Importance of Mammalian Nuclear-Envelope Nucleoside Triphosphatase in Nucleo-Cytoplasmic Transport of Ribonucleoproteins

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(Received 21 May 1979)

The nucleoside triphosphate-stimulated efflux of RNA from isolated nuclei was studied under a range of conditions, and the effects of these conditions on the process were compared with the properties of the nucleoside triphosphatase located in the pore complex. A marked similarity between the rate of efflux and the rate of nucleoside triphosphate hydrolysis was apparent, in terms of substrate specificity, sensitivity to treatment with insolubilized trypsin, kinetics and the effects of increased ionic strength and of many inhibitors. These results are taken, in view of earlier evidence, to suggest that the activity of the nucleoside triphosphatase is a prerequisite for nucleo-cytoplasmic RNA transport *in vivo*. There are some indications that the nuclear-envelope lipid is also involved in regulating the efflux process.

Several studies of the efflux of RNA from isolated mammalian nuclei *in vitro* have indicated that ATP, or some other nucleoside triphosphate, is essential for this process (Ishikawa *et al.*, 1969, 1970*a,b,c*, 1972; Schumm & Webb, 1972, 1974; Schumm *et al.*, 1973*a,b*; Raskas, 1971; Raskas & Bhaduri, 1973). The ATP is hydrolysed during the efflux (Blanchard *et al.*, 1975; Agutter *et al.*, 1976; Sauermann, 1976), and there is evidence (Agutter *et al.*, 1976) to suggest that the hydrolysis occurs at the pore complex, where an NTPase is located (Yasuzumi & Tsubo, 1966; Yasuzumi *et al.*, 1968; Kartenbeck *et al.*, 1973). RNA transport almost certainly occurs at this site (Stevens & Swift, 1966; Franke, 1974). Further circumstantial evidence for a functional relationship between ATP hydrolysis and RNA efflux from nuclei has been given by Agutter *et al.* (1977), who demonstrated that some RNA preparations, particularly poly(A) and poly(G) sequences, stimulate the NTPase in isolated nuclear envelopes. Moreover, it has been shown that under certain conditions this stimulation

is prevented by cordycepin, an inhibitor of RNA transport (Agutter & McCaldin, 1979).

The suggestion that ATP hydrolysis is involved is not universally accepted, since there are some reports of RNA efflux from nuclei in the absence of ATP (Heilmann & Hardeland, 1977; Stuart *et al.*, 1977). The present paper is therefore devoted to a more detailed evaluation of the role of the NTPase in this process, as studied in rat liver and cultured SV40-3T3-fibroblast nuclei. The properties of the NTPase in nuclear envelopes isolated from these sources have been described in a previous paper (Agutter *et al.*, 1979).

Measurement of the efflux of labelled RNA from isolated nuclei requires an incubation medium in which these organelles are stable and in which normal restriction of RNA and other nuclear contents can be demonstrated. In a detailed evaluation carried out in this laboratory of six incubation media described previously (Ishikawa *et al.*, 1969, 1970*a,b,c*, 1972; Schumm & Webb, 1972, 1974; Schumm *et al.*, 1973*a,b*; Blanchard *et al.*, 1975; Heilmann & Hardeland, 1977), only one medium was found to meet these criteria satisfactorily (P. S. Agutter, unpublished work). This medium was therefore used in the present investigation.

Materials and Methods

Materials

ATP (disodium salt, grade II), dATP (disodium salt), GTP (disodium salt, grade II), CTP (trisodium

Abbreviations used: SV40-3T3 cells, simian-virus-40-transformed 3T3 mouse-embryo cells; NTPase, nucleoside triphosphatase (EC 3.6.1.3); [β -methylene]ATP, adenosine 5'-[β -methylene]triphosphate; [γ -thio]ATP, adenosine 5'-[γ -thio]triphosphate.

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salt, grade II), UTP (disodium salt, grade III), ADP (disodium salt), AMP (sodium salt), cyclic AMP (sodium salt), actinomycin D, atractyloside, colchicine, 2,4-dinitrophenol, glucose 6-phosphate (sodium salt), gramicidin, oligomycin, ouabain, proflavin, quercetin, *p*-hydroxymercuribenzoate and *p*-chloromercuribenzenesulphonate were obtained from Sigma Chemical Co., London S.W.6, U.K. Yeast RNA, *N*-ethylmaleimide and phenyl arsenoxide were obtained from BDH Chemicals, Poole, Dorset, U.K. [β -Methylene]ATP and [γ -thio]ATP were obtained from Boehringer, Mannheim, Germany. Dulbecco's modification of Eagle's medium, calf serum, trypsin and Swiss-mouse SV40-3T3 cells were obtained from Flow Laboratories, Irvine, Ayrshire, Scotland, U.K. Gentamycin was obtained from Roussel Laboratories, London N.W.10, U.K. [5 - 3 H]Uridine was obtained from The Radiochemical Centre, Amersham, Bucks., U.K. Atoxyl (*p*-aminophenyl arsenoxide) was kindly given to the authors by Dr. I. A. Flynn. All other reagents used were of AnalaR grade from BDH Chemicals.

