Role of cytosol in the stimulation of RNA transport *in vitro* during cardiac hypertrophy in rats

Arun SUBRAMANIAM, Meenakshi THIRUNAVUKKARASU and Chellam RAJAMANICKAM*

Department of Biochemistry, School of Biological Sciences, Madurai Kamaraj University, Madurai 625 021, Tamil Nadu, India

The 100 000 *g* supernatant isolated from hypertrophic hearts on fractionation by $(\text{NH}_4)_2\text{SO}_4$ and DEAE-cellulose chromatography showed an enhanced RNA-transport activity when incubated with isolated nuclei from sham-operated hearts *in vitro*. Proteins of M_r 73 000, 68 000, 43 000 and 32 000 are enriched in the DEAE-cellulose fractions exhibiting maximal transport activity, and they are phosphorylatable. Pretreatment of the cytosol with antibodies to the M_r 68 000 and 32 000 proteins decreases the transport activity of the cytosol from 14 % to 4.25 %. Proteins of M_r 73 000, 68 000, 43 000 and 32 000 are translocated from the cytosol to the nuclear envelope under conditions of RNA transport *in vitro*. Our results here suggest that at least two of these proteins, those of M_r 68 000 and 32 000, play an indispensable role in the nucleocytoplasmic RNA transport *in vitro*. By making use of a specific myosin heavy-chain B-gene probe and hybridization, we have also shown the effect of cytosol on the transport of myosin heavy-chain mRNA from nucleus to cytosol.

INTRODUCTION

RNA transport *in vitro* has been studied extensively by using isolated nuclei in a cell-free system which permits normal RNA processing and transport under conditions stimulating the environment *in vivo*. Transport of RNA involves intranuclear processing and subsequent transport by an energy-dependent process [1–5] in which hydrolysis of ATP by the NTPase located at or near the pore complexes of the nuclear envelope has been shown to occur [5–8].

Previously we have reported the stimulation of RNA transport *in vitro* from normal nuclei upon incubation with cytosol obtained from hypertrophic hearts [9]. The cytosolic effect of stimulating RNA transport *in vitro* from isolated nuclei has been reported by several other workers [10–14], and it involves phosphorylation and dephosphorylation of proteins, endogenous protein kinases, NTPases and phosphoprotein phosphatases [2,3,7,15,16]. Besides the involvement of these enzymes, other cytosolic proteins have been shown to play a role in RNA transport [17–20].

In the present study, we have demonstrated that the cytosolic proteins of M_r 68 000 and 32 000 play an indispensable role in RNA transport. These two cytosolic proteins are bound to the nuclear envelope during RNA transport *in vitro*. The role of specific proteins in this process requires investigation. We have also shown the cytosolic stimulation of transport of RNA with respect to specific myofibrillar messages.

EXPERIMENTAL

Materials

Female albino rats of a Wistar-derived strain were used. Animals in the weight range 120–150 g were chosen for surgical aortic constriction. The animals were fed with Gold Mohur rat feed, from Hindustan Lever Ltd., Bangalore, India.

ATP (sodium salt), spermidine, dithiothreitol, phosphocreatine, creatine kinase, acrylamide, bisacrylamide and Coomassie Brilliant Blue R 250 were purchased from Sigma Chemical Co., St. Louis, MO, U.S.A. Radioactive chemicals were purchased through BARC, Bombay, India. All other chemicals used were of AnalaR grade.

Induction of cardiac hypertrophy

This was done by the method of Rakusan & Poupa [21] with slight modifications [9]. Female white albino rats were used for the experiments under ether anaesthesia; a left thorocotomy in the third interspace was performed and the ascending aorta was carefully lifted free. A tantalum haemoclip (Edward Weck and Co.; cat. no. 523135) was applied on the ascending aorta, just distal to the coronary ostia, with a specially designed applicator. Sham operation was performed in a similar way, but with no clips on the aorta. The incision was rapidly closed with surgical thread and then with 12 mm stainless-steel wound suture clips. The mortality during operation was about 10–12 %, but no death occurred during the post-operative days. The increase in the ratio of heart weight to body weight was calculated by using the formula $100 \times \text{heart weight/body weight}$. The percentage hypertrophy was calculated from the changes in the above ratio in aorta-constricted animals compared with sham-operated controls.

Hearts from animals killed on day 15 after the surgical operation were found to have 40 % hypertrophy. A minimum of three hearts was pooled for each experiment.

