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Activity of Specific mRNAs in Early Development of *Xenopus* and *Rana* Embryos

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Abstract: After transcription, processing and transport to the cytoplasm the mRNA may become a subject of various factors which activate or repress its translation. This work aimed to determine the start time of translation of mRNAs stored in the egg or transcribed from the zygote genome. The polyribosome/informosomes ratio served as a criterion of mRNA involvement in translation. Here we used cesium chloride density gradient centrifugation to study the distribution of *Xwnt-11*, *Xnot-2*, *Xbra* and *Xgsc* mRNAs between polyribosomes and informosomes in the eggs and early embryos of *Xenopus laevis* and *Rana temporaria*. Present results gave evidence that specific mRNAs which encode proteins participating in dorsoventral differentiation, follow individual dynamics of activation and inactivation.

Key words: Informosomes, mRNP, polyribosomes, regulation of translation, density gradient centrifugation

INTRODUCTION

Most of modern studies on gene expression during embryonic development assume without comments that the presence of a specific mRNA in the embryo suggests its translation into a functional protein. Spirin *et al.*^[1] showed that in the embryos of loach and sea urchin most of newly synthesized mRNA is not present in polyribosomes but in ribosome-free mRNPs which they named informosomes. The concept of masked mRNA was formulated for the first time and the existence of the regulation of protein biosynthesis on the level of translation was proposed. Later such mRNPs were discovered in all Eukaryote studied, both in embryonic and differentiated cells. During recent 10-15 years the regulation of gene expression on the level of translation has become a subject of intense investigation. Now it is clear that the expression of many genes in the developing embryo is regulated not only transcriptionally, but also translationally^[2,3]. Individual mRNAs in embryos have their specific place, time and mechanism of translational repression and activation. In our previous works we showed that some mRNAs produced at the start of zygote genome functioning in middle blastula come to polyribosomes much later, only in middle gastrula^[4]. The most part of *Xvent-2* mRNA was detected in informosomes at all developmental stages examined and only its minor fraction was associated with polyribosomes^[5]. Translational regulation was also demonstrated for several *Xenopus* genes involved in the

BMP signaling pathway^[6]. Stored mRNAs come to polyribosomes at different times: translation starts in oocyte maturation with *Smad1* and *ALK2* mRNAs, in early embryo development with *MBP-4* and *XSTK9* mRNAs and only in early gastrula with *ALK3* mRNA.

Here we examined the distribution of some mRNAs which participate in dorsoventral differentiation between active (polyribosome) and inactive (informosomes) forms in the period from the egg to early neurula stage. We gave preference to the method of CsCl density gradient centrifugation over sucrose gradient centrifugation because some inactive mRNAs in early embryos exist in the form of heavy informosomes which sediment in the sucrose gradient together with the polyribosomes^[1,7,8]. The buoyant density of polyribosomes from *Xenopus* in CsCl is 1.54 g cm⁻³ which has been demonstrated by labeling the nascent peptides by ³H-amino acids. The buoyant density of *Xenopus* mRNPs is 1.40-1.45 g cm⁻³^[4]. Thus, the buoyant densities of *Xenopus* polyribosomes and informosomes do not differ from those of other organisms^[1].

MATERIALS AND METHODS

Animals and embryos: *Xenopus laevis* frogs were injected subcutaneously with human chorionic gonadotropin at doses of 600 and 300 U per female and male, respectively. The embryos were treated with 2% cysteine, pH 8.0, for 10 min for dissolution of jelly coats, washed four times with distilled water and transferred into 0.1x Barth solution

(8.8 mM NaCl, 0.1 mM KCl, 8 mM MgSO₄, 0.03 mM Ca(NO₃)₂, 0.04 mM CaCl₂, 1 mM HEPES, pH 7.6) where they developed.