Cell culture

The SV40-3T3 cells were grown in roller culture in Dulbecco's modification of Eagle's medium supplemented with calf serum (10%, v/v) and gentamycin (40 μ g/ml). Trypsin (0.025%) was used to harvest the cells for experimentation when the cultures had attained densities of 5×10^5 cells/cm 2 . The trypsin was then neutralized by the addition of 50 ml of Krebs buffer, pH 7.4 (Andrew, 1972). The cellular material was pelleted by centrifugation for 10 min at 1500g at 4°C in the J20 rotor of a Beckman J21B centrifuge. For RNA efflux experiments, 50 μ Ci of [5 - 3 H]uridine per roller bottle was added to the cells 6 h before harvesting. For some experiments, male albino rats (100–150 g body wt.) were injected intraperitoneally with 50 μ Ci of [5 - 3 H]uridine 1 h before they were killed.

Isolation of nuclei

SV40-3T3-cell nuclei were isolated as described previously (Agutter *et al.*, 1979). Rat liver nuclei were prepared by the method of Blobel & Potter (1966).

Measurement of RNA efflux rate

The labelled nuclei were washed twice by resuspension in 20 vol. of incubation medium (0.25M-sucrose, 50mM-Tris/HCl buffer, 25mM-KCl, 5.0mM-spermidine, 5.0mM-2-mercaptoethanol, 2.0mM-MgCl $_2$, 0.5mM-CaCl $_2$, 0.3mM-MnCl $_2$ and 300 μ g of yeast RNA/ml, pH 7.6) and resuspended in the same buffer (pH adjusted to 7.6–7.7 at 37°C) at a concentration of about 10^7 nuclei/ml (0.5mg of protein/ml). ATP (or other nucleoside triphosphate) was added to a concentration of 2.5mM. The use of other

reagents is described in the Results and Discussion section. In experiments in which the ATP concentration was varied, EDTA was added to make the total concentration of chelating agent 2.5mM. The suspension was incubated routinely for up to 10 min at 37°C, cooled in ice and centrifuged at 1500g for 10 min at 0°C. Ice-cold trichloroacetic acid was added to the supernatant to a concentration of 10% (w/v). The precipitate was pelleted by centrifugation at 3000g for 15 min and was digested with 0.1 ml of 0.1M-NaOH at 50°C for 30 min. The digested material was diluted with 0.9 ml of water and mixed with 15 ml of NE-250 scintillator fluid (Nuclear Enterprises). The radioactive disintegrations were counted with a Packard Tri-Carb liquid-scintillation counter with the usual quench correction. Total radioactivities in the nuclear fraction were determined by digesting a 0.1 ml sample of unincubated nuclei with NaOH in the same way and proceeding as described above.

Nuclear envelope

This was isolated from rat liver nuclei by the method of Harris & Milne (1974) and from SV40-3T3 cell nuclei by the modification of this method (Agutter *et al.*, 1979).

Assay of NTPase activity

NTPase activity was assayed as previously described (Agutter *et al.*, 1979), with 2.85mM-ATP + 2.85mM-MgCl $_2$ except where otherwise stated. This gave a substrate (MgATP $^{2-}$) concentration of 2.5mM (Phillips, 1966). It was necessary to assay the enzyme in isolated envelopes, since intranuclear enzymes contribute significantly to the total ATP hydrolysis observed in intact nuclei.

Inhibitor studies

Nuclear envelopes in 25mM-triethanolamine/HCl buffer, pH 8.0, and nuclei in the incubation medium described above, were preincubated for 10 min at 0°C in the presence of the inhibitor. At the end of this time the effect of all inhibitors was maximal (Agutter *et al.*, 1979). When the inhibitor was added in ethanolic solution, 95% (v/v) ethanol was added to the controls. The NTPase activity or RNA efflux rate were then measured as described above.

Trypsin pretreatment of isolated nuclei

Agarose-insolubilized trypsin (a gift to the authors from Dr. W. E. Hornby) was incubated with labelled nuclei in the incubation medium for 3 min at 20°C. The suspension was then cooled to 0°C and the nuclei were re-purified by centrifugation through 58% (w/v) sucrose and washing with 2×20 vol. of fresh incubation medium. For controls, the nuclei were incubated with agarose beads in which the trypsin had been inactivated by treatment with 0.1M-NaOH.

Determination of protein

Protein was determined by the modification by Maddy & Spooner (1970) of the method of Lowry *et al.* (1951).

Significance testing

Correlations between alterations of NTPase activity and alterations of the rate of RNA transport were determined by multiple linear regression, and the significance of inhibitions was determined by analysis of variance (Freund, 1967).

Results and Discussion

The total amounts of label incorporated into SV40-3T3 cell and rat liver nuclei under the con-

ditions used were $12(\pm 2) \times 10^3$ and $17(\pm 3) \times 10^3$ d.p.m./ 10^7 nuclei respectively. In SV40-3T3 cells in the first 3 h exposure to $[5\text{-}^3\text{H}]\text{uridine}$, the incorporation of label into the nuclei was negligible, but incorporation was rapid over the interval 3–6 h.