Labelling and isolation of nuclei

$^{32}\text{P}\text{IP}_1$ (0.75 mCi) was injected into each animal via the intracardiac route, and labelling was allowed for 1 h. The heart nuclei were isolated and purified as described by the method of Muramatsu & Busch [22] with slight modifications.

For each experiment, five hearts, each weighing about 500–600 mg, were taken. The tissue was homogenized in 8 vol. of homogenizing buffer [0.3 M-sucrose, 0.01 M-Tris/HCl (pH 7.5), 0.01 M-NaCl, 0.001 M-MgCl₂, 0.005 M-CaCl₂, 0.1 % (v/v) diethyl pyrocarbonate, 400 μg of heparin/ml, 40 μg of polyvinyl sulphate/ml], with a motor-driven Teflon/glass homogenizer, and the homogenate was filtered through four layers of cheese-cloth. The homogenate was centrifuged at 1000 *g* for 10 min at 4 °C. The crude nuclear pellet was purified by pelleting through a step gradient of 2.4 M- and 1.6 M-sucrose in TEN buffer (0.01 M-Tris/HCl, pH 7.5, 0.002 M-EDTA and 0.01 M-NaCl) at 90 000 *g* for 1 h at 4 °C. The pellet was suspended in 1 M-sucrose

* To whom correspondence should be sent.

containing 0.001 M-CaCl₂ and washed twice with the same buffer by centrifuging at 11 000 *g* for 10 min at 4 °C. The pure nuclear pellet was used for the transport assays. The nuclear envelopes were prepared by the method of Kaufmann *et al.* [23].

Cytosol preparation and fractionation

Hearts isolated from sham-operated and hypertrophic animals (15 animals each) were homogenized in 8 vol. of homogenizing buffer (0.25 M-sucrose containing 10 mM-Tris/HCl, pH 7.5, and 2.5 mM-MgCl₂) as described above. The homogenate was centrifuged at 11 000 *g* for 15 min, and the supernatant was centrifuged at 100 000 *g* for 60 min. The 100 000 *g* supernatant was fractionated with (NH₄)₂SO₄. The 40–80 %-satd.-(NH₄)₂SO₄ fraction obtained from sham-operated and 40 %-hypertrophic-heart cytosols were further fractionated through DEAE-cellulose columns (1.5 cm × 12 cm), after dialysis against 100 vol. of 10 mM-Tris/HCl (pH 7.5) buffer at 4 °C with two changes. The column was equilibrated with equilibration buffer (50 mM-Tris/HCl, pH 7.5, 25 mM-KCl and 5 mM-MgCl₂) and a 25–300 mM-KCl gradient was used to develop the column. Fractions were collected and each fraction was checked for its *A*₂₈₀ and ionic conductance. All operations were carried out at 0–4 °C.

RNA transport *in vitro*

This was performed by the method of Schumm & Webb [10], with modification. The incubations for transport studies were performed in 1 ml of medium containing 4.5 × 10⁶ nuclei/ml, 50 mM-Tris/HCl, pH 7.5, 25 mM-KCl, 2.5 mM-MgCl₂, 0.5 mM-CaCl₂, 2.5 mM-Na₂HPO₄, 150 mM-sucrose, 0.5 mM-MnCl₂, 5 mM-NaCl, 5 mM-spermidine, 2 mM-dithiothreitol, 2 mM-ATP, 5 mM-phosphocreatine, 35 units (1 unit = 1 μmol/min) of creatine kinase, 500 μg of yeast tRNA/ml, and about 1 mg of the 40–80 %-satd.-(NH₄)₂SO₄ fraction/ml or 300 μg of DEAE-cellulose-fractionated cytosols/ml, after dialysis against 10 mM-Tris, pH 7.5. After incubation for 1 h at 25 °C, the nuclei were pelleted down by centrifugation at 5000 *g* for 10 min, and RNA was extracted from the supernatant. DNAase treatment of the supernatant removed less than 10 % of the radioactivity. The radioactivity associated with the RNA released into the assay medium in the absence of cytosolic fractions from the sham-operated heart nuclei (*x*) and that released into the supernatant in the presence of cytosolic fractions (*y*) were measured. In a parallel experiment, the radioactivity in RNA isolated from the same number of nuclei was determined (*z*). *x/z* × 100 and *y/z* × 100 give the percentage of RNA transport in the absence and presence of cytosolic fractions respectively.

