Differences in sequence content of nuclear and cytoplasmic polyribosomal RNA from adenovirus-infected cells

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RNA molecules from nuclear and cytoplasmic polyribosomes of adenovirus-infected HeLa cells were compared by hybridization to analyse the sequence content. Nuclear polyribosomes were released by exposure of intact detergent-washed nuclei to poly(U) and purified. Cytoplasmic polyribosomes were also purified from the same cells. To show that nuclear polyribosomes contain ribosomes linked by mRNA, polyribosomes were labelled with methionine and uridine in the presence of actinomycin D in adenovirus-infected cells. Purified nuclear polyribosomes were treated with EDTA under conditions which dissociate polyribosomes into ribosomes and subunits with a simultaneous release of mRNA, and sedimented. The treatment dissociated these polyribosomes, releasing the mRNA from them. Radiolabelled total RNA from each polyribosome population was fractionated in sucrose gradients into several pools or hybridized to intact adenovirus DNA to select virus-specific RNA. Sucrose-gradient-fractionated pool-3 RNA (about 28S) and virus-specific RNA were then hybridized to fragments of adenovirus DNA cleaved by restriction endonucleases *Sma*I, *Hind*III and *Eco*RI by the Southern-blot technique and by filter hybridization. The results showed that nuclear RNA contained sequences, from about 0 to 18 map units, which were essentially absent from cytoplasmic RNA. Furthermore, the amount of virus-specific RNA for a particular sequence was also different in the two populations.

Goidl *et al.* (1975) isolated a unique class of nuclear polyribosomes by treating detergent-washed HeLa nuclei with several polyanions, such as poly(U) or poly(A). These particles were characterized as ribosomal on the basis of their ultrastructure, their sedimentation behaviour and their ability to support protein synthesis when supplemented with S-30 or S-100 extracts from Ehrlich ascites-tumour cells. Nuclear polyribosomes were distributed between 75S and 100S. Various control experiments, including electron microscopy, ruled out contamination of the nuclear preparations with cytoplasmic polyribosomes. Goidl *et al.* (1975) concluded that translatable mRNA was associated with nuclear polyribosomes. The nature of the mRNA molecules was not investigated, however.

Gozes *et al.* (1977) reported the synthesis of tubulin and actin in a cell-free system by nuclear preparations from developing rat brain. Various control experiments and electron microscopy indi-

cated that nuclear preparations were free of nuclear outer membranes and adhering ribosomes. The authors concluded that the polyribosomes which directed protein synthesis were naturally associated with the nuclei and were not cytoplasmic polyribosomes adsorbed as artifacts during isolation. The nature or the complexity of the nuclear mRNA molecules was not studied by Gozes *et al.* (1977).

In a similar study we (Chatterjee *et al.*, 1977; N. K. Chatterjee, C. Tuchowski, G. E. Eagan & T. M. Haley, unpublished work) found a distinct population of polyribosomes containing functional mRNA in adenovirus-infected HeLa-cell nuclei. These polyribosomes were released from detergent-washed intact nuclei by poly(U) or other polynucleotides. They displayed in sucrose gradients a number of peaks in the polyribosome region, with a prominent peak at about 75S, and exhibited a buoyant density of about 1.31 in CsCl gradients. mRNA in these polyribosomes directed protein synthesis efficiently when the polyribosomes were incubated in Ehrlich-ascites-cell S-30 or S-100 extracts at low Mg^{2+} concentrations.

Abbreviation used: SDS, sodium dodecyl sulphate.

The possibility that the nuclear polyribosomes might be cytoplasmic contaminants was ruled out by data from several experiments and electron microscopy. The products of protein synthesis by nuclear polyribosomes were different from the products by cytoplasmic polyribosomes. Only the virus capsid proteins, including p-VII (the precursor to capsid protein VII), but not VII, were synthesized by cytoplasmic polyribosomes. Nuclear polyribosomes, on the other hand, synthesized many proteins, including both p-VII and VII. Thus the conversion of p-VII into VII appeared to occur only in the nuclear population. The observation by Bhatti & Weber (1979) that a proteinase which converts p-VII into VII is present in nuclei of adenovirus-infected HEp-2-cells supports our findings.

