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Transition of *Xwnt-11* mRNA from Inactive Form to Polyribosomes in Frogs During Early Embryogenesis

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Abstract—The distribution of *Xwnt-11* mRNA between polysomes and informosomes was studied in *Xenopus laevis* and *Rana temporaria* during early embryogenesis. The ratio between polysomes and informosomes suggests their involvement in translation of these mRNAs. In eggs and immediately after fertilization the *Xwnt-11* mRNAs are mostly positioned in informosomes. During the cleavage stage, these mRNAs have also been recognized in polysomes. Just before the onset of zygote genome functioning (at the stage of mid blastula), *Xwnt-11* mRNA rapidly appears in polysomes of *Rana* embryos. However, in *Xenopus*, *Xwnt-11* mRNA appears in polysomes only at the end of gastrula. Before this stage, the *Xwnt-11* mRNA in *Xenopus* can be found mostly in informosomes.

Key words: informosomes, polyribosomes, regulation of translation, dorsoventral differentiation, *Xwnt-11*, *Xenopus laevis*, *Rana temporaria*, density gradient centrifugation

Xwnt-11 is a secretory protein that binds to extracellular matrix. It is involved into dorsoventral differentiation of frogs [1, 2]. *Xwnt-11* transcript is detected in oogenesis. In the egg, these templates are localized on the vegetative pole. After fertilization and cytoplasmic rotation, *Xwnt-11* mRNA undergoes polyadenylation followed by polysome formation only on the dorsal side of embryos [3]. At the stage of late blastula, i.e., after the beginning of zygote genome functioning, *Xwnt-11* mRNA is located in a dorsal marginal zone; during gastrulation it also appears in lateral and ventral marginal zones of embryos. Subsequently this transcript is also recognized in somites and in the first bronchial arch. However, it remains unclear whether these mRNAs are translated in embryos or as in the case of the egg they exist in inactive form for some time. So, in this study we investigated distribution of *Xwnt-11* mRNA between polysomes and informosomes. This distribution suggests involvement of the studied mRNAs in translation.

MATERIALS AND METHODS

Preparation of embryos of *Xenopus laevis* and *Rana temporaria*, their homogenization, preparation of cytoplasmic extract, CsCl density gradient centrifugation, and dot-hybridization of the gradient fractions with a radioactive probe were described previously [4, 5]. Number of stages are given by Newcop and Faber tables for *Xenopus laevis* [6] and by Dabagyan and Sleptsova table [7] for *Rana temporaria*.

Radioautographs were scanned and calculated using Totallab software.

RESULTS

In mature amphibian eggs, mRNA is stored in inactive form as the informosome [8]. After fertilization transition of these mRNAs from informosomes to polyribosomes should occur. At the stage of mid blastula, mRNA synthesis begins on the zygote genome. These newly synthesized mRNAs should also transit to polysomes. We monitored the time course of *Xwnt-11* mRNA transition from informosomes into polyribosomes using ribonucleoprotein fractionation in CsCl density gradient followed by subsequent dot-hybridization with ^{32}P-probe to *Xwnt-11* mRNA. In these gradients, polyribosomes and informosomes have buoyant density of 1.54 and 1.40-1.45 g/cm^3, respectively [4]. Figures show distribution of mRNP containing *Xwnt-11* mRNA in CsCl density gradients. Figure 1 shows results obtained in experiments with *Xenopus* embryos at various stages of their development. Figure 2 shows results of similar experiments with *Rana* embryos.