Rana temporaria adult frogs were collected in nature at hibernation and kept at 4°C. To obtain embryos, the frogs were injected with a suspension of dried *R. temporaria* hypophyses (1-5 hypophyses per frog depending on the season) and were put in tanks with water at room temperature. To remove jelly coats, embryos were incubated in 1% cysteine, 1% papain at pH 8.0 with stirring for 30-60 min, thoroughly washed with tap water and transferred into Holtfreter's solution (0.7% NaCl, 0.01% KCl, 0.02% CaCl₂, 0.04% NaHCO₃) prepared with distilled water purified by passing through a column with activated charcoal. Staging of *Xenopus laevis* embryos was done using tables by Nieuwkoop and Faber^[9] and that of *Rana temporaria* according to Dabagyan and Sleptsova^[10].

Cytoplasmic extract preparation and CsCl density gradient centrifugation: The embryos were washed with a homogenation buffer (0.01 M triethanolamine-HCl, pH 7.8, 0.25 M sucrose, 0.1 M KCl, 0.005 M MgCl₂, 0.005 M β-mercaptoethanol, 0.1% diethyl pyrocarbonate, 0.005% cycloheximide), then frozen and stored in liquid nitrogen. Homogenations were done in three volumes of this buffer by 60 movements of a glass pestle. The homogenate was centrifuged at 13,000 rpm for 1.5 min. The supernatant of 50 embryos was supplemented with Triton-X100 to 0.5% and was diluted by 5 mL of following buffer: 0.01 M triethanolamine-HCl, pH 7.8, 0.1 M KCl, 0.005 M MgCl₂, 4% formaldehyde. This fixed material was stored at 4°C for one day and then was centrifuged in a CsCl gradient. The CsCl density gradient centrifugation was performed in preformed gradients in a Ti65 rotor (Beckman) at 40,000 rpm at 4°C for 24 h. After centrifugation the gradient material passed through the Uvicord and fractions were collected. Solution densities in the fractions were determined by measuring the index of refraction, corrections being made for the index of the buffer with formaldehyde.

Dot-hybridization: Syntheses of radioactive probes for individual mRNAs were performed using the Klenow fragment of DNA polymerase I, ³²P-deoxy-ATP (Obninsk, Russia) and cDNA inserts, restricted from plasmids *pBS(KS)* and *pRN3* that contained inserts of cDNAs *Xbra*, *Xnot2*, *Xwnt11* or *Xgsc*^[11]. For hybridization with labeled DNA, fractions from CsCl gradient were applied onto nylon membranes (Hiyu Kalur, Estonia) using a Minifold device (Schleicher and Schuell, USA). Before

application, the filters were washed successively with 2% Na dodecylsulphate, bidistilled water and 20xSSC (SSC: 0.15 M NaCl, 0.015 M sodium citrate, pH 7.0). After application, the filters were washed with 6.7 SSC, dried and were heated in vacuum at 80°C for 2 h. It was shown in special experiments that during this procedure no less than 90% of the RNA was immobilized onto filters and was not washed off by a hot Na dodecylsulphate solution. Hybridization and washing of filters were performed as described earlier^[12]. Radioautographs were scanned and the radioactivity was calculated using Total lab software.

RESULTS AND DISCUSSION

The distribution of ribosomes and polyribosomes in CsCl density gradients: The ribosomes tested by UV absorption have a buoyant density of 1.56 g cm⁻³, while the polyribosomes containing nascent labeled peptides are concentrated in the zone of 1.52-1.55 g cm⁻³ with a peak at 1.54 g cm⁻³ (Fig. 1). This experiment was repeated three times with the same outcome. That the labeled material in this zone is represented by polyribosomes was confirmed in experiments with pretreatment of the extract with ethylenediaminetetraacetic acid or incubation of the embryos with puromycin which in both cases led to the disappearance of the label from this zone (data not shown).

