ON THE EXISTENCE OF FREE INFORMOSOMES IN THE CYTOPLASM

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Received 7 March 1978

1. Introduction

Free cytoplasmic informosomes have been described often (reviewed [1,2]). However, the conclusion that these particles are really localized in the cytoplasmic compartment of the cell and are not nuclear contaminants remains to be confirmed, especially for the heavy cytoplasmic informosomes. Earlier, when nuclei of *Chironomus tentants* salivary gland cells were removed from the cytoplasm by a microsurgical technique, it was shown that high-molecular weight 75 S RNA was localized in the cytoplasmic compartment of the cell [3,4]. Unfortunately, this method cannot be applied to other biological objects, in particular to embryonic cells. The problem arose [5–10] with sea urchin embryos. It was discussed whether leakage of labelled RNA from the nucleus takes place during homogenization. At the early blastula stage, labelled RNA could be observed in the nuclear fraction only, suggesting the absence of RNA leakage from the nuclei during homogenization. However, this result cannot be extrapolated to the later stages of the development without a special investigation, since the structure of embryonic nuclei may change with the age of the embryo.

We describe here experiments where the distribution of the labelled RNA between the nucleus and the cytoplasm was studied both in vivo (in intact loach gastrula cells) by an autoradiographic technique and in vitro, in extracts obtained by homogenization. Experiments of this kind can be performed on any type of cells. The data obtained show that in loach gastrula cells there is essentially no leakage of messenger-containing ribonucleoproteins from the nuclei during homogenization. It is also shown that cytoplasmic ribonucleoproteins are not contaminated with nuclear ribonucleoproteins which could be released into the cytoplasm during mitosis.

2. Materials and methods

Embryos of loach (*Misgurnus fossilis* L) at the gastrula stage were used. Blastoderms isolated from the yolk as in [11] were incubated in the presence of [^{3}H]uridine [12]. All autoradiographic procedures were as in [13,14].

Embryos were homogenized in buffer containing 0.01 M triethanolamine–HCl, pH 7.8, 0.01 M KCl, 0.001 M $MgCl_2$, 0.005 M β-mercaptoethanol and 0.25 M sucrose [11,12]. The homogenate was centrifuged at 10 000 × g for 20 min. The supernatant obtained was considered as the 'cytoplasmic extract' [12,15]. The cytoplasmic extract was analyzed by CsCl gradient centrifugation and by sucrose gradient centrifugation as in [11,12,15].

The pellet containing nuclei and mitochondria was suspended in the homogenization buffer and centrifuged at 2500 rev./min for 20 min. The procedure was repeated 2 or 3 times. The final pellet was considered as the 'nuclear fraction'. The purity of the isolated

 Elsevier/North-Holland Biomedical Press

nuclei was checked microscopically. The combined 2500 rev./min supernatants were considered as the 'mitochondrial fraction' (though they contained other cytoplasmic material besides mitochondria).

The nuclear pellet was suspended in the homogenization buffer and heparin was added to final conc. 20 mg/ml, to destroy the nuclei and so to prevent quenching during radioactivity determination. Heparin was added also to all other fractions to ensure standard experimental conditions. Aliquots were taken from all the fractions, diluted to final conc. 0.1–0.5 A_{260} units and the trichloroacetic acid-insoluble radioactivity was measured on nitrocellulose filters by a routine procedure [15].

In the experiments with colchicine treatment the embryos were incubated with the drug (10^{-4} M) for 3 h at 20°C and, in addition, for 17 h at 12°C. The embryos were fixed with ethanol–acetic acid and stained with acetocarmine.

3. Results and discussion

The cytoplasmic extract of loach embryos was analysed by CaCl gradient centrifugation (fig.1). It is seen that the main labelled component in the cytoplasmic extract has the buoyant density typical of informosomes (1.39 g/cm^3). The labelled material is absent from the bottom of the gradient (free RNA) and from the band of ribosomal density (1.55 g/cm^3). This confirms [1,11,15] that at the blastula and gastrula stages the loach embryos do not yet synthesize ribosomal RNA so that the radioactive uridine is incorporated only into mRNA, in the form of informosomes, and some into tRNA.