Our subsequent studies (Chatterjee *et al.*, 1979; Sarma & Chatterjee, 1980) showed several differences between the RNA and protein moieties of nuclear and cytoplasmic polyribosomes. For example, the distributions and amounts of virus-specific RNA appeared to be different in the two populations. Nuclear virus-specific RNA was considerably less susceptible to ribonucleases. Cyclic AMP-independent protein kinase activity was more than twice as high in nuclear as in cytoplasmic polyribosomes. The endogenous protein substrates for the kinase activity were also different in the two populations.

To characterize these nuclear polyribosomes further, and to understand their possible function, we have analysed the sequence content of adenovirus RNA present in the two polyribosome populations by hybridization. Our results show that nuclear virus-specific RNA contains sequences which are essentially absent from cytoplasmic virus-specific RNA. It appears that nuclear virus-specific RNA may not be completely processed.

Materials and methods

Virus-infected cells: preparation of nuclei and post-nuclear supernatant

Procedures have been described for the growth of HeLa cells (S3) in suspension cultures, the growth of type-2 adenovirus in these cells, and purification of the virus (Maizel *et al.*, 1968), as well as for infecting HeLa cells with 3000–4000 adenovirus particles/cell and preparing detergent (Triton N101)-washed nuclei and the postnuclear cytoplasmic supernatant (Chatterjee *et al.*, 1977).

Labelling, isolation and purification of nuclear and cytoplasmic polyribosomes

HeLa cells were grown in a phosphate-free Joklik-modified F-13 medium (Grand Island Bio-

logical) overnight, infected with the virus, and grown in the same medium. At 16–17h after infection the infected cells were concentrated to about 0.75×10^7 cells/ml of medium and labelled with carrier-free [32 P]P_i (8–10 μ Ci/ml) for 2 or 4h at 37°C. In some experiments the labelling was performed in the presence of actinomycin D (3 μ g/ml). To evaluate the growth and production of adenovirus in these experiments, a small portion of the infected cell culture was incubated for 47h and examined for virus yield. Where indicated, infected cells were also labelled with [3 H]uridine (40 Ci/mmol) and/or [35 S]methionine (1100 Ci/mmol).

The procedure of Goidl *et al.* (1975) was modified (Chatterjee *et al.*, 1977) to release nuclear polyribosomes from the detergent-washed nuclei. Briefly, nuclei (equivalent to 15–20mg of protein) were incubated in 6ml of 10mM-Tris/HCl (pH 7.5)/125mM-sucrose containing 1mg of poly(U) for 10min at 0°C. After low-speed centrifugation (3500g for 10min) the nuclear-residue pellet was discarded, and the supernatant was adjusted to 5mM-magnesium acetate and centrifuged at about 170000g for 2.5h at 2°C. This pellet was used immediately for extraction of RNA. The yield of polyribosomes was about $3.5 A_{260}$ units/ 4×10^8 – 5×10^8 cells. Goidl *et al.* (1975) found poly(U) very efficient in releasing nuclear polyribosomes, probably by disruption of nucleoli and dispersion of chromatin. This effect is similar to a mechanism suggested by other workers (Kraemer & Coffey, 1970) in which polyanions disrupt DNA–histone binding sites to reveal previously masked DNA template sites. According to Goidl *et al.* (1975) and in our own studies, essentially no poly(U) was found in the polyribosome pellet after high-speed centrifugation of the poly(U)-released material.

To isolate cytoplasmic polyribosomes, the post-nuclear supernatant was adjusted to 100mM-KCl, 7mM-magnesium acetate, 50mM-Tris/HCl, pH 7.5, 1mM-dithiothreitol and 0.5% (v/v) Nonidet P40 and homogenized gently in a Dounce homogenizer. The homogenate was centrifuged at 10000g for 15min. Cytoplasmic polyribosomes in the resulting supernatant were pelleted by centrifugation through a 6ml layer of 2M-sucrose containing 100mM-KCl, 7mM-magnesium acetate, 50mM-Tris/HCl, pH 7.5, and 1mM-dithiothreitol at about 150000g for 8h at 2°C.

Extraction of RNA from nuclear and cytoplasmic polyribosomes

To extract RNA, a polyribosomal pellet was suspended in a small volume (1–2ml for nuclear, 5–8ml for cytoplasmic) of 50mM-sodium acetate/(pH 5.0)/100mM-NaCl/5mM-EDTA containing

0.5% (w/v) SDS. RNA was extracted from the resulting mixture with phenol/chloroform/3-methylbutan-1-ol (24:24:1, by vol.) (Anderson *et al.*, 1974). RNA from the aqueous phase was precipitated with 150 mM-NaCl and 3 vol. of ethanol at -20°C overnight. The precipitated RNA was dissolved in autoclaved distilled water.