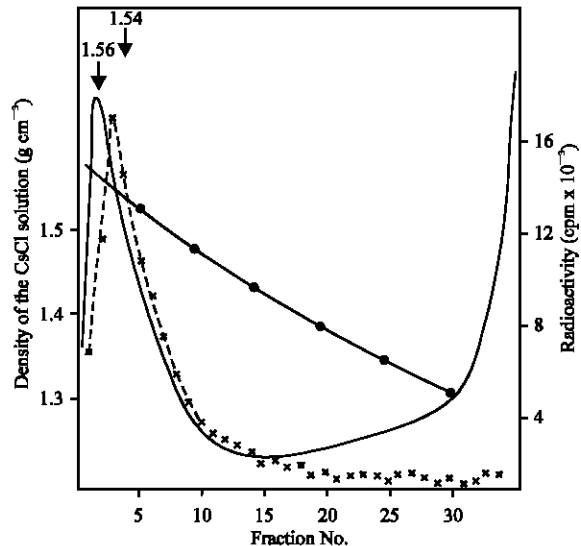


Fig. 1: The distribution in CsCl density gradient of ribonucleoproteins from *X. laevis* cytoplasm labeled by ³H-amino acids at the blastula stage. Solid line shows UV absorption at 260 nm, crosses - ³H radioactivity, closed circles - CsCl density