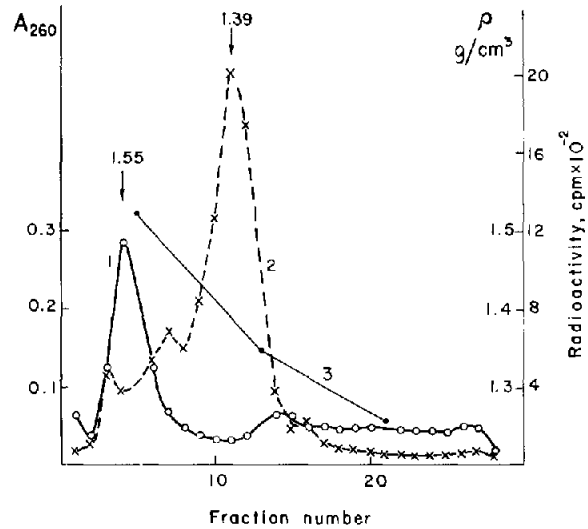


Fig.1. Density distribution in a CsCl gradient of the [^{3}H]-uridine-labelled loach embryo cytoplasmic extract. The cytoplasmic extract was diluted with buffer containing 0.01 M triethanolamine, 0.01 M KCl, 0.001 M $MgCl_2$, 0.001 M β-mercaptoethanol, pH 7.8, to final conc. 5 A_{260} units and was fixed with 4% formaldehyde. The pre-formed CsCl gradient was prepared in the same buffer. Centrifugation was done in a high-speed 65 (MSE) SW-65 rotor at 53 000 rev./min for 10 h at 20°C. (1) A_{260}. (2) Radioactivity. (3) Buoyant density.

Table 1
Radioactivity of RNA in nuclear and cytoplasmic fractions of the loach embryo extract obtained at the gastrula stage

Fraction	Experiment 1		Experiment 2	
	Total radioact. (cpm × 10^{-6})	% Total radioact. homogenate	Total radioact. (cpm × 10^{-6})	% Total radioact. homogenate
Total homogenate	4.62	100	2.80	100
Nuclei	1.65	36	1.10	40
Cytoplasm	2.48	54	1.08	39
Mitochondria	0.45	10	0.60	21

Incubation with [^{3}H]uridine for 1 h

Table 2
Amount of labelled RNA over nuclei and cytoplasm in loach gastrula cells as determined by autoradiography

Cell compartment	Grain counts/ section[a]	Grain counts/vol. cell compartment	% labelled RNA
Nucleus	46 ± 2	276	39
Cytoplasm	9 ± 1	425	61

[a] Mean value of 60 determinations

Incubation with [^{3}H]uridine for 1 h

The amount of labelled RNA in the nuclear and the cytoplasmic fractions of the extract are presented in table 1. Table 2 shows the amount of labelled RNA determined as the number of the silver grains over the nuclei and the cytoplasm in autoradiographs of the intact cells. Grain counts were recalculated per cytoplasm and nuclei volumes. The data on cell and nuclei volumes were taken from [13,14]. A comparison of the results from tables 1 and 2 shows that in both cases the nuclei comprise 39–40% of the labelled RNA. The coincidence of the results obtained by different methods shows that leakage of radioactive material from the nuclei during the homogenization procedure was virtually absent.

While working with embryos one must take into consideration that in dividing cells the nuclear DNA-like RNA may be dispersed throughout the cytoplasm and thus increase the amount of heavy cytoplasmic informosomes. To check this possibility the number of mitotically-dividing cells in the embryos was increased by colchicine treatment and the sedimentation distribution of ribonucleoproteins in the cytoplasmic extract was determined in the colchicine-treated embryos, and compared with that of the controls (without colchicine). The embryos of the mid-gastrula stage were incubated for 3 h in the presence of [^{3}H]uridine, then the label was washed off with double-strength Holtfreter solution [12,15] and the labelled embryos were divided into 3 groups. Embryos of the first group were assayed immediately after labelling (control 1). Embryos of the second and the third groups were further incubated, either in the presence (experimental group), or in the absence (control 2) of colchicine (see also section 2). In all the groups one portion of the embryos was used for mitotic index determination (fig.2a–c, table 3) and another one was homogenized to assay the informosome distribution profile after centrifugation in a sucrose density gradient (fig.3a–c). Table 3 shows that after colchicine treatment the mitotic index

