Nature of the Facilitated Messenger Ribonucleic Acid Transport from Isolated Nuclei

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Cytoplasmic macromolecules were previously identified which regulate both qualitatively and quantitatively the release of messenger-like RNA from isolated nuclei. These macromolecules are now shown to be denatured at 45–50°C and their synthesis is sensitive to pactamycin or cycloheximide. The putative regulatory proteins are essentially quantitatively precipitated with high specificity from the cytosol by streptomycin at a concentration 10-fold higher than that used to precipitate RNA. The nuclear concentration-dependence of RNA transport from successive samples of nuclei strongly suggests that the regulatory factors are recycled. Quantitative changes in the sequences transported at various dilutions of the cytosol suggest that not all the different classes of the putative regulatory macromolecules are present in an effective concentration at any one dilution.

Earlier papers from this laboratory (Schumm & Webb, 1972, 1974a,b; Schumm *et al.*, 1973a,b) described a cell-free system which supported the release of mRNA from isolated nuclei under conditions approximating to those of the intact cell. Studies utilizing this system, which consisted of pre-labelled nuclei in fortified homologous or heterologous cytosol (i.e. surrogate cytoplasm), indicated that the release of mRNA in informosome (i.e. ribonucleoprotein) particles had an absolute requirement for both ATP and cytoplasmic macromolecules. Within the limits of the hybridization procedures used, the RNA sequences transported to homologous cytosol were qualitatively similar to those transported *in vivo*, whereas those transported to heterologous cytosol were not.

The present investigation is directed towards elucidating the mechanism whereby cytoplasmic macromolecules facilitate the release of messenger-like RNA from isolated nuclei on the assumption that this phenomenon represents a basic nucleocytoplasmic control of eukaryotic cell function. The apparent qualitative differences in the RNA transported from normal liver nuclei in response to surrogate cytoplasm from normal and neoplastic liver (Schumm *et al.*, 1973a,b) is particularly intriguing in view of the reported modification of nuclear RNA restriction in the cancer cell (Shearer, 1974). Here we report on the nature of the nucleocytoplasmic controls responsible for the phenomenon of nuclear RNA restriction, including the observation that the regulatory factors, which appear to be proteins, are recycled.

Materials and Methods

Preparation of subcellular fractions

Nuclei and cytosol were prepared from the livers of 250 g male rats of the Sprague–Dawley strain (Laboratory Supplies, Indianapolis, IN, U.S.A.). The rats were maintained on Purina chow, but deprived of food 15 h before use. Nuclei for the cell-free system were purified from liver homogenate by 60 min centrifugation of the homogenate through concentrated sucrose (Schumm & Webb, 1972; Muramatsu & Busch, 1967). The nuclear RNA was pre-labelled *in vivo* by the intraperitoneal injection of 60 μ Ci of [6-¹⁴C]orotic acid (specific radioactivity 323 μ Ci/mg; New England Nuclear Corp., Boston, MA, U.S.A.) 30 min before the removal and processing of the liver. Cytosol, which contained the transport factors, was prepared (Schumm & Webb, 1972; Schumm *et al.*, 1973b) from a 1:3 (w/v) homogenate by centrifuging the latter at 105000g for 90 min, then dialysing the supernatant against buffer [50 mM-Tris/HCl (pH 7.6)/25 mM-KCl/2.5 mM-MgCl₂] at 4°C for 16 h.