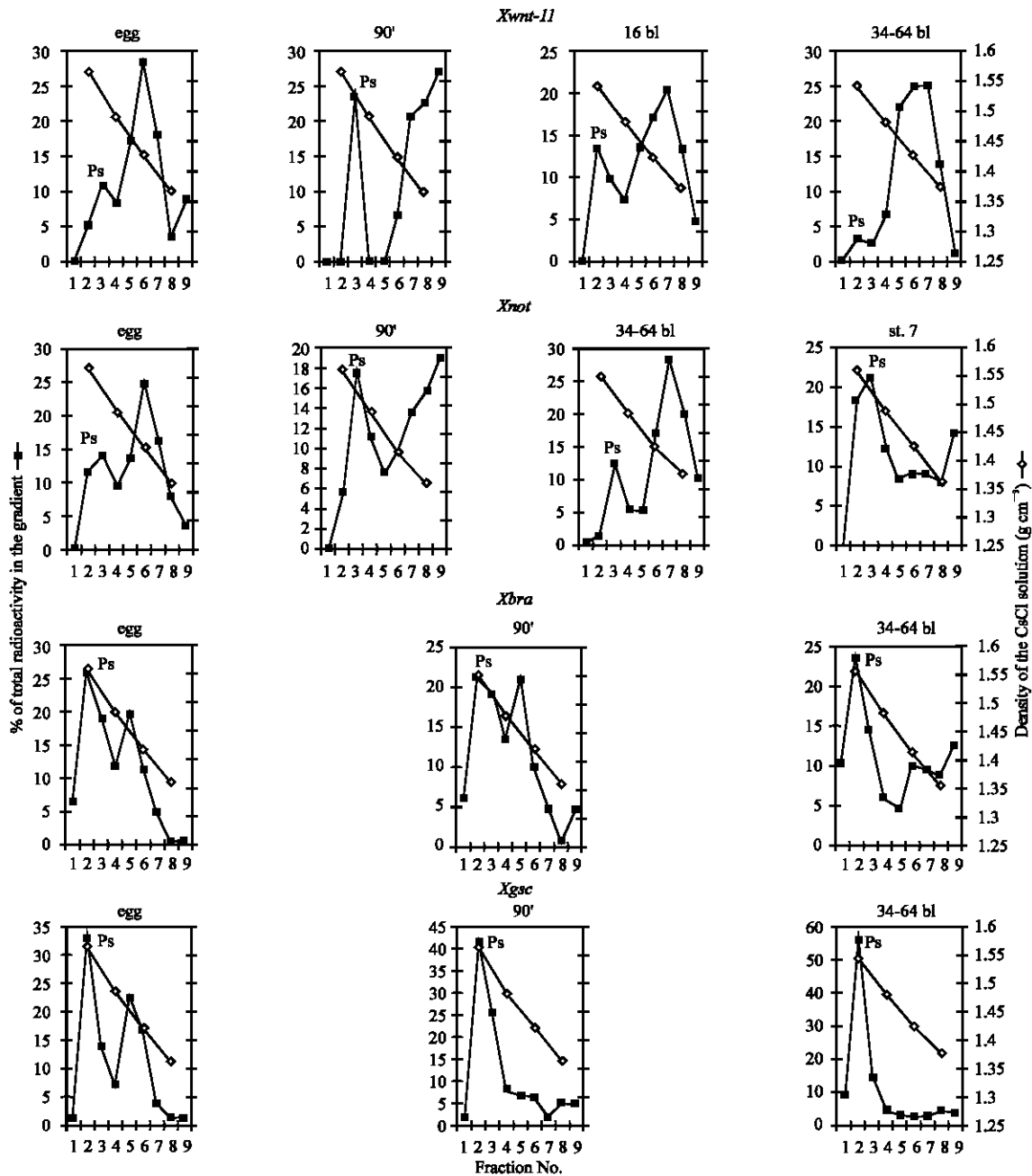


Fig. 2: The distribution in CsCl density gradient of maternal ribonucleoproteins from *X. laevis* embryos. Closed circles show results of hybridization of fractions with ³²P-probe to the *Xwnt-11* (first line), *Xnot* (second line), *Xbra* (third line) and *Xgsc* (forth line) mRNAs. Open circles show CsCl density. Ps, polyribosomes. The developmental stage is shown on the top

Activity of maternal mRNAs before midblastula transition: We investigated the distribution in CsCl gradients of four *Xenopus laevis* embryo templates: *Xwnt-11*, *Xnot-2*, *Xbra* and *Xgsc*. Figure 2 shows that in the eggs all these mRNAs are distributed as two peaks with buoyant densities of 1.54-1.56 g cm⁻³ and

1.40-1.45 g cm⁻³ which correspond to densities of polyribosomes and informosomes, respectively^[4].

Hence, in mature eggs poor synthesis of *Xwnt-11* and *Xnot-2* occurs compared to more intense synthesis of *Xbra* and *Xgsc* proteins. Ninety min. after fertilization, just before the first cell division, all *Xgsc* mRNAs are seen

