hr- and 6.5 hr-blastoderms (2 ml of each) were incubated in 2 ml solution A for 1 hr with [^{3}H]uridine (15 μCi/ml). ss RNA preparations were obtained and analysed as described in fig. 1. (——) O.D.; (o—o—o) radioactivity.

cubation of blastoderms with labeled uridine, nucleic acids were extracted by phenol procedure and treated by 1.5 M NaCl; ss NA and si RNA fractions were obtained. The results of the ss NA analysis are represented in figs. 1 and 2. From fig. 2 one can see that at the mid blastula stage the loach embryos synthesize mature tRNA. In contrast to this, in the early blastula embryos (fig. 1) the amount of label incorporated into the mature tRNA fraction is very small as compared to the label present in the RNA fraction which was eluted between 5 S and 4 S RNA and that appearing in the position of 5 S RNA. Labeled RNA present in the 5 S fraction should not be regarded as the 5 S rRNA, since the rRNA synthesis is absent at such an early stage of loach development [8, 9].

Recently we have demonstrated that after 150 min chase the label from both labeled fractions mentioned above is found in the mature tRNA fraction [7]. That made it possible to postulate that this RNA fraction seemed to correspond to so-called pre-tRNA [1–6].

The low rate of the maturation process and the absence of rRNA synthesis at the blastula stage allows one to reveal all possible tRNA precursors and to follow their fate in the cell. It is noteworthy that, in addition to the pre-tRNA present in the ss NA fraction, a low molecular weight si RNA was synthesized in the blastula embryos. This si RNA sediments as a single peak with sedimentation coefficient 6–7 S. In fig. 3 the results of a sucrose gradient sedimentation analysis of the si RNA fraction from mid blastula embryos are

represented. One can see that about 60% of label corresponds to the 6–7 S RNA peak, the other 40% sedimenting as heterogeneous material. From the distribution patterns of UV-absorbing material in fig. 2 and 3 it is obvious that 5 S rRNA and tRNA were quantitatively extracted by 1.5 M NaCl (ss NA fraction). However, the level of ^{14}C-incorporation into 6–7 S RNA is quite similar to incorporation into the ss RNA fraction. This result indicates that the radioactivity in the 6–7 S peak does not correspond to the 5 S rRNA and tRNA contaminants. Recently we have shown that this 6–7 S RNA is metabolically unstable and can be methylated by the methionine methyl group [16]. The concurrent synthesis of 6–7 S RNA and tRNA at such an early stage of embryonic development in the absence of rRNA synthesis and no (or at most low) DNA-like RNA synthesis led us to the inference that 6–7 S RNA is possibly a high molecular precursor of tRNA. In contrast to the known pre-tRNA, this precursor could not be extracted by 1.5 M NaCl suggesting the absence of a regular secondary structure similar to that of tRNA or 5 S-rRNA.

Comparison of tRNA synthesis at the early and mid blastula stages (fig. 1 and 2, respectively) shows that synthesis of mature tRNA molecules is activated due to the activation of pre-tRNA processing in loach embryos at the mid blastula stage.

To elucidate the factors controlling the activation of pre-tRNA processing at the mid blastula stage, the following experiments were performed.

The pecularities of the tRNA synthesis in isolated blastoderms at the early blastula (5 hr) and mid blastula (6.5 hr) stages have been investigated.

An aliquot of the 5 hr-blastoderms was cultivated *in vitro* in Goltfreter's solution for 1.5 hr (cult. 5 hr-blastoderm). 5 hr-Blastoderms are unable to differentiate; however, mitosis proceeds normally [17], and at the end of the cultivation period, the cell number is equal to that of 6.5 hr-blastoderms [17].

All three types of blastoderms (5 hr-, cult. 5 hr-, 6.5 hr) were incubated with labeled cytidine for 1 hr.

The ss RNA fraction from these blastoderms was subjected to gel-filtration analysis, the results of which are represented in fig. 4. One can see that the 5 hr-blastoderms incorporate the label mainly in the pre-tRNA fraction.

The cult. 5 hr-blastoderms seem to follow the same pattern of synthesis. The increase in the radioactivity level of the pre-tRNA reflects the increase in number of nuclei in each blastoderm. The 6.5 hr-blastoderms display an enhanced radioactivity of the ss RNA fraction and an increase in relative synthesis of mature tRNA molecules.

The results obtained indicate that the pre-tRNA maturation process is activated at the mid blastula – 6.5 hr – stage.

In order for the activation to be manifested at this stage the embryos must be kept intact at the earlier stages.

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References

[1] R.H. Burden, B.T. Martin and B.M. Lal, J. Mol. Biol. 28 (1967) 357.
[2] R.H. Burden and A.E. Clason, J. Mol. Biol. 39 (1969) 113.
[3] B.B. Mowshowitz, J. Mol. Biol. 50 (1970) 143.
[4] S. Altman, Nature New Biology 229 (1971) 19.
[5] S. Altman and J.D. Smith, Nature New Biology 233 (1971) 35.
[6] B.K. Choe and M.W. Taylor, Biochim. Biophys. Acta 272 (1972) 275.
[7] I.A. Solovjeva, M.Ja. Timofeeva and I.E. Sosinskaya, Ontogenesis (1973) Vol. 5, in press.
[8] M.A. Ajtkhoghin, N.V. Belitsina and A.S. Spirin, Biochimiya 29 (1964) 169.
[9] M.Ja. Timofeeva and C.A. Kafiani, Dokl. Akad. Nauk SSSR 164 (1965) 1183.
[10] A.A. Neyfakh, Zh. Obshch. Biol. 22 (1961) 202.
[11] A.A. Kostomarova and A.A. Neyfakh, Zh. Obshch. Biol. 25 (1964) 386.
[12] K. Scherrer and J.E. Darnell, Biochim. Biophys. Acta 7 (1962) 486.
[13] M.Ja. Timofeeva, A.A. Neyfakh and C.A. Kafiani, in: Structure and functions of cell nucleus, ed. I.B. Zbarsky (Nauka, Moscow, 1967) p. 201.
[14] I.A. Solovjeva, M.Ja. Timofeeva and I.E. Sosinskaya, Ontogenesis (1973) Vol. 4, in press.
[15] F. Gilbert, C.J. Larsen, J.C. Lelong and M. Boiron, Nature 207 (1965) 1039.
[16] I.A. Solovjeva and M.Ja. Timofeeva, Molekul. Biol., in press.
[17] A.A. Kostomarova, J. Embryol. Exptl. Morphol. 22 (1969) 407.