Fig.2a

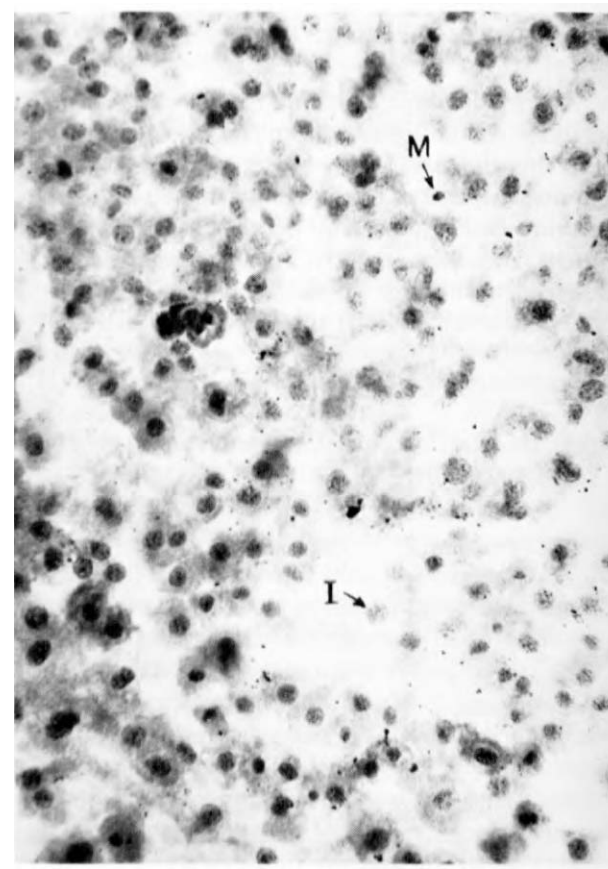


Fig.2b

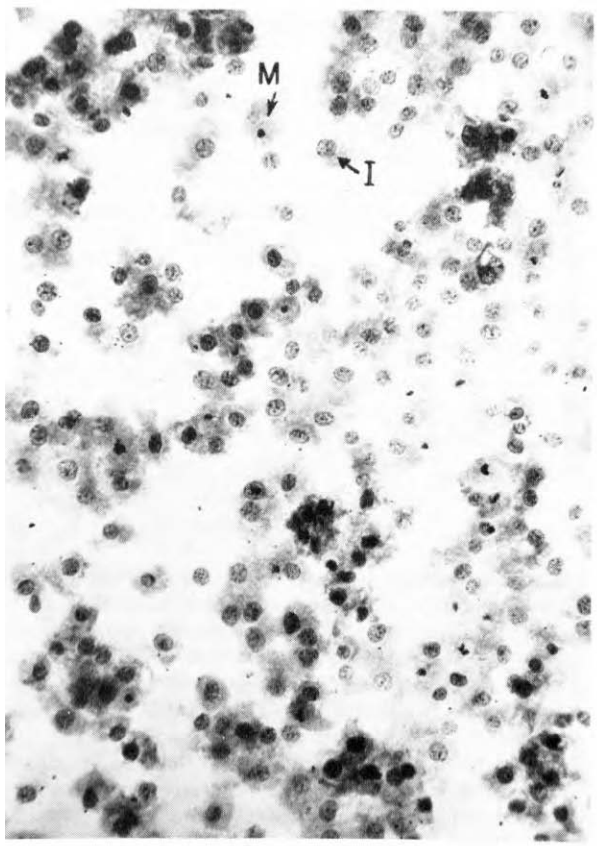


Fig.2c

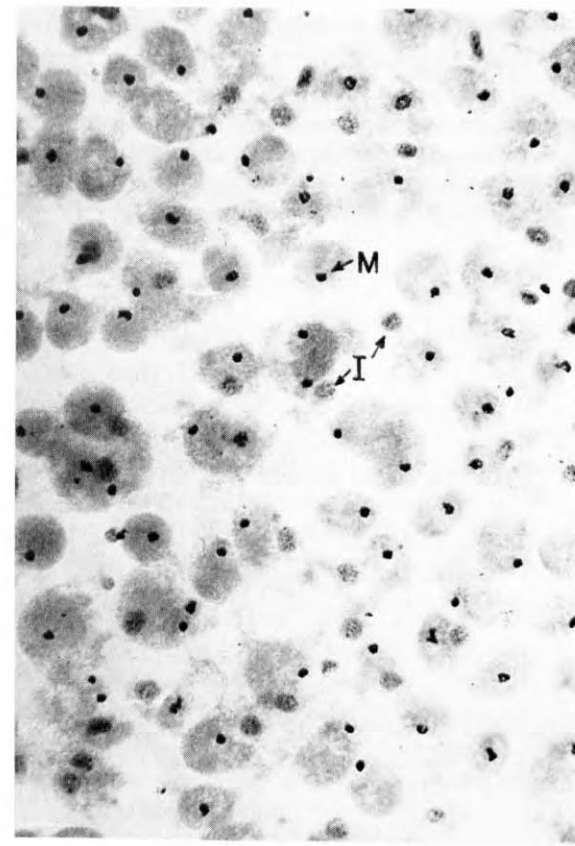


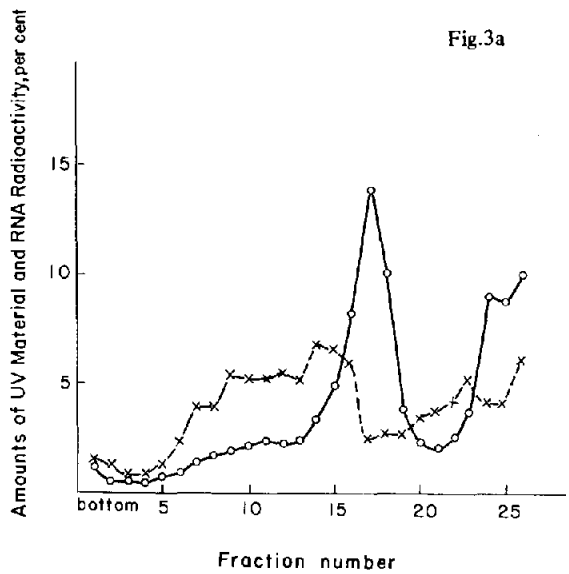
Fig.2. Squashed preparations of loach blastoderms at the mid-gastrula stage. (a) Control 1. (b) Control 2. (c) Experimental group (after colchicine treatment). (M) Mitosis. (I) Interphase nuclei.

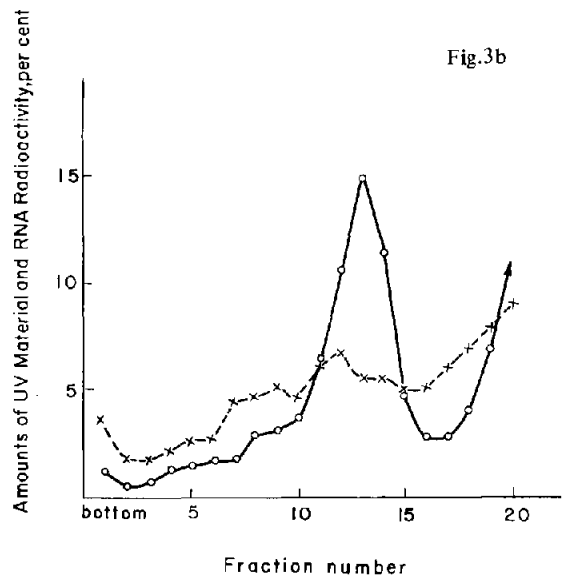
Table 3
Mitotic index in loach gastrula cells after colchicine treatment

Material	Total nuclei studied	Interphase nuclei	Dividing nuclei	Mitotic index (%)
Control 1[a]	1998	1967	31	1.55
Control 2[a]	1919	1898	21	1.09
Colchicine treatment	2163	1438	725	33.5

[a] Details in the text

increased from 1.55–33%, i.e., 20 times. At the same time fig.3 shows that the sedimentation distribution of labelled ribonucleoproteins does not markedly differ in the experimental and control groups. From the data presented it can be concluded that neither the pre-ribosomal nor the post-ribosomal informosomes observed in the cytoplasm can be considered as an artifact due to release of pre-messenger containing ribonucleoproteins from the dividing nuclei.