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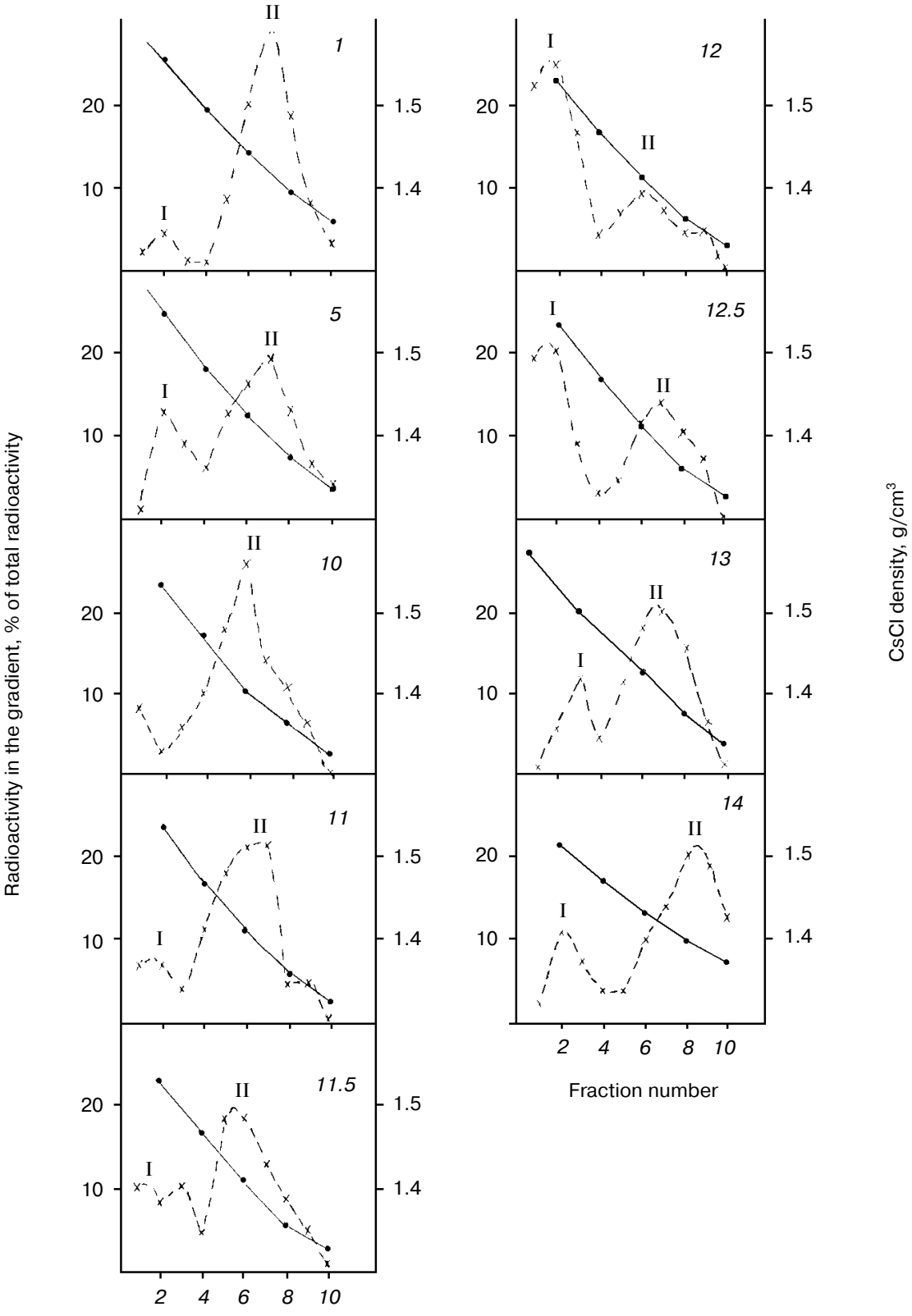

Fig. 1. Distribution of ribonucleoproteins from *X. laevis* embryos in CsCl density gradient: I) polyribosomes; II) informosomes. Solid line shows CsCl density, broken line shows results of hybridization of fractions with ^{32}P-probe to *Xwnt-11* mRNA. Number of the developmental stage is shown in the right upper corner.

