APPEARANCE OF CYTOPLASMIC INFORMOSOMES IN CULTURED CHINESE HAMSTER CELLS IN THE ABSENCE OF PROTEIN SYNTHESIS

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1. Introduction

Cytoplasm of cultured animal cells contains approximately equal amounts of messenger RNA associated with rebosomes (in polysomes) and messenger-like RNA, or mlRNA, free of ribosome attachment but associated with protein to form nucleoprotein particles, referred to as informosomes [1, 2]. Polysomal messenger and informosomal messenger-like RNAs are similar in size, composition, hybridization properties, and stability [1]. Their poly A contents are comparable [Los Alamos Scientific Laboratory, unpublished results]. The role of informosomes in polysome formation is uncertain; possibly they represent the form in which messenger RNA exists in transit from the nucleus to the cytoplasm and during association with ribosomes [2]. Alternatively or in addition, they are inactive or storage forms of cytoplasmic messenger, possessing protein moieties which differ from those in ribosome-associable messenger nucleoprotein [1, 3].

Similar nucleoproteins exist in the nucleus [4]. The relationship of cytoplasmic to nuclear informosomes is uncertain; their protein moieties have not yet been compared, and the mechanisms involved in nuclear-to-cytoplasmic transport of informosomes remain to be defined [5]. Shiokawa and Pogo [6] showed that nuclear-to-cytoplasmic transport of poly A containing RNA in yeast requires the initiation of (membrane-associated) protein synthesis. If extended to animal cells, their results would indicate a possible involvement of and a requirement of protein synthesis in the appearance of cytoplasmic informosomes. Thus, the effect of inhibited initiation of protein synthesis on the appearance of cytoplasmic free informosomes in animal cells is of interest and is the subject of the studies reported herein.

In the present communication it will be demonstrated that nascent free informosomes appear in the cytoplasm of Chinese hamster cells during culture at 42°C, a condition which causes rapid and essentially complete inhibition of translation initiation.

2. Materials and methods

Chinese hamster ovary cells, line CHO, were cultured in Ham's F-10 medium without calcium. The initiation of protein synthesis was selectively terminated by culture at 42°C [7].

Formaldehyde-fixed informosomes band in cesium chloride at a density of 1.4 g/cc and polysomes at 1.52 g/cc. Pretreatment of cultures with a low concentration of actinomycin D selectively reduces incorporation of nucleosides into rRNA and tRNA, with little effect on mRNA or mlRNA incorporation. Thus, the relative incorporation of labelled nucleosides into informosomal mlRNA and polysomal mRNA was determined by isopycnic analysis of formaldehyde-fixed cytoplasmic material from cells labelled in the presence of 0.05 μg/ml actinomycin. This procedure and the techniques for cell fractionation, gradient resolution of cytoplasm, RNA extraction, etc., have been described in detail elsewhere [8–10].

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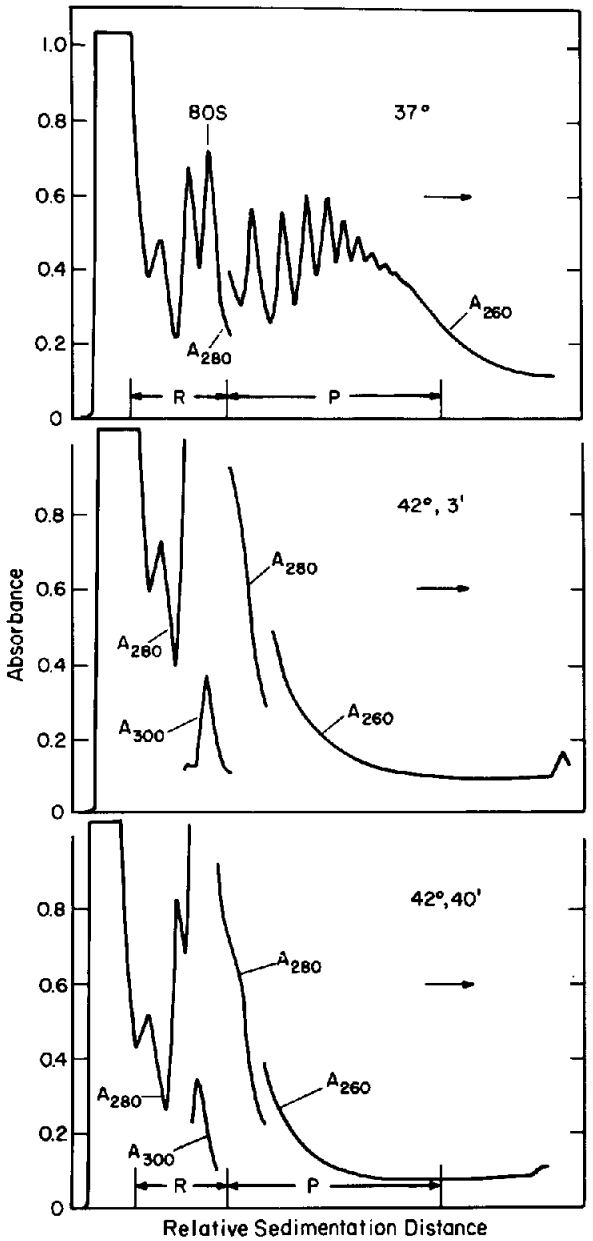
3. Results and discussion