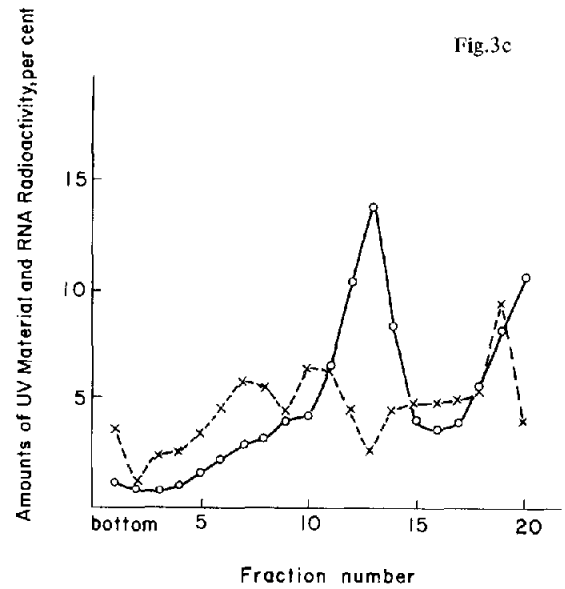


Fig.3. Sedimentation distribution in a sucrose gradient of the ribonucleoproteins of the loach embryo cytoplasmic extract. Sucrose gradients (15–55%) were prepared in buffer containing 0.01 M triethanolamine, 0.01 M KCl, 0.001 M $MgCl_2$, 0.001 M β-mercaptoethanol, 4% formaldehyde, pH 7.8. Centrifugation in a Spinco L SW-25 rotor at 20 000 rev./min for 7 h. (1) A_{260}. (2) Radioactivity. (a) Control 1. (b) Control 2. (c) Experimental group (after colchicine treatment). Total amount of ultraviolet absorption and radioactivity in gradient fractions considered as 100% comprised: for (a), 14.2 A_{260} units and 19 000 cpm; for (b), 15.2 A_{260} units and 30 200 cpm; for c, 16.5 A_{260} units and 30 300 cpm, respectively.

Acknowledgements

The authors wish to thank Professor A. S. Spirin for helpful suggestions and criticism during preparation of the manuscript.

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