The effect of preincubated cytosol was determined by incubating an already incubated cytosol with freshly labelled nuclei. During the second incubation, the ionic concentrations are adjusted so that the overall ionic concentration of the incubation mixture remained the same.

Polyacrylamide-gel electrophoresis

Phosphorylated nuclear-envelope proteins and the 40–80 %-satd.-(NH₄)₂SO₄ cytosolic fraction from sham-operated and hypertrophic hearts were analysed by SDS/10 %-polyacrylamide-gel electrophoresis [24]. Gels of 1.5 mm thickness were used for analytical purposes, and those of 3 mm thickness for preparative purposes.

Raising of antisera

Antisera were raised in rabbits against the *M*_r-68 000 and -32 000 proteins by the method of Stick & Hausen [25]. Up to 5 mg of the proteins precipitated by 40–80 %-satd.-(NH₄)₂SO₄ from the hypertrophic-heart cytosol were loaded on to the

preparative gels. The gels were stained for 1 h in 0.1 % Coomassie Brilliant Blue R-250, and the required bands were cut out, homogenized in a minimum volume of running buffer without SDS and injected subcutaneously [26]. After 2 weeks, a booster injection was administered intravenously for 3 consecutive days. The antibody titre was checked after 1 week by Ouchterlony double diffusion.

Immunoprecipitation

Immunoprecipitation of the nuclear-envelope and cytosolic proteins was carried out with the antisera raised against the *M*_r-68 000 and -32 000 proteins. The immunoprecipitates were analysed by electrophoresis [27].

Peptide mapping analysis

The parallel proteolytic digestion of *M*_r-73 000, -68 000 and -43 000 proteins of cytosol and nuclear envelope was carried out with papain, in polyacrylamide/SDS gels by the method of Cleveland *et al.* [28]. Bands from SDS gels stained with Coomassie Blue were conveniently digested by placing the gel slices containing these bands in the sample wells of a second SDS gel and then overlaying each slice with papain. Digestion proceeded directly in the stacking gel during subsequent electrophoresis. The digested peptides were resolved by electrophoresis on a 20 %-acrylamide gel.

Transport-inhibition assay

This was performed by the method of Schumm & Webb [10], with some minor modifications. The cytosols from hypertrophic heart were treated with excess of antibodies against *M*_r-73 000, -68 000, -43 000 and -32 000 proteins and incubated at 4 °C for 12 h. The mixture was then centrifuged at 10 000 *g* for 15 min. This supernatant was used in the subsequent transport assays. A sample of the hypertrophic-heart cytosol was treated with pre-immune rabbit serum and used as control.

Slot-blot of transported RNA

The plasmid pcMHC 5 was nick-translated [29] and used for hybridization. Transported RNA was isolated from unlabelled nuclei and DEAE-cellulose-purified cytosol by the method of Schumm & Webb [10]. The RNA samples were denatured by heating at 65 °C for 10 min and cooled on ice. Samples of equal volume and amount were spotted directly on to dry nitrocellulose filters. The filters were baked at 80 °C under vacuum for 2 h, pre-hybridized, hybridized and washed as described in the method of Thomas [30]. After drying, the blots were autoradiographed with intensifying screens at -80 °C.

RESULTS

Previous work from this laboratory [9] has shown that the cytosol (100 000 *g* supernatant) isolated from hypertrophic hearts stimulated RNA transport from nuclei of sham-operated controls. This stimulation was maximal with supernatants obtained from 40 %-hypertrophic hearts. It was also shown that the (NH₄)₂SO₄ fractionation of the cytosol resulted in an enrichment of these stimulating factors, which were heat-labile [9]. The 40–80 %-satd.-(NH₄)₂SO₄ fraction of the 100 000 *g* supernatant was further fractionated on a DEAE-cellulose column. The DEAE-cellulose column fractions were checked for transport activities. The transport activity was separated into three different peaks, with a single peak having the maximum activity (Fig. 1). The transport activities in these three peak fractions were much higher for hypertrophic-heart than for the corresponding sham-operated cytosol fractions.