Components of the cell-free system

The standard cell-free system developed to support RNA transport from isolated nuclei with near-normal nuclear restriction (Schumm & Webb, 1974b) consisted of 4 $\times$ 10⁶ pre-labelled nuclei/ml (5123 c.p.m./10⁶ nuclei) in medium containing 12 mg of dialysed cytosol protein/ml, 50 mM-Tris/HCl (pH 7.5), 25 mM-KCl, 2.5 mM-MgCl₂, 0.5 mM-CaCl₂, 0.3 mM-MnCl₂, 5.0 mM-NaCl, 2.5 mM-Na₂HPO₄, 5.0 mM-spermidine, 2.0 mM-

dithiothreitol, 2.0mM-ATP, 2.5mM-phosphoenolpyruvate, 35 units (1 unit is the conversion of 1 μ mol of substrate/min at pH7.6 and 37°C) of pyruvate kinase (EC 2.7.1.40) and 300 μ g of yeast RNA/ml. The labelled RNA transported as nucleoprotein during the standard incubation at 30°C for 30min has the properties of mRNA (Schumm & Webb, 1972, 1974a,b).

In some experiments the concentration of nuclei and/or cytosol was varied from those specified below. Where indicated the length of the incubation period was also modified.

The messenger-like RNA released from the nuclei was precipitated from the nuclei-free incubation medium (Schumm & Webb, 1972) with 5% (w/v) trichloroacetic acid; the precipitate was washed with ethanol, then solubilized and assayed for radioactivity in liquid scintillant (Schumm & Webb, 1974b). Protein concentrations were determined with biuret reagent (Schumm & Webb, 1972) and the concentration of nuclei was determined in a counting chamber. All procedures, except for the incubations, were carried out rapidly at 0–4°C.

Serial incubation of nuclei

Nuclei undiminished in their capacity to process and transport RNA were recovered after the initial incubation in fortified cytosol by layering the incubation mixture over an equal volume of 1.0M-sucrose/1.0mM-calcium acetate, then centrifuging at 3000g for 5min. The nuclei were gently resuspended in 1.0M-sucrose/1.0mM-calcium acetate for reincubation in fresh fortified cytosol.

Heat- and streptomycin sulphate-treatment of the cytosol

Portions of the cytosol prepared directly from the 1:3 (w/v) homogenate were preincubated at either 40° or 50°C for 5min and the precipitate was removed by centrifuging at 3000g for 5min. Equivalent portions of the control and heat-treated samples were tested in the standard transport system, the final cytosol protein concentration in the cytosol being 12mg/ml.

The transport factors were precipitated from the cytosol by adding either 0.05 or 0.5 vol. of neutralized 10% (w/v) streptomycin sulphate to the cytosol prepared from the 1:3 (w/v) homogenate. The mixture was stirred for 30min at 4°C, then centrifuged at 15000g for 30min. The resulting supernatant was dialysed for 15h at 4°C against 0.05M-Tris/HCl buffer, pH7.5, to remove excess of streptomycin.

DNA-RNA competitive hybridization

For the preparation of the RNA, labelled or unlabelled nuclei were incubated in the fortified incuba-

tion medium containing 4 or 12mg of cytosol protein/ml for 30min at 30°C. After removal of the nuclei by centrifugation the supernatant was extracted with 3 $\times$ 1 vol. of phenol/chloroform (1:1, v/v) and the RNA in the aqueous layer was precipitated with ethanol. Hybridization reaction mixtures contained 10 μ g of rat liver DNA (Schumm & Webb, 1974b). The RNA-DNA hybridization was carried out with labelled RNA from the equivalent of 1.0ml of transport reaction and where indicated unlabelled RNA was obtained from the equivalent of a 20ml transport reaction. Although the RNA concentration could not be estimated, owing to the presence of yeast RNA used to suppress the small amount of ribonuclease activity not sequestered by the natural liver inhibitor (Schumm & Webb, 1972), hybridization curves were run to ensure that the latter had essentially reached a plateau. Hybridizations were carried out in 0.3ml of 0.12M-sodium phosphate buffer, pH6.8, 0.001M-EDTA and 0.1% sodium dodecyl sulphate at 65°C for 18h. The reactions were analysed by trapping the duplex molecules on HA Millipore filters. The filters were thoroughly washed with water and dried, then counted for radioactivity in liquid scintillant (Schumm & Webb, 1974b).