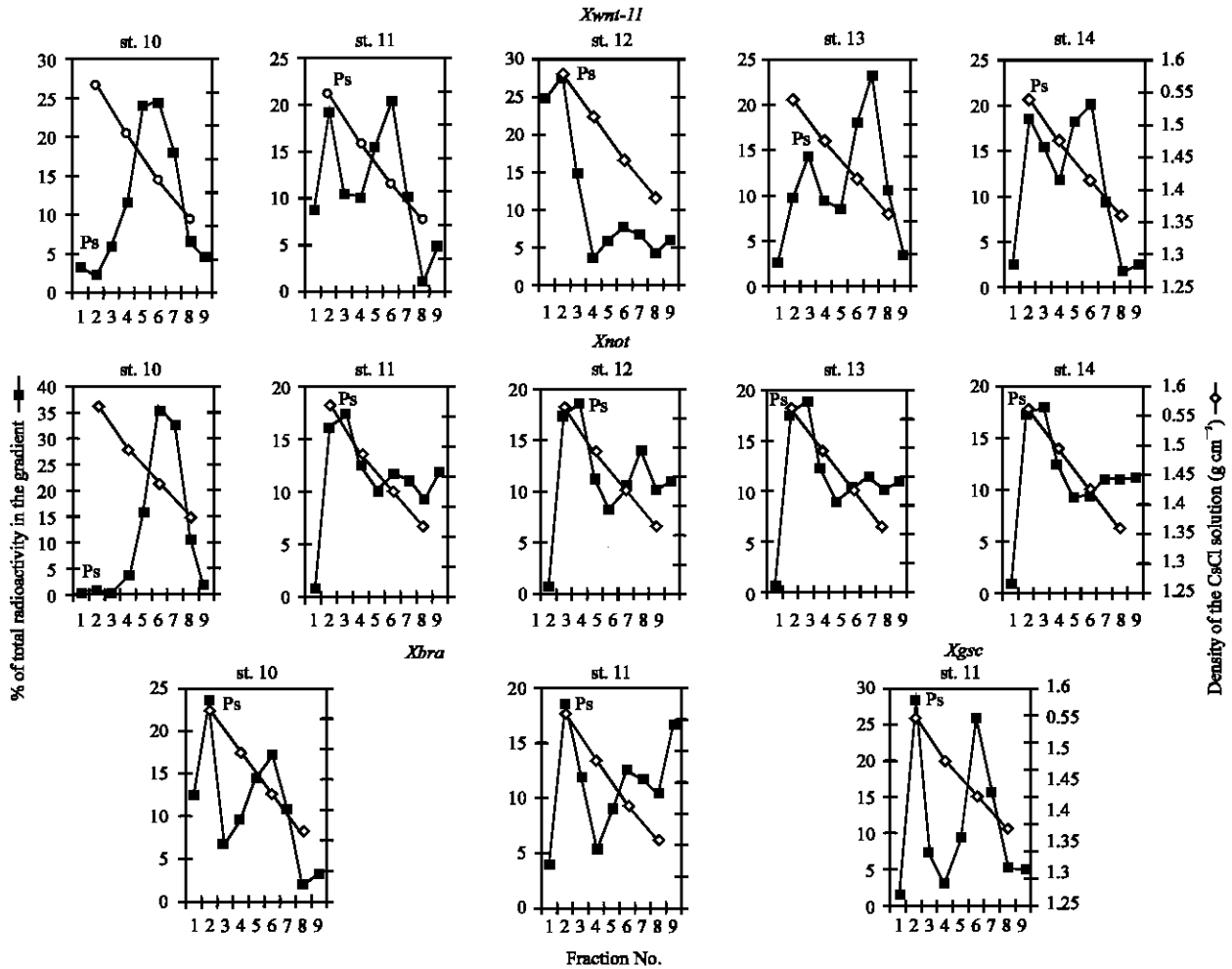


Fig. 3: The distribution in CsCl density gradient of embryonic ribonucleoproteins from *X. laevis* embryos after midblastula transition. Designations as in Fig. 2

in polyribosomes. The polysome/informosomes ratio is not changed significantly in the case of *Xbra* mRNA and increases slightly in the case of *Xwnt-11* and *Xnot-2* mRNAs. The buoyant density of *Xwnt-11* and *Xnot-2* mRNPs is reduced at this stage presumably as a result of their association with some additional proteins during cytoskeleton transformation after fertilization. The translation of *Xwnt-11* templates is terminated at 32-64 blastomer stage and most of these mRNAs remain in informosomes. This agrees with earlier observations that after fertilization the *Xwnt-11* mRNA is polyadenylated and forms polyribosomes on the dorsal side of the embryo only^[13]. The other mRNA fraction located in the vegetal hemisphere is probably suppressed. The translation of *Xnot-2* decreases at the stage of 32-64 blastomers but increases again at early blastula (stage 7). Most of *Xbra* and *Xgsc* mRNAs are actively translated at the 32-64 blastomer stage. We conclude that each of the

stored templates demonstrates its own dynamics of translational activation and repression after fertilization.

Activity of mRNAs after midblastula transition: The transcription of zygote genes in *Xenopus* starts at midblastula. The distribution of mRNAs between polyribosomes and informosomes after the midblastula transition is shown in Fig. 3.

At early gastrula, *Xwnt-11* and *Xnot-2* mRNAs appear only in informosomes. They are functionally masked as it was in the egg. The translation of these templates starts at midgastrula and continues at neurula. However, a fraction of these mRNAs remains in an inactive form, in the informosomes. It is interesting that at the end of gastrulation most of *Xwnt-11* mRNAs are translated in polyribosomes but at the beginning of neurulation the fraction of informosomes increases. *Xbra* and *Xgsc* mRNAs are detected both in polyribosomes and