We studied the nature of these factors present in the DEAE-

Fig. 1. Effect of partially purified DEAE-cellulose-column-fractionated supernatants from normal and hypertrophic hearts on RNA transport *in vitro* from sham-operated heart nuclei

Each point represents the transport capability of a single fraction. Equal amounts of cytosolic protein from DEAE-cellulose-column-eluted fractions were used for incubation. Transport activity (○; a, cytosol from sham operated hearts; b, cytosol from hypertrophic hearts) is expressed as a percentage; ●, A_{280} ; ○, KCl concn. gradient.

cellulose-fractionated cytosol. The electrophoretic pattern of the DEAE-cellulose column fractions which have maximum stimulatory effect on RNA transport *in vitro* from normal nuclei is shown in Fig. 2. Fractions 7 and 8, which showed maximum stimulation, are enriched with proteins of M_r 73000, 68000, 43000 and 32000 (Fig. 2b). Comparison of the electrophoretic patterns of the proteins of the 40–80 % -satd.-(NH_4) $_2$ SO $_4$ fractions obtained from normal and hypertrophic-heart cytosol showed an enrichment of the above-mentioned proteins in the hypertrophic-heart cytosol (Fig. 2a). The strong correlation between the enrichment of these proteins of the above M_r values and the maximal stimulation of transport activity in the DEAE-cellulose column fractions suggest that these proteins may play a role in RNA transport.

Moffett & Webb [17] have reported that a protein of M_r 32000 from liver cytosol is involved in mRNA transport. These reports, along with our results of enrichment of proteins of the above M_r values in DEAE-cellulose fractions, suggested that antibodies should be raised against these proteins and the effects of specific immunoprecipitation of these proteins on the transport activity

should be checked. The cytosol was treated with antibodies against the 73000-, 68000-, 43000- and 32000- M_r proteins as described in the Experimental section. The pretreated cytosols were then used in the transport experiments *in vitro*, and the results are presented in Fig. 3. For these experiments, cytosol from hypertrophic hearts was used, as these showed greater stimulation of transport activities and had enriched amounts of these proteins. Under identical conditions of incubations, sham-operated nuclei showed about 14.75 % of RNA transport in the presence of hypertrophic-heart cytosol. However, with the cytosol pretreated with antibodies to 68000- and 32000- M_r proteins, the transport activity declines from 14.75 % to 4.25 % and 6.3 % respectively. But pretreatment with antibodies to the 73000- and 43000- M_r proteins did not decrease the transport activity. When pre-immune rabbit serum was used in a control experiment, the transport activity of the cytosol was unaltered.

Moffett & Webb [17] have shown that the M_r -32000 protein involved in mRNA transport is phosphorylatable. To see if the proteins which are enriched in DEAE-cellulose fractions and suspected to play a role in RNA transport were phosphorylated, [32 P]P $_i$ was injected through the intracardiac route *in vivo*, and the cytosol was isolated and fractionated. The phosphorylation profile of the 40–80 % -satd.-(NH_4) $_2$ SO $_4$ -fractionated cytosol is given in Fig. 4(a). Among several other proteins, those M_r 73000, 68000, 43000 and 32000, which are enriched in the DEAE-cellulose column fractions, were also found to be phosphorylated *in vivo*. Figs. 4(b) and 4(c) represent the scanning profiles of the phosphorylation patterns. From the calculated areas of the peaks in the scanning profile, the phosphorylation of proteins of M_r 73000, 68000, 43000 and 32000 is considerably greater in aorta-constricted hearts (Fig. 4c) than in sham-operated hearts (Fig. 4b).

The fate of the cytosol was investigated to see if the cytosol could be deprived of its stimulatory effect. Non-radioactive sham-operated heart nuclei were preincubated with sham-operated and 40 % -hypertrophic heart cytosols under conditions of RNA transport *in vitro*. At the end of the incubation, the nuclei were pelleted down and the cytosol was re-used for an assay of *in vitro* RNA transport with fresh labelled nuclei. The energy-regenerating systems were replenished, and care was taken to prevent any alteration in the ionic concentrations. The results in Table 1 indicate that there is a 31 % fall in the stimulation of RNA transport, suggesting that the protein factors involved in RNA transport *in vitro* are exhaustible. The decrease in the transport activity observed when preincubated hypertrophic-heart cytosol was used along with fresh sham-operated heart nuclei suggested the depletion of the cytosolic factors during preincubation, and this subsequently raised the possibility of a nuclear translocation of these factors during RNA transport.