Results

Dependence of mRNA release on the concentration of nuclei

In all of the following experiments the nuclear RNA in rat liver nuclei was pre-labelled for 30min with [14 C]orotic acid, before isolation and incubation of the nuclei in fortified liver cytosol. Under these conditions over 85% of the labelled nuclear RNA released during the first 30min of incubation at 30°C was messenger-like, with detectable labelled rRNA being transported beyond 45min of incubation (Schumm & Webb, 1972; Racevskis & Webb, 1974). Putative mRNA transport is expressed as percentage of total nuclear radioactivity (c.p.m.) released as RNA.

Fig. 1 shows the time-course of release of messenger-like RNA from liver nuclei to surrogate cytoplasm at nuclear concentrations 4×10^6 and 8×10^6 /ml and at a cytosol protein concentration of (a) 4.0mg/ml and (b) 12.0mg/ml. Incubation medium containing 12.0mg of cytosol protein/ml supported the release of more than twice as much labelled RNA as medium containing 4mg/ml, at a nuclear concentration of 4×10^6 /ml. In both instances the putative mRNA transport was essentially complete after 20–30min incubation; the release of labelled rRNA became detectable 15–20min later if extra phosphoenolpyruvate is supplied (Schumm & Webb, 1972; D. E. Schumm & T. E. Webb, unpublished work).

A comparison of the kinetics of RNA release indicates that they are very similar at the two nuclear

Fig. 1. Time-course of labelled RNA release as a function of nuclear and cytosol protein concentration

Cytosol protein in medium is (a) 4 mg/ml and (b) 12 mg/ml. ●, 4×10^6 nuclei/ml; ○, 8×10^6 nuclei/ml. The composition of the cell-free system and the conditions of incubation are described in the Materials and Methods section.

Fig. 2. Labelled RNA release as a function of nuclear concentration at two concentrations of cytosol protein

●, Cytosol protein 12 mg/ml, incubated 30 min; ■, cytosol protein 12 mg/ml, incubated 60 min; ▲, cytosol protein 4 mg/ml, incubated 30 or 60 min. Other conditions as for Fig. 1.

concentrations when the cytosol protein concentration was 4 mg/ml, but markedly different when the latter was 12 mg/ml. Thus when the concentration of nuclei was increased from 4×10^6 to 8×10^6 /ml in medium containing 12 mg of protein/ml the total mRNA release after 30 min incubation was decreased by 33%. Further, this decrease is directly attributable to a lower rate of release, equivalent amounts of RNA being released at both nuclear concentrations if the incubation period was extended to 60 min. The addition of further 2.5 mM-phosphoenolpyruvate after

10 and 20 min incubation did not influence the time-course of release up to 30 min (D. E. Schumm, unpublished work), ruling out the possibility that the slower release at 8×10^6 nuclei/ml was due to a depletion of the energy supply. However, as shown previously (Racevskis & Webb, 1974) the extra energy supported significant transport of labelled rRNA beyond 45 min incubation; no labelled rRNA is transported up to 60 min when phosphoenolpyruvate and ATP is added only at the beginning of the incubation period.

Fig. 2 shows labelled RNA transport as a function of nuclear concentration at the two concentrations of cytosol protein. The results confirm that RNA release during the initial 30 min incubation to medium containing 12 mg of protein/ml falls significantly (i.e. 35% decrease at 10^7 nuclei/ml), as the concentration of nuclei is increased. However, this fall is nullified when the incubation period is increased to 60 min.