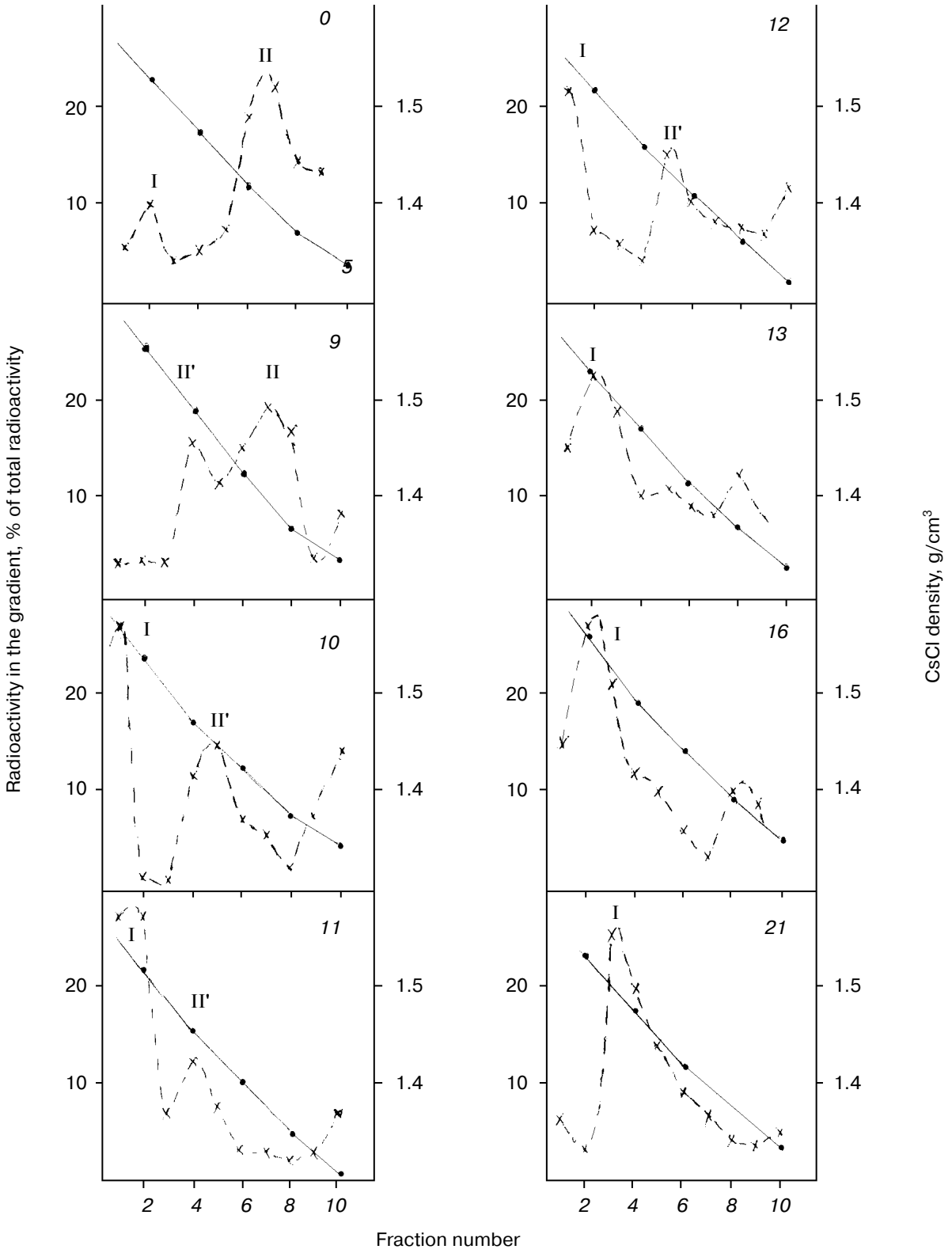

Fig. 2. Distribution of ribonucleoproteins from *R. temporaria* embryos in CsCl density gradient: I) polyribosomes; II) informosomes; II′) complex with intermediate buoyant density (1.45-1.48 g/cm^3). Solid line shows CsCl density, broken line shows results of hybridization of fractions with ^{32}P-probe to *Xwnt-11* mRNA. Number of the developmental stage is shown in the right upper corner.