To confirm this, [32 P]P $_i$ -labelled 40–80 % -satd.-(NH_4) $_2$ SO $_4$ -fractionated cytosols from sham-operated and hypertrophic hearts were incubated with the non-radioactive nuclei isolated from sham-operated hearts. At the end of the incubation, the nuclei were pelleted down and their envelopes isolated. The nuclear envelopes were solubilized and the M_r -68000 and -32000 proteins were immunoprecipitated with antibodies raised against the respective proteins of cytosolic origin. The immunoprecipitates were resolved by electrophoresis on a 10 % -acrylamide gel containing SDS, and autoradiographed. Fig. 5 shows that the proteins of similar M_r , i.e. 68000 and 32000, were immunoprecipitated by the antibodies from the nuclear-envelope proteins isolated from the nuclei incubated with the labelled cytosol (lanes 3 and 4 and lanes 7 and 8 respectively). Although the comparison of the autoradiographic signals in lanes 1 and 2 and lanes 5 and 6 strongly favours the previous observation of increased phosphorylation of the M_r -32000 and -68000 proteins

Fig. 2. Polyacrylamide-gel electrophoresis of DEAE-cellulose-column fractions

SDS/10% polyacrylamide-gel profile of chromatographic fractions having maximum RNA-transport activity. (a) 40–80% satd. $(\text{NH}_4)_2\text{SO}_4$ fraction from (S) sham-operated and (H) hypertrophic heart. (b) Equal amounts of protein from fractions (Fig. 1) showing RNA-transport activity were loaded on to the gel. The numbers of the fractions showing transport activity are as indicated. Standard protein M_r markers (S) used were BSA (68000), ovalbumin (43000) and cytochrome *c* (12500); ---- indicates the proteins involved in RNA transport.

Fig. 3. Transport of RNA *in vitro* with immunoprecipitated cytosols

RNA transport *in vitro* was performed in the absence of M_r -68000 and -32000 proteins respectively. Total cytosol and preimmune rabbit serum were used as controls. Experimental procedures are as given in the Experimental section.

Transport *in vitro* using cytosol:

1. treated with preimmune rabbit serum;
2. untreated;
3. treated with anti-73000 antibody;
4. treated with anti-68000 antibody;
5. treated with anti-43000 antibody;
6. treated with anti-32000 antibody.

respectively in hypertrophic-heart cytosol, quantitative conclusions cannot be drawn on the basis of these results. Also it was seen that the antibodies against M_r -68000 and -32000 proteins cross-reacted with the respective antigens. A similar cross-reactivity between these two proteins was observed in immunodiffusion slides (results not shown).

Fig. 6 shows the presence of phosphorylatable proteins of similar M_r values to those above in the nuclear envelope during labelling *in vivo* with $[^{32}\text{P}]\text{P}_i$, among many other phosphorylatable proteins. The extent of phosphorylation was greater in nuclear envelopes isolated from hypertrophic hearts compared with those of sham-operated controls. These observations, along with the immunoprecipitation data, suggest that the cytosolic phosphorylated proteins may be translocated to the nuclear envelope and become part of the envelope complex under conditions of RNA transport, and perhaps are involved in the formation of an active pore complex. To confirm the similarity of the proteins of the above M_r values in the cytosol and the nuclear envelope, partial peptide digests of the proteins which were depleted in the cytosol during RNA transport were carried out. Fig. 7 shows densitometric scans of the partial digest profile of the M_r -73000, -68000 and -43000 proteins from both the cytosol and the nuclear envelope. The similarity of the peptide profiles further suggests that these proteins in the cytosol and the nuclear envelope are similar.

Next, we wanted to see if the stimulation of RNA transport by the hypertrophic-heart cytosol included increased transport of a muscle-specific mRNA. Fig. 8 shows the slot-blot analysis of the transported RNA with respect to the myosin heavy-chain mRNA. An increased transport of the myosin heavy-chain mRNA is seen from hypertrophic-heart nuclei as compared with

Fig. 4. Phosphorylation profile *in vivo* of the 40–80%-satd.-(NH₄)₂SO₄-fractionated cytosol

(a) Labelling *in vivo* was performed and cytosol (1, from sham-operated hearts; 2, from hypertrophic hearts) was isolated as given in the Experimental section. The autoradiogram was scanned with an LKB Ultrascan XL: (b) (sham-operated) and (c) (hypertrophic) represent the scanning profiles of the phosphorylation patterns. The relative areas of the different peaks as obtained from the scanning data are given in parentheses.

that from sham-operated heart nuclei (compare blots 1 and 2). Further, the DEAE-cellulose-fractionated hypertrophic-heart cytosol could stimulate the transport of myosin heavy-chain mRNA from sham-operated heart nuclei (blot 3). The endogenous myosin mRNA levels of the DEAE-cellulose fractionated cytosol from hypertrophic hearts was almost negligible, as there were no visible signals.