The data in Figs. 1 and 2 are consistent with the theory that the transport factors are recycled and consequently at high nuclei/cytosol protein ratios their interaction with each nucleus becomes the rate-limiting step. The apparent independence of RNA transport of nuclear concentration at 4 mg of cytosol protein/ml suggests that there are multiple classes of transport proteins and that some of these are below a critical physiologically effective concentration at the lower cytosol protein concentration. In this respect it is clear that increasing the nuclei/protein ratio by holding the cytosol protein at 12 mg/ml while increasing the nuclear concentration threefold is not equivalent to achieving the same ratio by diluting the cytosol threefold. When the nuclei are increased the rate of release is lower, with no decrease in the amount of RNA transported, whereas when the cytosol is

Table 1. *Sequence homology of RNA molecules transported in response to two concentrations of cytosol proteins*

Standard transport assays were carried out with either labelled or unlabelled nuclei and the indicated concentration of cytosol in the incubation medium. The transported RNA was extracted with phenol/chloroform and precipitated with ethanol. Hybridization-competition reactions were carried out in 0.012M-sodium phosphate buffer, pH 6.8, 0.001M-EDTA, 0.1% sodium dodecyl sulphate and 10 µg of DNA at 65°C for 18 h. Hybrids were analysed by trapping the duplex molecules on Millipore filters, followed by radioassay. A background of 10–12 c.p.m. was subtracted from all samples. The percent inhibition of hybrid formation between DNA and labelled RNA by unlabelled competitor RNA is shown in parentheses. The data represent the average of two experiments which agreed to within 10%.

Unlabelled competitor RNA	Labelled RNA release by cytosol protein measured as radioactivity in hybrid (c.p.m.)	
	12mg/ml	4mg/ml
None (control)	297 (0)	242 (0)
Released by 12mg of cytosol protein/ml	14 (95)	12 (94)
Released by 4mg of cytosol protein/ml	103 (65)	7 (97)

diluted, both the rate of transport and the amount of RNA transported are decreased.

The results of DNA-RNA competitive-hybridization experiments (tabulated in Table 1) support the theory that many of the sequences of RNA transported at 12mg of cytosol protein/ml are not transported at 4mg/ml. Owing to the low amount of ¹⁴C-labelled RNA released it was possible to measure primarily only the reiterated sequences, which represent an unknown fraction of the total mRNA sequences. Thus, although 94% of these sequences released into the medium containing 4mg of protein/ml are also released into medium containing 12mg/ml, only 65% of the total sequences released into medium containing 12mg/ml are also released at 4mg/ml. Another undetermined parameter is the amount of unlabelled RNA released from the nuclei which may act as competitor. Despite the indicated limitations, these results, together with evidence for the specificity of the transport proteins for different RNA sequences (Schumm *et al.*, 1973a,b), support the proposal that a significant fraction of the transport proteins are below an effective concentration when the cytosol protein is decreased to 4mg/ml of incubation medium.

Analysing RNA transport by serial incubation of nuclei

Experiments were designed to establish further that putative regulatory proteins for mRNA transport

Table 2. *Serial incubation of fortified cytosol with fresh nuclei as a test for the recycling of transport factors*

Pre-labelled nuclei (4×10^6 /ml) were incubated in fortified medium, containing either 4 or 12mg of cytosol protein/ml, for 20 min at 30°C. The nuclei were removed by centrifugation at 500g for 5 min before the addition of an equal number of fresh nuclei, after which the incubation was continued for an additional 20 min. Samples of the medium freed of nuclei (3000g for 5 min) were analysed after the first and second incubations to estimate the net percentage of nuclear radioactivity released as acid-precipitable RNA during each 20 min incubation period. Before the second incubation the medium was supplemented with 2.0mm-ATP and 2.5mm-phosphoenolpyruvate. The data represent the average of two experiments which agreed to within 10%.

Cytosol protein in medium	Percentage of nuclear radioactivity released	
	First incubation	Second incubation
4mg/ml	2.3	2.5
12mg/ml	4.2	4.5

were recycled, rather than sequestered, for example, through permanent association with RNA, during active RNA transport. Thus nuclei were incubated for 30 min at 30°C in fortified cytosol, after which the used cytosol was recovered for reincubation with fresh nuclei. Since the energy supply was depleted during the initial incubation, before the second incubation with fresh nuclei the medium was supplemented with 2.0mm-ATP and 2.5mm-phosphoenolpyruvate. The results in Table 2 are consistent with the recycling of the transport factors, the amount of putative messenger transported during the first and second incubations being essentially equal. In addition to indicating that the factors are not sequestered, the results further confirm that the cessation of transport after 20–30 min incubation is not due to a depletion of transport factors.