In eggs (*Rana*, Fig. 2, stage *0*) or right after fertilization but before formation of the first cleavage furrow (*Xenopus*, Fig. 1, stage *1*), the main proportion of *Xwnt-11* mRNA was detected in informosomes. However, in both cases there is a small but significant peak in the region of buoyant density of polysomes. This suggests the existence of some basal synthesis of Xwnt-11 protein or some proportion of mRNA is located in inactive or weakly elongating polysomes. At stage *16* blastomeres (stage 5 in *Xenopus*), significant (but not all) *Xwnt-11* mRNA is detected in polyribosomes. This is consistent with data by Schroeder et al. [3] that synthesis of Xwnt-11 protein triggered after fertilization only at the dorsal side of embryo, although mRNA is also present on the ventral side. After the onset of zygote genome and during the whole gastrulation, *Xwnt-11* mRNA in *Xenopus* embryos is detected mainly in informosomes (stages *10-11.5*). At the end of gastrulation (stage *12*) a sharp increase in polysome proportion occurs due to regulated triggering of translation of these templates. At the stage of early neurula (stages *13-14*) polysome proportion in *Xenopus* reduced due to accumulation of newly synthesized templates in informosomes or due to decrease in translation of pre-existing *Xwnt-11* mRNA. A rather different situation was observed in *Rana* embryos (Fig. 2). From the stage of mid blastula (stage *9*) and up to early gastrula (stage *12*) a significant proportion of *Xwnt-11* mRNA was found in ribonucleoproteins with buoyant density of 1.45-1.48 g/cm^3 (II′, Fig. 2), which probably represents the initiation complex. It is also possible that informosomes containing newly synthesized (on zygote genome template) mRNAs have higher buoyant density than those stored in the egg. In contrast to *Xenopus*, *Xwnt-11* mRNA in *Rana* embryos appears in polysomes at the stage of late blastula (stage *10*) and to mid gastrula (stage *13*) and the major part of these mRNAs is translated. At all subsequent stages up to mid neurula (stage *21*, Fig. 2) *Xwnt-11* mRNA is detected mainly in polysomes. Thus, activation of translation of *Xwnt-11* mRNA synthesized on the zygote genome template was observed in both frog species, but in *R. temporaria* embryos this activation occurs earlier than in *X. laevis* embryos and at the stage of mid gastrula almost all *Xwnt-11* mRNA is involved in translation.

DISCUSSION

At the stage of mid blastula transcription of genes responsible for gastrulation and dorsoventral differentiation begins in amphibian embryos. Some of previously studied templates are initially accumulated in cytoplasm of *Xenopus* embryos in the form of informosomes and only at the stage of mid gastrulation they are involved in protein synthesis [4]. Each template (or group of templates) is characterized by its own time course of transition from the inactive form into polysomes, i.e., activity of each template is individually regulated [9]. Study of mechanisms of such regulation requires elucidation of concrete developmental stage at which activation of synthesis of each protein occurs. Our approach allows this stage to be detected.

These results are consistent with data by Neifakh [10] on investigation of embryonal development by means of inhibition of cell nuclei functioning. Radioactive irradiation of *Rana* embryos at the stage of early gastrula caused arrest of development at the stage of late gastrula. In other words, all processes of gastrulation have been programmed by genome functioning from the stage of mid blastula to early gastrula. According to a model proposed by Spirin [11], this information is stored in cytoplasm as masked mRNAs in form of informosomes. Unmasking these templates occurs due to signals that are independent of nuclear activity [9].

According to our data, activation of Xwnt-11 protein synthesis in *Xenopus* embryos occurs later than that in *Rana* embryos. It should be noted that these two species are related to different sub-orders of anourous amphibians. They live in different climatic zones and are characterized by large differences in biology of reproduction. It is possible that due to cooler climate the development of *Rana* embryos requires earlier and more intensive synthesis of certain proteins, including extracellular matrix proteins.

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