Denaturation of RNA and sucrose-gradient sedimentation

RNA was dissolved in RNA buffer (10 mM-Tris/HCl, pH 7.5, 20 mM-NaCl, 1 mM-EDTA and 0.5% SDS) and denatured by heating for 5 min at 65°C . This is a satisfactory procedure for denaturation of nuclear and cytoplasmic polyribosomal RNA, comparable with denaturation by dimethyl sulphoxide (Chatterjee *et al.*, 1979). The denatured RNA was centrifuged in a linear 15–30% (w/v) sucrose gradient in the RNA buffer for 18 h at 20000 rev./min in a Spinco SW25.1 rotor at 25°C . Marker RNA species (Chatterjee *et al.*, 1979), denatured, were centrifuged in a parallel gradient. RNA from selected fractions was pooled and precipitated with NaCl and ethanol as above.

Selection of adenovirus-specific RNA by RNA–DNA hybridization; cleavage of DNA by restriction endonucleases and Southern blot

Adenovirus was purified by at least two CsCl-gradient centrifugations (Maizel *et al.*, 1968) and dialysed against 10 mM-Tris/HCl, pH 8.1, at 2°C to extract DNA, by a published procedure (Bello & Ginsberg, 1969). Hybridization between RNA and alkali-denatured DNA (5 μg /filter) was performed by the procedure of Gillespie & Spiegelman (1965). On each filter 20–30 μg of ^{32}P -labelled polyribosomal RNA was hybridized for 20 h at 66°C . The filter was then washed exhaustively, as follows: (i) five washes (5 ml each) with $2\times\text{SSC}$ (300 mM-NaCl/30 mM-sodium citrate, pH 7.2) containing 0.1% SDS at room temperature; (ii) three washes with $0.2\times\text{SSC}$ containing 0.1 SDS at 65°C ; (iii) one wash with $0.2\times\text{SSC}$ only at 65°C ; (iv) one wash with 2 mM-EDTA only at room temperature; and (v) one wash with distilled water. This procedure removes all of the non-specificity adhering RNA from DNA filters (N. K. Chatterjee, C. Tuchowski, G. E. Eagan & T. M. Haley, unpublished work).

The washed filters were cut into 1 mm pieces in a small volume of distilled water containing yeast tRNA (15 $\mu\text{g}/\text{ml}$) as carrier, boiled at 100°C for 5 min, quenched rapidly, and centrifuged at 3000 rev./min for 10 min. The supernatant was kept. The residual pellet was boiled twice more as above with small amounts of water and tRNA solution, and then re-centrifuged. The supernatants were combined, and RNA was precipitat-

ed with NaCl and ethanol. This boiling procedure released more than 70% of the hybridized RNA from the filters. The hybrid-selected RNA was highly sensitive to ribonuclease, which indicated that essentially no RNA–DNA hybrids remained in the preparation.

Fragments of adenovirus DNA for Southern blot and filter hybridization were prepared by digestion with restriction endonucleases *Sma*I, *Hind*III and *Eco*RI, followed by electrophoresis in 1% (w/v) agarose (Sugden *et al.*, 1975). The fragments were immobilized on a nitrocellulose membrane and hybridized to different RNA populations by the Southern-blot technique (Southern, 1975). The hybrids were examined by autoradiography.

For filter hybridization, agarose-gel slices containing DNA fragments were crushed in a small volume of 10 mM-Tris/HCl (pH 7.9)/1 mM-EDTA, incubated for 2 h at 37°C , and then centrifuged at 4000 rev./min for 30 min. To precipitate DNA with ethanol, the supernatant containing DNA was adjusted to 50 mM-Tris/HCl (pH 9.0)/200 mM-NaCl and extracted with 88% (v/v) phenol in the above buffer. The fragments immobilized on each filter were equivalent to about 3 μg of whole DNA.