In a second series of experiments, essentially the reverse of those outlined above, nuclei (5×10^6 /ml) were incubated for 20 min in a medium containing either 4 or 12mg of cytosol protein/ml, then transferred to fresh medium for reincubation. The maximum amount of labelled RNA released during each 20 min incubation period was estimated and the results are summarized in Table 3. In all experiments the time-course of release of messenger-like RNA was measured to ensure that transport was complete (i.e. release had ceased) within the 20 min incubation period. When nuclei were first incubated in media containing 4mg of cytosol protein/ml, then transferred to media containing 4 or 12mg/ml, 50 and 100% respectively of additional labelled RNA was released during the second incubation compared with

Table 3. *Reincubation of nuclei in fresh cytosol as a test for completeness of transport of potential mRNA*

Pre-labelled nuclei were incubated for 20 min at 30°C in media containing 4 or 12 mg of cytosol protein/ml; the nuclei recovered by centrifugation through 1.0 M-sucrose were then reincubated for an additional 20 min in fresh media containing either 4 or 12 mg of cytosol protein/ml. The data represent the percentage of nuclear radioactivity released to the incubation medium during each incubation. Blanks of 1 and 0.2% have been subtracted from the observed transport during the first and second incubations respectively. The data shown represent the average of two experiments which agree to within 10%.

First incubation		Second incubation	
Cytosol protein (mg/ml)	RNA transport (%)	Cytosol protein (mg/ml)	RNA transport (%)
4	2.1	4	0.8
		12	2.1
12	4.5	4	0.5
		12	1.0

the first incubation. A third incubation was impractical owing to the release of significant amounts of rRNA. Conversely, when the initial incubation was in media containing 12 mg of cytosol protein/ml, reincubation in media containing 4 or 12 mg/ml led to additional release of only 22 and 13% respectively. The results suggest that a significant amount of potential mRNA, normally transported at 12 mg of cytosol protein/ml, is not transported when the cytosol protein concentration is lowered to 4 mg/ml.

Protein nature of putative transport factors

The size, stability to storage and behaviour on salt fractionation suggested that the cytosol factors were proteins (Schumm *et al.*, 1973a). As indicated in Table 4, either cycloheximide (Sigma Chemical Co., St. Louis, MO, U.S.A.) or pactamycin (Upjohn Co., Kalamazoo, MI, U.S.A.), two inhibitors of protein synthesis in eukaryotic cytoplasm (Grollman & Huang, 1973), markedly decreased the capacity of the cytosol to support RNA transport *in vitro*. In these experiments the rats received 1.0 mg of cycloheximide or pactamycin/kg at 5.0 h and again at 2.5 h before removal of the liver for the standard preparation of the cytosol. The capacity of the cytosol for transport was tested in the cell-free system at a dialysed cytosol protein concentration of 12 mg/ml and the duration of the incubation was 30 min.

It is important to note that the cycloheximide and pactamycin pretreatment led to a 33 and 55% fall respectively in the capacity of the cytosol to support RNA transport, despite the fact that such pretreatment did not detectably alter the cytosol protein

Table 4. *Effect of pretreating rats with inhibitors of protein synthesis on the capacity of the cytosol to support RNA transport*

Rats were given intraperitoneal injections of either 0.9% NaCl (controls) or 1.0 mg of cycloheximide or pactamycin/kg at 5.0 h and again at 2.5 h before removal of the liver for cytosol isolation. The cytosol was tested at a protein concentration of 12 mg/ml. The data are expressed as percentage of total nuclear radioactivity transported as labelled RNA and represent the average of three or more rats $\pm$ S.E.M. The protein concentration of the cytosol as prepared was the same for the cytosol from both control and treated rats, i.e. 30 mg/ml.

