Cytoplasmic Extraction: Polyribosomes and Heterogeneous Ribonucleoproteins without Associated DNA

Abstract. *Two methods are described for preparing cytoplasmic extracts from sea urchin embryos. One method, involving homogenization, yields DNA structures that cosediment with polyribosomes and subribosomal ribonucleoproteins. In addition this method also yields extraneous structures containing RNA that cosediment with polyribosomes. The DNA is not associated with polyribosomes, as shown by buoyant density analysis. Furthermore, this DNA appears to be spurious, because its release into a cytoplasmic extract does not occur when a different method of cell disruption, involving passage of embryos through a hypodermic needle, is used. With this second method, polyribosomes are obtained without extraneous cosedimenting RNA structures and subribosomal ribonucleoproteins are obtained in the virtual absence of DNA.*

The identification of extracted cellular constituents as cytoplasmic may be obscured by contamination from nuclei. The avoidance of such contamination is necessary in efforts to characterize purely cytoplasmic elements and to ascertain the mechanism of transfer of genetic information from nucleus to cytoplasm. Some investigators claim that labeled DNA is detected in association with cytoplasmic microsomes (*1*) and in association with subribosomal material as well as with polyribosomes (*2*). We question the method of cell disruption used in each of these cases, and challenge the proposed conjunction of these cytoplasmic structures with DNA. We describe how two different methods of cell disruption result in different degrees of presumably nuclear contamination of cytoplasmic extracts. A method for the lysis of sea urchin embryos is presented that does not allow the appearance in the cytoplasm of either nonmitochondrial DNA or extraneous structures containing RNA, such as those shown to cosediment with polyribosomes (*3*). Under these conditions newly synthesized RNA appears in polyribosomes and in heterogeneous subribosomal ribonucleoproteins (*4, 5*). Our results offer a method designed to yield pure cytoplasmic extract for a particular biological material and, moreover, furnish a rationale for determining the purity of such an extract.

The DNA and RNA of sea urchin embryos at the early blastula stage were labeled with [^{14}C]thymidine and [^{3}H]-uridine, respectively, and the embryos were divided into two portions. The first portion was homogenized by one to four complete strokes in a Dounce homogenizer (method 1). The second portion of labeled embryos was passed once through a hypodermic needle, according to a modification of the method of Hinegardner (*6*) (method 2). Supernatants (S15), freed of mitochondria and nuclei, were centrifuged through sucrose gradients (Fig. 1). As judged by the absorbance at 260 mμ (A_{260}) of the monoribosomes, the gradients were divided into a subribosomal region and a polyribosomal region, which extended into a sucrose cushion. A large amount of labeled DNA appeared in the gradient of method 1, whereas approximately only 20 percent of similar label appeared in the gradient of method 2. In equivalent amounts of cytoplasmic extract derived by the two methods, similar quantities of labeled RNA appeared throughout the gradient. The appearance of labeled DNA in material smaller than mitochondria depends on the method of extraction. The major portion of the labeled DNA, most likely of nuclear origin, corresponds to approximately 20*S*, but a significant amount is spread throughout the gradient into the polyribosomal region. The sedimentation of this DNA-containing material resembles that of the labeled DNA detected by Bell in cytoplasmic extracts of embryonic chick tissues (*2*).

Analysis of the labeled RNA in the polyribosomal region of the gradient of method 1 indicates that part of it belongs to cosedimenting nonpolyribosomal structures (Fig. 2); the polyribosomes were either resedimented without treatment (Fig. 2a) or they were resedimented after treatment with ethylenediaminetetraacetate (EDTA) (Fig. 2b). Although EDTA caused dissociation of ribosomes and a shift of most of the labeled RNA to slowly sedimenting material (*3*), a substantial portion of the labeled RNA was resistant to this treatment. The polyribosomes were fixed with formaldehyde and thus stabilized by intramolecular cross-linking (*5*). They were then analyzed in CsCl density gradients (*5, 8*) and most of the labeled RNA appeared coincident with the A_{260} peak of the polyribosomes (Fig. 2c). However, a small portion of labeled RNA, similar in amount to that which was resistant to EDTA, displayed buoyant densities different from that of the polyribosomes. This nonpolyribosomal material with labeled RNA resembled structures that can be extracted from nuclei (*9*).