Results

Kinetics and extent of labelling of polyribosomes

To obtain a suitable labelling protocol, we analysed first the pattern of labelling of total RNA in nuclear or cytoplasmic polyribosomes. In this pilot experiment adenovirus-infected whole cells at 18–20 h after infection were labelled with [^3H]-uridine (3 $\mu\text{Ci}/\text{ml}$) for 2, 3 or 5 h. The nuclear or cytoplasmic polyribosomes were purified, and acid-precipitable radioactivity in these polyribosomes was measured. Incorporation of radioactivity increased more or less linearly with the increase in time of labelling (results not shown). However, less radioactivity was consistently recovered from the nuclear than from the cytoplasmic polyribosomes.

To show that nuclear polyribosomes contain ribosomes linked by mRNA, adenovirus-infected cells were labelled with [^{35}S]methionine (1.3 $\mu\text{Ci}/\text{ml}$). At 18–20 h after infection, the cells were concentrated to about 1.6×10^7 cells/ml and labelled with [^3H]uridine (20–25 $\mu\text{Ci}/\text{ml}$) for 2 h in the presence of actinomycin D. The nuclear or cytoplasmic polyribosomes were purified. Half of the nuclear polyribosomes were treated with EDTA under conditions that dissociate polyribosomes into ribosomes and subunits (Martin *et al.*, 1969) with a simultaneous release of mRNA. Fig. 1 shows the sedimentation profiles of the nuclear polyribosomes in sucrose gradients before (a) and after (b) EDTA treatment. The treatment dis-

Fig. 1. Sedimentation of nuclear polyribosomes before and after EDTA treatment

Equivalent amounts of [^{35}S]methionine- and [^3H]-uridine-labelled polyribosomes were treated with buffer containing 50 mM-Tris/HCl, pH 7.8, 10 mM-KCl and 12.5 mM-MgCl₂ (a) or exposed to 20 mM-EDTA in the above buffer with 80 mM-KCl (b) and analysed by sedimentation in linear 15–30% (w/v) sucrose gradients with a 0.5 ml 2 M-sucrose cushion. The gradient solutions were made in the above buffer with low salt for (a) or with high salt and without MgCl₂ for (b). The samples were centrifuged for 2.5 h at 38000 rev./min in a Spinco SW40 rotor at 4°C. A sample from each fraction was used to measure A_{260} and to count acid-precipitable radioactivity.

sociated these polyribosomes, releasing the mRNA from them (note the decrease in A_{260} and uridine radioactivity in fractions 1–9 in b). Treatment of the cytoplasmic polyribosomes with EDTA also produced essentially the same effect (results not shown). The yield of mRNA from nuclear polyribosomes of 2×10^9 cells was approx. 1.67×10^6 c.p.m., compared with 13.3×10^6 c.p.m. from cytoplasmic polyribosomes.

RNA was labelled with [^{32}P]P_i for 2 or 4 h. The specific radioactivity of the nuclear RNA labelled for 4 h (after correction for a trace amount of DNA contamination) was 4800–6000 c.p.m./μg, com-

Fig. 2. Sedimentation of denatured total RNA from nuclear (a) and cytoplasmic (b) polyribosomes

A sample of each RNA from ^{32}P -labelled adenovirus-infected HeLa cells was denatured by heating and centrifuged in a linear 15–30% (w/v) sucrose gradient containing a low salt concentration (see the Materials and methods section). A sample from each fraction was counted for acid-precipitable radioactivity. RNA from a number of equivalent fractions from each gradient was pooled (as shown by bars) and precipitated. Marker RNA species labelled with [^3H]uridine were mixed, denatured, and centrifuged in a parallel gradient.

pared with 10000–12000 c.p.m./μg with cytoplasmic RNA. The reason for this difference in specific radioactivity is not clear. The nucleus is known to contain RNA of different metabolic stabilities (Herman & Penman, 1977). Possibly the nuclear and cytoplasmic polyribosomes contained RNA of different metabolic stabilities, and their rates of association with the two polyribosome populations may have been different, which could account for the difference in specific radioactivity.