Pretreatment of cytosol donors	Percentage of nuclear radioactivity transported	Decrease (%)
None (control)	4.5 $\pm$ 0.13	0
Cycloheximide	3.0 $\pm$ 0.29	33
Pactamycin	2.2 $\pm$ 0.28	51

Table 5. *Effect of heat- or streptomycin sulphate-treatment of the cytosol on its capacity for RNA transport*

Samples of the cytosol were treated by either heating or the addition of neutralized 10% streptomycin sulphate with stirring at 4°C. After removal of the precipitates the supernatants were dialysed for 16 h, before assay. The control cytosol was tested at a protein concentration of 12 mg/ml and after correction for changes during treatment, an equivalent volume of the heat- or streptomycin-treated supernatants was tested in the assay. The results represent the average of two experiments which agreed to within 10%.

Treatment of cytosol	Percentage of nuclear radioactivity transported	Decrease (%)
Control	4.3	0
Heat		
40°C for 5 min	1.6	63
50°C for 5 min	1.0	77
Streptomycin sulphate		
0.5%	3.3	23
5.0%	1.0	77

concentration. The significant decrease in transport activity in the absence of a change in total cytoplasmic protein emphasizes the high degree of specificity of the transport phenomenon.

The heat-stability of the transport factors is also consistent with their protein nature. As was shown by the results in Table 2, incubation of the transport system for 20 min at 30°C had no effect on the ability of the fortified cytosol to support further transport as long as the energy supply was replenished. However, as indicated in Table 5 the transport activity of the cytosol was decreased by 59 and 74% by preincubating

the cytosol for 5 min at 40° and 50°C respectively before testing it in the cell-free system.

The effect of pretreating the cytosol with streptomycin sulphate, also shown in Table 5, is particularly noteworthy. The pretreatment of the cytosol with 0.5 vol. of neutralized 10% streptomycin sulphate, followed by removal of the precipitate and dialysis, led to a 75% fall in the transport activity. Only a slight fall in activity was detected after treatment with 0.05 vol. of 10% streptomycin sulphate, which is optimal for the removal of RNA during enzyme purification (Krystal & Webb, 1971). Although the higher concentration of streptomycin sulphate (a final concentration of 3.3%) removed at least 75% of the transport activity, only 1.8% of the cytosol protein was precipitated. Further, the residual 25% of the transport activity appears to be derived from proteins in the nucleus, since increasing the concentration of the streptomycin-treated cytosol in the assay no longer gave an increase in RNA transport. Also the re-addition of purified cytoplasmic RNA in place of cytosol did not support RNA transport (A. Yannarell, unpublished work). The decreased transport is not due to residual streptomycin sulphate remaining after dialysis since increasing the time of dialysis or addition of small amounts of streptomycin to the assay had no effect; indeed, the yeast RNA in the assay would react with any free streptomycin. These results show not only that the transport factors possess unusual physicochemical properties, but also that they constitute a specific minor fraction of the cytosol. To date it has not been possible to recover the factors from the streptomycin sulphate-treated precipitate in an active form.

Discussion

In eukaryotic cells the cytoplasmic mRNA molecules appear to be derived from large precursor molecules in the nucleus by a processing mechanism; only a small fraction of the precursor RNA is conserved for nucleocytoplasmic transport (Darnell *et al.*, 1971; Attardi *et al.*, 1966; Spohr *et al.*, 1974). The post-transcriptional nuclear processing and/or nucleocytoplasmic transport of most, but not all, messenger sequences is preceded by, and may depend on, the addition of a poly(A) tract to the 3'-end of the precursor molecule (Darnell *et al.*, 1971). It is also known that the messenger is transported to the cytoplasm as a ribonucleoprotein complex called an informosome and that the heterogeneous nuclear RNA (pre-messenger RNA) also exists in the nucleus as a nucleoprotein complex (Samarina *et al.*, 1968; Kwan & Brawerman, 1972). Limited methylation (Both *et al.*, 1975) and passage through a nuclear-pore complex with a characteristic substructure (Faberge, 1974) are also thought to be important post-transcriptional events. Aside from these findings very little is known

about the regulation of mRNA processing and transport.