The labeled RNA associated with polyribosomes derived by method 2 was analyzed in the same way. The polyribosomes were resedimented (Fig. 2a′), and, when treated with EDTA,

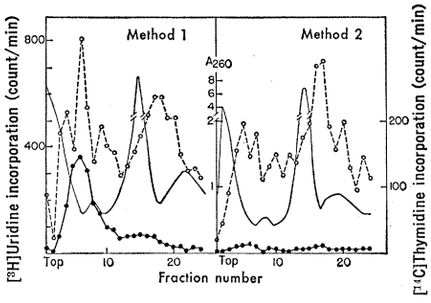

Fig. 1. Sedimentation diagrams of supernatants prepared by different methods of cell disruption. The fertilization membranes of *Lytechinus pictus* embryos were removed immediately after fertilization by passage through bolting silk (No. 16), and the embryos were developed at 18°C in artificial seawater (*7*). The embryos were incubated with [2-^{14}C]thymidine (5 μc/ml; 52.8 mc/mmole, New England Nuclear Corp., Boston, Mass.) for 180 minutes and with [5-^{3}H]uridine (50 μc/ml; 23.7 c/mmole, New England Nuclear Corp.) for 45 minutes before the 9-hour stage of development. Equal aliquots of embryos in seawater were layered onto, then centrifuged through, ice-cold 1*M* dextrose in distilled water. The pelleted embryos were resuspended in five volumes of TK medium (0.24*M* KCl, 0.005*M* $MgCl_2$, 0.01*M* triethanolamine HCl buffer, *p*H 7.8). The two aliquots were submitted to different methods of cell disruption. Method 1. Bentonite was added to a concentration of 0.2 mg/ml, and the embryos were quickly homogenized by four complete strokes in an all-glass Dounce homogenizer. Method 2. The same amount of bentonite was added, and the suspension was passed gently through a 20-gauge hypodermic needle. In each case the cell lysate was centrifuged for 15 minutes at 15,000*g*, and the supernatant (S15) was layered onto a 15 to 30 percent sucrose gradient in TK medium underlaid with 2 ml of 50 percent sucrose as a cushion. The gradients were centrifuged for 90 minutes in a Spinco rotor SW 50.1 at 50,000 rev/min. The A_{260} (———) was continuously monitored by a recording spectrophotometer, and gradient fractions were collected for assay of [^{14}C]thymidine (●) and [^{3}H]uridine (○) incorporation in material adhering to membrane filters (*5*). Radioactivity was normalized to equal yields of ribosomes for each preparation.

they yielded no resistant labeled RNA (Fig. 2b′). At this point, further analysis was necessary to establish the purity of these polyribosomes, because nonpolyribosomal structures sensitive to EDTA cannot be excluded. Fixation and analysis of buoyant density in CsCl allowed a test for further polyribosomal properties. All of the labeled RNA had the same buoyant density as that of the polyribosomal fraction at A_{260} (Fig. 2c′). Method 2 not only diminished the amount of DNA in the cytoplasmic extract but also allowed the isolation of polyribosomes, free of contaminating cosedimenting structures. These two methods have been juxtaposed to determine whether or not certain constituents are integral parts of a cytoplasmic extract. Another approach (*2*) might be to mix purified, labeled nuclei with unlabeled cytoplasm or unlabeled cells, submit the mixture to a given method of cell disruption, and determine the extent of release of labeled nuclei. However, such a test might not be valid, because the purified nuclei would have themselves been derived by the very method of cell lysis under question and might have experienced specific loss of constituents deemed to be contaminants of the cytoplasmic extract.