These results indicate that hypertrophic-heart nuclei are capable of transporting more myosin heavy-chain mRNA than are normal sham-operated heart nuclei. The latter nuclei showed stimulated myosin heavy-chain mRNA transport upon in-

cubation with the DEAE-cellulose-fractionated cytosol from hypertrophic hearts.

DISCUSSION

We have previously reported [9] that the cytosol from hypertrophic hearts is capable of stimulating RNA transport *in vitro* from sham-operated heart nuclei. Several other workers have shown that the cytosolic proteins play an important role in RNA transport *in vitro*. Moffett & Webb [17] have suggested the involvement of a cytosolic protein of *M_r* 32000–35000 in the transport of mRNA in rat liver. Schroder *et al.* [5] have purified two proteins of *M_r* 31000 and 58000 and shown them to be involved in mRNA transport.

Our results on fractionation and partial purification of heart cytosol have shown a preferential enrichment of proteins of *M_r* 73000, 68000, 43000 and 32000 in the DEAE-cellulose-eluted fractions. These fractions stimulate maximally the RNA transport activity *in vitro*. As the hypertrophic-heart cytosol is capable of stimulating RNA transport from normal nuclei *in vitro*, it suggests that the stimulation may be due to increases in these protein factors. The hypertrophic-heart cytosol contains elevated levels of these proteins as compared with its sham-operated counterpart (Fig. 2a). The transport induced by the cytosol was linear up to 60 min. This increase is not likely to be due to any alteration in RNA breakdown. Analysis of the RNA by electrophoresis on a 3.5%-acrylamide gel at different time

Table 1. Fate of the cytosols from sham-operated and hypertrophic hearts

RNA transport *in vitro* was performed as detailed in the Experimental section, by using cytosol from sham-operated and hypertrophic hearts, and nuclei from sham-operated hearts only. The percentage transport was calculated by taking the control transport value as 100% (nuclei and cytosol from sham-operated hearts).

Source of cytosol	Transport (%)
Sham-operated heart	100
Hypertrophic heart	160.3 ± 3
Preincubated hypertrophic heart	111.9 ± 1

Fig. 5. Electrophoretic analysis of the M_r -32000 and -68000 immunoprecipitates

[32 P] P_i -labelled cytosol was incubated with cold nuclei as described in the Experimental section. Antibodies against M_r -68000 and -32000 proteins were used to immunoprecipitate the respective antigens from the cytosol and the nuclear envelope. The immunoprecipitates were resolved by SDS/PAGE (10% gel), dried and autoradiographed at -70°C . Immunoprecipitation with anti-(M_r -32000 protein) antibody, lanes 1-4, and with anti-(M_r -68000 protein) antibody, lanes 5-8: 1 and 5, sham-operated heart cytosol; 2 and 6, hypertrophic-heart cytosol; 3 and 7, sham-operated heart nuclear envelope after incubation with labelled sham-operated heart cytosol; 4 and 8, sham-operated heart nuclear envelope after incubation with labelled hypertrophic-heart cytosol.

intervals of RNA transport shows an accumulation of RNA in the mRNA region up to 30 min and high labelling of 28 S and 18 S rRNA species at 60 min [31]. Further, we have observed (Rajamanickam, Selvamurugan, Subramaniam & Siddiqui, unpublished work) an efficient translation of the RNA transported *in vitro*, and this suggests that the RNA transport observed is unlikely to be non-specific leakage owing to breakdown or nuclease activity. The decreased transport activity exhibited by cytosol from which the M_r -68000 and -32000 proteins were

Fig. 6. Phosphorylation profile *in vivo* of nuclear envelopes (sham-operated and hypertrophic hearts)

The labelling *in vivo* and analysis of proteins were done as described in the Experimental section, with nuclear envelopes from: (lane 1) hypertrophic heart and (lane 2) sham-operated heart.

removed by immunoprecipitation by their respective antibodies substantiates the important role of at least those two proteins in RNA transport *in vitro*.