When HeLa cells are grown at 42°C, elongation continues in the absence of initiation, and the polysomes quickly disaggregate [7]. Similarly, the culture of CHO cells at 42°C for 3 min results in the conversion of polysomes to ribosomes (fig. 1). Continued incubation at 42°C eventually results in the appearance of a heat-stable initiation factor and corresponding reappearance of polysomes [7]. However, as seen from the data in fig. 1, polysomes remain disaggregated for at least 40 min after temperature elevation. Thus, one may look for the appearance of cytoplasmic informosomes in the absence of protein synthesis during this time interval.



CHO cells cultured at 37°C were pretreated with 0.05 μg/ml actinomycin D for 30 min to selectively reduce nucleoside incorporation into tRNA and rRNA. Part of the culture was brought to 42°C, and the 37°C and 42°C cultures were exposed to [5-^{3}H]uridine for 40 min (beginning 3 min after culture at 42°C). The cells were harvested, and the cytoplasm was prepared and resolved through sucrose gradients as shown in fig.1 (top, 37°C; bottom, 42°C). Regions of the gradients designated R (ribosomes) and P (polysomes) were taken for isopycnic analysis of formaldehyde-fixed aliquots (fig.2) and sucrose zone sedimentation analysis of sodium dodecyl sulfate–chloroform–phenol extracted RNA (fig.3).

The sedimentation regions occupied by informosomes and polysomes overlap, with informosomes occupying the region from 30S through that occupied

Fig.1. Zone sedimentation analysis of cytoplasm prepared from cells cultured at 37°C, for 3 min at 42°C, and for an additional 40 min at 42°C. One liter of CHO cells at a concentration of 370 000/ml was treated with 50 μg of actinomycin D for 30 min. During this time, the cells were spun down and suspended in 15 ml of medium; at the end of the 30 min period, 5 ml was added to 200 ml of F-10 medium at 37°C, and 10 ml was added to 400 ml F-10 medium at 42°C (both containing 0.05 μg/ml actinomycin D). After 3 min at 42°C, 100 ml was harvested over frozen 0.25 M sucrose, and the cells were washed with cold 0.25 M sucrose and suspended in 1.6 ml buffer (100 mM KCl, 10 mM Tris, pH 7.4 at 25°C, 1.5 mM Mg^{++}, 0.2 mM dithiothreitol). The suspension was frozen and stored at −80°C. Three ml of [5-^{3}H] uridine (Schwarz/Mann, 500 μCi/ml) was added to the remaining 300 ml of 42°C culture after 3.5 min at 42°C, and 2 ml of label was added to the 37°C culture (200 ml). After 40 min, 100 ml of each of the labeled 42°C and 37°C cultures was harvested and treated as above. Upon thawing, the suspended cells were disrupted with Nonidet P–40 and sodium deoxycholate [8], the nuclei were removed by centrifugation, and the cytoplasm was resolved by zone sedimentation through 10–50% sucrose gradients for 3 hr at 25 000 rev/min in an SW 25.2 rotor. Fractionation of the gradients produced the patterns shown.