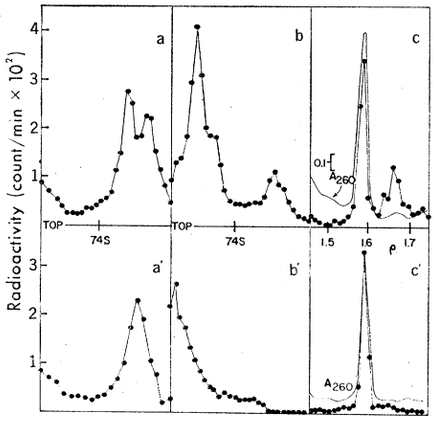

Fig. 2. Polyribosomes and cosedimenting RNA structures. Embryos of *Strongylocentrotus purpuratus* were developed and incubated with [^{3}H]uridine, and S15 extracts were prepared and sedimented as in Fig. 1. The material in the polyribosomal region was dialyzed against TK medium for 2 hours to remove sucrose and then divided into three portions for further processing. (a,a′) Resedimentation under conditions of Fig. 1 without further treatment; (b,b′) resedimentation after addition of EDTA to a concentration of 0.1*M*; and (c,c′) fixation for 24 hours with 6 percent formaldehyde and centrifugation for 14 hours in CsCl gradients (*5*). The series (a,b,c) was derived from extracts prepared by method 1; the series (a′,b′,c′) was derived by method 2. This experiment with embryos of *L. pictus* yielded the same results.

The DNA and the rapidly sedimenting, nonpolyribosomal structures containing RNA, observed in method 1, each have their nuclear counterparts (*9*) and may be presumed to be of nuclear origin. An unlikely alternative is that method 2 fails to release both of these constituents from cytoplasmic structures. If we allow this possibility, then we should examine whether or not the released DNA is attached to additional polyribosomes as proposed (*1, 2*). A further analysis of the labeled DNA cosedimenting with polyribosomes was made by examining its buoyant density. Material such as that in the polyribosomal region of Fig. 1 (method 1) was fixed with formaldehyde and centrifuged in CsCl density gradients. Essentially all of the labeled DNA banded at a density ($\rho = 1.50$) distinctly different from that of the band of polyribosomes ($\rho = 1.59$) (Fig. 3). Furthermore, although most of the labeled RNA coincided with the A_{260} of the polyribosomes, a substantial portion of this labeled RNA was spread throughout the gradient—most was more dense than the polyribosomes, but some was less dense and in association with the labeled DNA. We may conclude that (i) the labeled DNA obtained by the homogenization method is not associated with polyribosomes and (ii) the extraneous structures cosedimenting with polyribosomes are of two categories, those which contain RNA but apparently no DNA and those which contain both DNA and RNA. These structures are similar to those observed in extracts of nuclei prepared by method 2 (*9*). If the released DNA does, nevertheless, arise from cytoplasmic structures, it cannot be linked significantly with ribosomes or polyribosomes. Similar tests might well be applied to other cases, where complexes between DNA and microsomes (*1*) or polyribosomes (*2*) have been postulated. This material may represent fragmented chromatin.

Besides being associated with polyribosomes, labeled RNA is also observed in heterogeneous subribosomal particles, characterized as unique ribonucleoproteins (*5*) and designated "informosomes" (*4*). A substantial yield of these particles is obtained under conditions that prevent the release of labeled DNA. We may thus conclude that there is no link between these ribonucleoproteins and the labeled DNA whose appearance in the sub-

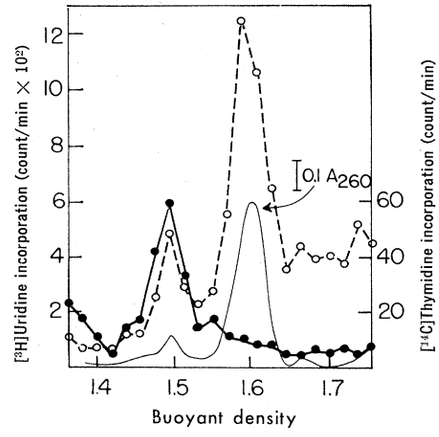

Fig. 3. Buoyant densities of labeled DNA and RNA cosedimenting with polyribosomes. The polyribosomal region (fractions No. 16 to 23) of a sucrose gradient parallel to that of Fig. 1 (method 1) was fixed with formaldehyde and centrifuged in a CsCl gradient as in Fig. 2c. All experiments were performed with both species of sea urchin, *S. purpuratus* and *L. pictus*, and the same results were obtained. [^{14}C]Thymidine, ●; [^{3}H]uridine, ○.

ribosomal region of sucrose gradients depends on the conditions of extraction. An assignment of a cytoplasmic role to this DNA, especially in conjunction with subribosomal particles containing RNA (*2*) would seem unwarranted. If indeed the subribosomal ribonucleoproteins do function in the transfer of genetic information from nucleus to cytoplasm, as hypothesized (*4, 5*), then they do so without the intervention of cytoplasmic DNA (*10*).

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References and Notes

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