Sequence analysis

The difference in the composition of nuclear and cytoplasmic polyribosomal RNA was analysed by using two types of RNA: sucrose-gradient-fractionated total polyribosomal RNA, and adenovirus-specific RNA. Total RNA extracted from purified nuclear or cytoplasmic polyribosomes from ^{32}P -labelled virus-infected whole cells in the

presence of actinomycin D was denatured and sedimented in sucrose gradients (Fig. 2). RNA from a number of equivalent fractions from each gradient was pooled and precipitated with ethanol. Pool 1 contained >45S RNA; pool 2, >28S; pool 3, about 28S; pool 4, about 18S; and pool 5, about 4S. Adenovirus mRNA consists of more than 12 species, from >30S to about 9S, most of which are derived from a single transcription unit between map units 16.45 and 99 on the genome (Parsons *et al.*, 1971; Lindberg *et al.*, 1972; Bachenheimer & Darnell, 1975; Weber *et al.*, 1977; Nevins & Darnell, 1978). The major late promoter at 16.45 map units is not used by some early mRNA species of the virus; their synthesis continues even in the late period (Spector *et al.*, 1978). These species should be distributed in pools 2–4.

RNA from these pools were analysed by T_1 -ribonuclease oligonucleotide fingerprinting, and those from nuclear pool 3 and cytoplasmic pool 3 by hybridization. The data from hybridization experiments are presented in this paper.

Southern-blot hybridization

In the first set of experiments, ^{32}P -labelled nuclear or cytoplasmic RNA from pool 3 and adenovirus-specific RNA (selected by prior hybridization of total polyribosomal RNA to virus DNA) were hybridized by the Southern-blot technique to fragments of virus DNA cleaved by restriction endonucleases *Sma*I and *Hind*III. These enzymes cleave adenovirus DNA at several sites (Fig. 3a; see Dunn & Hassell, 1977), generating several fragments (A–M with *Sma*I, A–L with *Hind*III) of decreasing size (Figs. 3a and 3b, lanes 1 and 6). Fragment A is the largest; fragment L is the smallest. Fragments J and K with *Sma*I and G and K with *Hind*III represent the two ends of the molecule. Fragments L and M with *Sma*I and L with *Hind*III, being extremely small, were not detected in the gel system used. Restriction endonuclease *Eco*RI generates six fragments (A–F) of decreasing size after cleavage of the DNA (Fig. 3a).

Nuclear RNA hybridized with almost all of the fragments (*Sma*I A–I and *Hind*III A–J) representing the entire genome (Fig. 3b, lanes 2, 4, 7, 9). Since *Hind*III fragments F and G (lane 6) could not be resolved well, the data did not clearly indicate hybridization with the terminal fragment G. However, hybridization of nuclear RNA with this terminal segment of the genome was evident from the results with *Sma*I fragment E (lanes 2, 4), which contains most of the sequences of *Hind*III fragment G. Cytoplasmic RNA, on the contrary, hybridized with all fragments except *Sma*I fragments E and F (lanes 3, 5) and *Hind*III fragments C and probably G (lanes 8, 10), which represent about 18% of the genome. These results suggest

that nuclear polyribosomal RNA contains sequences from a section of the genome, comprising about 18 map units, which are not present in cytoplasmic polyribosomal RNA.

Filter hybridization

To examine further whether sequences from a section of adenovirus genome are missing from cytoplasmic RNA and whether the amount of virus-specific RNA for a particular sequence is different in the two populations, the next experiment was performed. [^3H]Uridine-labelled pool-3 RNA was hybridized to various restriction fragments by filter hybridization. The fragments were *Sma*I J, E, F, D, H and C, *Hind*III C; and *Eco*RI F.

The results (Table 1) show that, compared with nuclear RNA, very little cytoplasmic RNA hybridized with *Sma*I fragments J, E and F and *Hind*III fragment C, which represent about 18 map units from one end of the genome. Although nuclear RNA hybridized well with *Sma*I fragment J in this experiment, no such hybridization was observed in the experiment shown in Fig. 3(b). Perhaps not enough *Sma*I fragment-J DNA was present on the nitrocellulose filter to detect this hybridization. Quantitative transfer from an agarose gel to a nitrocellulose filter is a difficult way to obtain a fragment as short as J. Significantly more cytoplasmic RNA hybridized with *Eco*RI fragment F, map units 70.7–75.9. Finally, hybridization of nuclear RNA was slightly higher with *Sma*I fragments H and C. These results support the conclusions that (i) nuclear RNA contains sequences, from about 0 to 18 map units, which are essentially absent from cytoplasmic RNA, and (ii) the amount of virus-specific RNA for a particular sequence is different in the two populations.