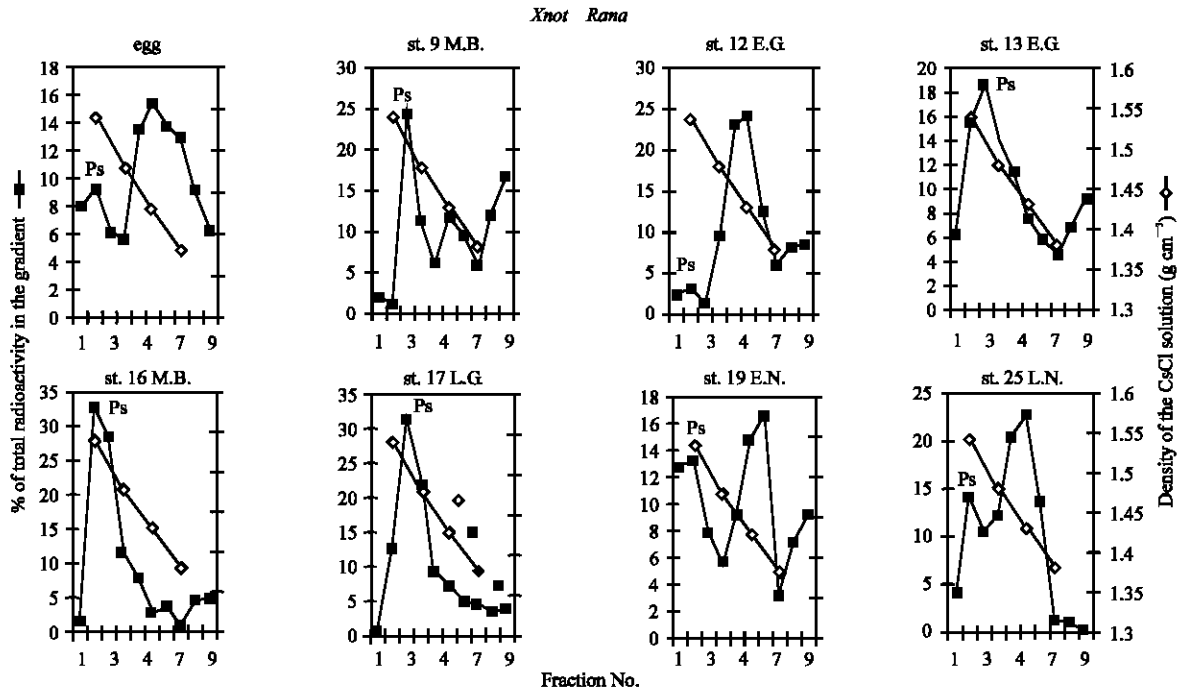


Fig. 4: The distribution in CsCl density gradient of *not* informosomes from *R. temporaria* embryos. Designations as in Fig. 2. MB – midblastula, EG – early gastrula, MG – midgastrula, LG –late gastrula, EN – early neurula, LN – late neurula

informosomes during gastrulation. In contrast to the blastula when all *Xgsc* mRNAs were translated, at gastrula about a half of these templates is masked in informosomes. Thus, each individual mRNA demonstrates its own dynamics of transition from informosomes to polyribosomes and back. We conclude that the regulation of translation of all these mRNAs takes place after midblastula transition.

Activity of *Xnot* mRNA in *Rana* embryos: We investigated whether this dynamics of mRNA transition is the same in another frog species, *Rana temporaria*. The distribution of the *not* mRNAs between polyribosomes and mRNPs in the embryos of *R. temporaria* is shown in Fig. 4.

Most of the mRNAs in the eggs are masked in the form of informosomes and at blastula these mRNAs are detected in both types of particles, as in *Xenopus*. After the midblastula transition the *not* mRNAs are accumulated in mRNPs and at early gastrula, between stages 12 and 13, all these templates are recruited to polyribosomes. In contrast to *Xenopus*, during gastrulation these mRNAs are detected only in polyribosomes and appear in informosomes only at neurula. At late neurula the majority

of *not* mRNAs are detected in informosomes. Thus, in *Rana* and *Xenopus* the mRNAs are accumulated in informosomes in the eggs and during early gastrulas, but later some differences were observed: more efficient recruitment of mRNAs to polyribosomes at gastrula was in *Rana* than in *Xenopus*. Similar data were obtained for *wnt-11* mRNA distribution in these two species^[14].

In this research we successfully used CsCl density gradient centrifugation to determine the time of activation and repression of translation of individual mRNAs during amphibia embryo development. The mRNAs accumulate in informosomes (functionally masked form) both in the egg and just after the onset of the embryo genome transcription. Each individual mRNA studied is translationally regulated during the early embryogenesis and displays its own temporary pattern. In the literature most investigations on gene expression in the development concentrate on transcriptional level. Here we emphasize that the presence of mRNAs in the cytoplasm does not necessarily mean their participation in translation. The data of *in situ* hybridization do not give complete evidence that genes of interest are expressed. For correct interpretation of such data the translational regulation should be taken into account.

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