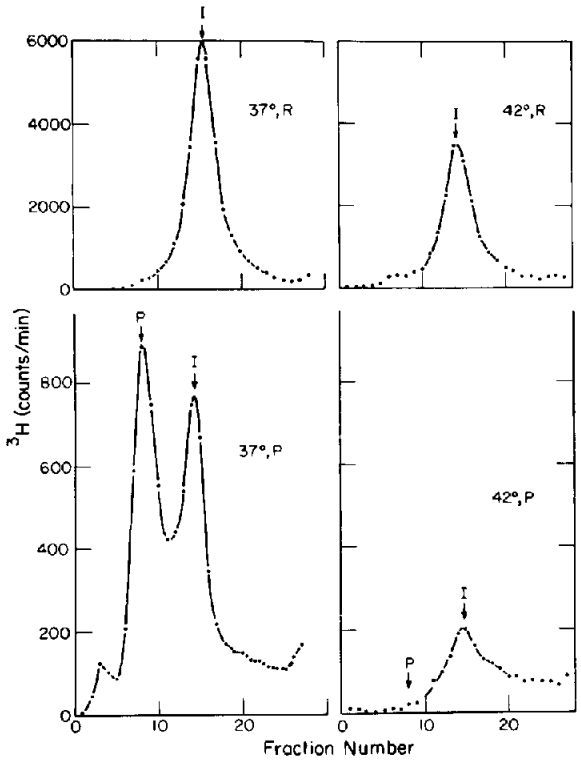


Fig.2. Isopycnic centrifugation of formaldehyde-fixed zone sedimentation fractions R (upper half) and P (lower half). Aliquots (1.6 ml) of sucrose gradient fractions were fixed for 24 hr with one-fourth volume of 30% HCHO in 0.1 M sodium cacodylate (pH 7.6), dialyzed, and then banded in cesium chloride gradients as previously described [8,9].

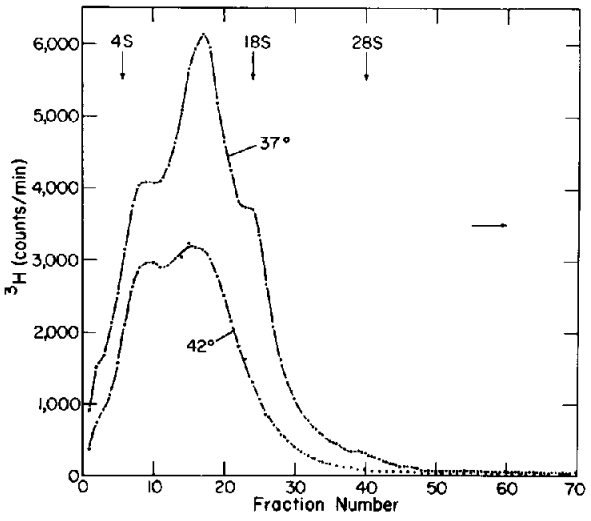


Fig.3. Zone sedimentation of RNA extracted from fraction R (from fig.1). Ten ml each of fraction R of cells labeled at 37°C and at 42°C were precipitated with ethanol, and the ethanol precipitate was dissolved in 0.05 M sodium cacodylate (pH 7.0), extracted with sodium dodecyl sulfate-chloroform-phenol, reprecipitated with ethanol, dissolved in buffer, and resolved on sucrose gradients as before [8].

by polysomes. This is evident in the isopycnic analysis shown in fig. 2. Uridine incorporated into mRNA and ml RNA of normal cytoplasm is found in informosomes (I, $\rho \cong 1.4$) in the region of the sucrose gradient (R), which contains ribosomes and ribosome subunits, and in informosomes and polysomes (P, $\rho = 1.52$) in the polysome (P) region. When the R and P regions of the cytoplasm from cells labelled during culture at 42°C were examined (fig. 2), two facts were observed. First, the cessation of protein synthesis at 42°C is reflected in an absence of label banding at the polysome density. Second, cytoplasmic label appears at the density of informosomes, with no indication of free-RNA (which would appear in the first few fractions). Fig. 3 shows that the incorporated label in RNA that is messenger-like in size distribution and that incorporation at 42°C is approx. one-half the 37°C level. Thus, informosomes appear in the cytoplasm in the absence of initiation of protein synthesis. This result suggests that one need not invoke models for nuclear-to-cytoplasmic transport of informosomes involving protein synthesis as a direct and requisite component.

Acknowledgment

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