Convincing evidence for the feedback regulation of nuclear genetic activity by the cytoplasm comes from cell-fusion and nuclear-transplantation experiments (cf. Sherbert, 1970). Although their nuclear site of action has not been unequivocally established, the glucocorticoid-binding proteins constitute an example of macromolecules involved in nucleocytoplasmic control (Kalimi *et al.*, 1973). This information together with evidence for nuclear restriction, i.e. that messengers are selected for transport to the cytoplasm (Shearer, 1974; Johnson *et al.*, 1975), raises the possibility that post-transcriptional nuclear activities are regulated in part by nucleocytoplasmic controls. The results to date obtained with the cell-free system for RNA release support the concept that nucleocytoplasmic controls regulate the mRNA release from the nucleus and further provide a molecular basis for the selection process. They suggest that mRNA release from isolated nuclei under conditions approximating to those *in vivo* depends on and indeed is modulated by macromolecules in the cytosol.

The results of the present investigation, together with those previously reported (Schumm *et al.*, 1973a), are consistent with the concept that the regulatory macromolecules are protein in nature. In the present study their effective cytoplasmic concentration is shown to be decreased by inhibitors of protein synthesis, and they are moderately heat-sensitive. Of particular note are the data outlined above, which strongly suggest that these transport factors are not sequestered, but rather recycled. Both the decreased RNA release per nucleus at higher nuclear concentrations, owing to a decrease in the ratio transport factors/nuclei, and the maintenance of complete transport capacity of cytosol serially incubated with fresh nuclei, are consistent with a recycling mechanism. The fact that these components are recycled, together with the inhibitor data, which indicate that they have a relatively long half-life *in vivo*, probably accounts for a previous failure to observe an effect of pactamycin on mRNA release in sea-urchin eggs (Duncan *et al.*, 1975). The fact that mRNA is transported as a nucleoprotein in itself suggests that protein synthesis must be a prerequisite for messenger transport.

The present data indicate that the dilution of the transport factors is not equivalent to a decrease in their effective concentration in the assay through an increase in the concentration of nuclei. This is supported by both the analysis of the amount of RNA transported according to these two protocols and an analysis of the sequences transported by DNA-RNA hybridization; despite the fact that mainly reiterated sequences were measured in the latter experiment, the differences observed were striking. It is concluded that the transport factors are present in different

effective concentrations and that they become essentially non-functional when their concentration is decreased below a critical value.

The demonstration that the proteins are virtually quantitatively precipitated from the cytosol by a concentration of streptomycin ten times that required to precipitate RNA provides strong evidence for the specificity of these factors. In fact the streptomycin-treated cytosol appears to be devoid of transport activity, the residual activity being contributed by the nuclei (A. Yannarell, unpublished work). Despite the marked fall in transport activity there is only a minimal change in the protein concentration of the cytosol after streptomycin treatment and dialysis. These results confirm the validity of earlier experiments (Schumm *et al.*, 1973a) purporting to demonstrate the absolute dependence of RNA release on cytosol protein. Although approx. 98% of the residual proteins in the streptomycin-treated cytosol are not required for RNA transport, it does still contain macromolecules necessary for the stabilization of the rat liver nuclei. As shown previously (Schumm *et al.*, 1973a), when the cytosol protein concentration is decreased to less than 2.0 mg/ml, RNA is released as a result of nuclear lysis rather than by controlled transport.

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