Table 1. Hybridization of pool-3 RNA from nuclear and cytoplasmic polyribosomes with various restriction fragments

Uridine-labelled (2h) RNA was hybridized with DNA fragments on nitrocellulose filters and with blank filters (see the Materials and methods section). Input RNA (c.p.m./filter): nuclear, 15750; cytoplasmic 15440.

DNA fragment	Nuclear-hybridized		Cytoplasmic-hybridized	
	(c.p.m.)	(%)	(c.p.m.)	(%)
<i>Sma</i> I J	320	2.03	20	0.13
E	595	3.77	42	0.27
F	442	2.80	124	0.80
D	517	3.28	573	3.71
H	423	2.68	379	2.45
C	685	4.34	610	3.95
<i>Hind</i> III C	769	4.88	113	0.73
<i>Eco</i> RI F	209	1.32	377	2.44

Fig. 3. Analysis of hybrids formed between nuclear or cytoplasmic polyribosomal RNA and *SmaI* and *HindIII* fragments of adenovirus DNA by the Southern-blot technique

^{32}P -labelled RNA (labelled for 2 h) from each population was hybridized with a series of nitrocellulose filter strips containing immobilized DNA fragments. The strips containing the hybrids were autoradiographed. (a) Restriction-enzyme maps showing cleavage positions of adenovirus DNA by the nucleases used (see Dunn & Hassell, 1977). (b) Photographs of ethidium bromide-stained DNA fragments after agarose-gel electrophoresis (lanes 1 and 6) and autoradiograms (lanes 2-5, 7-10).

Discussion

We have demonstrated that the sequence complexity of adenovirus RNA present in poly(U)-released nuclear polyribosomes exceeds that present in cytoplasmic polyribosomes. The results of the hybridization experiments with restriction fragments of the viral DNA indicate that nuclear RNA contains sequences from almost all of the genome, whereas cytoplasmic RNA contains sequences from most of the genome except a short segment of about 18 map units at one end.

The possibility seems unlikely that the more complex profile seen with nuclear polyribosomal RNA was due to a mixture of infected and non-infected cells, since the cytoplasmic polyribosomal RNA, used in parallel experiments, was isolated from the same preparations. It is also unlikely that sequences specific to nuclear RNA arose through contamination, by aggregation or non-specific association with nuclear polyribosomes, since these polyribosomes were purified sedimenting through sucrose solution. Also, the observation by Goidl *et al.* (1975) and by us (Chatterjee *et al.*, 1979) that essentially no poly(U), a polyanion similar to RNA in many respects, was found to associate with nuclear polyribosomes after high-speed centrifugation (see the Materials and methods section) argues against this notion of contamination.

Adenovirus late mRNA species are generated from precursor RNA molecules by multiple splicing, followed by intramolecular joining of short sequences which are not contiguous on the viral DNA (see Klessig, 1977). The short sequences at positions 16.6, 19.6 and 26 on the unit map are spliced on to the body of the mRNA to form a tripartite leader (Berget *et al.*, 1977; Chow *et al.*, 1977). On the basis of these studies, our finding of hybridization of processed cytoplasmic RNA to DNA sequences distal to map unit 18 is expected. However, the hybridization of nuclear polyribosomal RNA with almost all of the genome, an observation which cannot be satisfactorily explained, suggests that these molecules may not be completely processed, and therefore may not function as cytoplasmic mRNA. Since only about one-fifth of the nuclear RNA is transported into the cytoplasm (Flint & Sharp, 1976), existence of such unprocessed or partially processed RNA in the nucleus is not unlikely.

The function of nuclear polyribosomes is not fully understood. Our observation of synthesis *in vitro* of both p-VII and VII proteins by nuclear polyribosomes, as well as the observation by Bhatti & Weber (1979) that a proteinase which converts p-VII into VII is nuclear in origin, show that nuclear polyribosomes take part in protein process-

ing. It is also possible that they synthesize nuclear-matrix proteins (Mariman *et al.*, 1982; Younghusband & Maundrell, 1982) implicated in processing of heterogeneous nuclear RNA (van Eekelen & van Venrooij, 1981). Experiments are required to investigate this possibility.

Finally, poly(U)-released polyribosomes and the cell-free system of protein synthesis may be of general use for development of a coupled system for processing of heterogeneous nuclear RNA and protein synthesis.

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