Agutter *et al.* [2,7] and Lam & Kasper [32] suggested the involvement of a phosphorylation/dephosphorylation mechanism in RNA transport *in vitro*. Studies in which phosphorylated nuclear envelopes have been subjected to SDS/PAGE have

Fig. 7. Peptide map profiles of cytosolic and nuclear-envelope proteins

Partial proteolytic digestions were carried out with papain as described in the Experimental section. The digests were fractionated by SDS/PAGE (20% gel). The Coomassie-Blue-stained gels were scanned at 546 nm. Proteins: M_r 73000 from cytosol (IC) and nuclear envelope (IN); M_r 68000 from cytosol (IIC) and nuclear envelope (IIN); M_r 43000 from cytosol (IIIC) and nuclear envelope (IIIN).

Fig. 8. Slot-blot analysis of transported RNA using a β -myosin heavy-chain-specific probe pCMHC5

RNA was extracted from: (1) sham-operated heart nuclei incubated with transport buffer (see the Experimental section); (2) hypertrophic-heart nuclei incubated with transport buffer; (3) sham-operated heart incubated with DEAE-cellulose-fractionated cytosol from hypertrophic hearts. Different concentrations of transported RNA (a, 5 μ g; b, 10 μ g; c, 20 μ g) were used for hybridization.

demonstrated the selective phosphorylation of an M_r -68000 protein [32]. Phosphorylation of several other nuclear-envelope proteins [33,34] along with the proteins of M_r 7000, 20000, 51000, 59000 and 70000 in the rat liver nuclear envelope have also been reported [35]. The results in the present paper suggest that the proteins of M_r 73000, 68000, 43000 and 32000 of the nuclear envelope are phosphorylated *in vivo*. An interesting observation of the phosphorylation studies *in vivo* is the phosphorylation of cytosolic proteins of M_r values similar to the above, which were suggested to be involved in RNA transport. The cytosolic proteins purified by Schroder *et al.* [5] and Moffett & Webb [17] are also phosphoproteins. Interestingly, we have found that the protein of M_r 68000 exhibits protein phosphatase activity, and the M_r -32000 protein modulates the NTPase activity of the nuclear envelope (A. Subramaniam & C. Rajamanickam, unpublished work). This rules out the possibility of these proteins acting as proteases degrading the nuclear-pore complex and triggering the non-specific release of RNA.

The presence of M_r -68000 and -32000 phosphorylated proteins in both the cytosol and the nuclear envelope suggested that they could be similar proteins and may be translocated from the cytosol to the nuclear envelope during RNA transport *in vitro*. This is rather substantiated by our observations of the depletion of the stimulatory factors from the cytosol (Table 1) and of the immunoprecipitation of the phosphoproteins of M_r 68000 and 32000 from the nuclear envelope. The antibodies used for the immunoprecipitation are against the M_r -68000 and -32000 proteins of cytosolic origin. The peptide-map analysis of the proteins of M_r 73000, 68000 and 43000 from the cytosol and nuclear envelope also contribute to the fact that they are similar proteins. However, the possibility that the cytoplasmic fraction contains nuclear materials which leaked out from the nuclei during cell fractionation in the hypo-osmotic buffer, leading to disturbance of their true cellular localization, cannot be ruled

out. Schroder *et al.* [5] have reported the possibility of proteins of cytosolic origin becoming translocated to the nuclear envelope and playing an important role in RNA transport *in vitro*.

The cytosolic effect observed on RNA transport *in vitro* from isolated nuclei holds good for the muscle-specific mRNAs too. A recombinant probe pcMHC5 corresponding to the β -myosin heavy-chain cDNA [36] has been used in the present study to quantify the myosin heavy-chain mRNA in the transported RNA. More of this mRNA is transported when sham-operated nuclei are incubated with partially purified hypertrophic-heart cytosol. The endogenous contribution of the partially fractionated cytosol towards the hybridization signal is negligible. Further work on the role of transport of muscle-specific mRNA through nucleo-cytoplasmic interactions towards the expression of different myosin isoenzymes under normal and hypertrophic conditions is required.

A.S. is the recipient of a Senior Research Fellowship from the Council of Scientific and Industrial Research, New Delhi. The financial assistance provided by the Department of Science and Technology and the Indian Council of Medical Research for the above work is gratefully acknowledged. We thank Professor B. Nadal-Ginard for giving us the myosin heavy-chain gene clone pCMHC5.

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Received 6 October 1989/8 November 1989; accepted 24 November 1989