Fig. 1(a) illustrates the time-course of release of trichloroacetic acid-precipitable label from SV40-3T3-cell nuclei; rat liver material gave similar results. The results shown in Fig. 1(a) differ in three important respects from those described in a previous publication (Agutter *et al.*, 1976), when a different incubation medium was used. First, only about 3.5% as opposed to 10% of the total nuclear label is eluted in 20 min in the presence of ATP. Secondly, the time-course is linear for only 10 min, as opposed to at least 30 min. In view of this, results described below are expressed as d.p.m. released/10 min per 10^6 nuclei. Thirdly, chelating agents such as EDTA and $[\beta\gamma\text{-methylene}]\text{ATP}$ do not promote any significant efflux of label. These results are consistent with the claim that the medium described in the present paper is superior to that previously used in terms of maintenance of nuclear stability (see the introduction), and indicate that the stimulation of RNA efflux by ATP is not attributable to chelation and that the $\beta\gamma\text{-anhydride}$ bond of the molecule is essential for its action. Fig. 1(b) indicates that the nuclei are less stable in the absence of the 2-mercaptoethanol (cf. Heilmann & Hardeland, 1977), but the stimulating effect of ATP is still highly significant ($P < 0.001$ by analysis of variance).

The RNA eluted under these incubation conditions appears to be messenger material, together with some

Fig. 1. Time-course of RNA release from isolated SV40-3T3-cell nuclei

Release of RNA by the nuclei into incubation medium was measured at 37°C in the presence of ATP (■), $[\beta\gamma\text{-methylene}]\text{ATP}$ (□) and EDTA (▲) at 2.5 mM concentration. Δ , Controls. The values given are means $\pm$ S.E.M. for five determinations (three determinations for $[\beta\gamma\text{-methylene}]\text{ATP}$). (a) Incubation medium described in the Materials and Methods section; (b) incubation medium without 2-mercaptoethanol. Rat liver nuclei gave similar results.

Fig. 2. Time-course of RNA release from SV40-3T3-cell nuclei after pretreatment with insolubilized trypsin

Release of RNA by the nuclei was measured as described in the text. Δ and $\square$, Nuclei pretreated with agarose-immobilized trypsin that had been inactivated by treatment with NaOH. Δ and Δ , 2.5 mM-ATP present in medium. $\blacksquare$ and $\square$, Controls. Values represent means $\pm$ S.E.M. for three determinations.

Fig. 3. Effect of increased ionic strength of incubation medium on RNA efflux rate and NTPase activity in SV40-3T3-cell nuclei. RNA efflux rate over a 10 min incubation of the nuclei was measured in the presence (■) and absence (□) of 2.5 mM-ATP. Experimental details are given in the text. Mean values $\pm$ S.E.M. for four experiments are shown. NTPase activity in isolated nuclear envelopes was also measured (▲). The standard errors are similar to those described previously for such experiments (Agutter *et al.*, 1979).

Table 1. Substrate specificities of activities of NTPase and RNA efflux rate in SV40-3T3-cell nuclei

Nuclear-envelope NTPase activity and RNA efflux rate over a 10 min incubation of the nuclei were measured in the presence of various substrates. Experimental details are given in the text. Results are means $\pm$ S.E.M. for five triplicate determinations. Neglecting the glucose 6-phosphate and ATP+GTP results, which for reasons discussed in the text do not belong to the same statistical population as the other sets of values quoted, the results in the two columns show a correlation coefficient of +0.91 by multiple linear regression. This correlation is significant at the 1% level.

Substrate	Sp. activity of NTPase (μ mol of P_i /h per mg of protein)	RNA efflux rate (d.p.m. released/10 min per 10^6 nuclei)
None (control)	—	83 $\pm$ 20
2.5 mM-ATP	7.1 $\pm$ 1.1	213 $\pm$ 25
2.5 mM-dATP	7.2 $\pm$ 1.4	200 $\pm$ 20
2.5 mM-GTP	7.7 $\pm$ 1.1	238 $\pm$ 25
2.5 mM-UTP	3.8 $\pm$ 0.9	142 $\pm$ 15
2.5 mM-CTP	3.5 $\pm$ 0.9	171 $\pm$ 15
2.5 mM-ADP	0.2 $\pm$ 0.1	105 $\pm$ 15
2.5 mM-Glucose 6-phosphate	13.5 $\pm$ 1.3	75 $\pm$ 10
2.0 mM-ATP+0.5 mM-GTP	7.3 $\pm$ 1.6	569 $\pm$ 90
1.0 mM-ATP+1.5 mM-UTP	6.7 $\pm$ 1.0	193 $\pm$ 30
1.0 mM-ATP+1.5 mM-CTP	6.7 $\pm$ 1.0	219 $\pm$ 30

low-molecular-weight material (probably including tRNA species) that diffuses out of the nuclei in the absence of ATP. This matter is not discussed in detail here. In brief, however, RNA released from nuclei in the absence of ATP is predominantly about 5S and has a negligible poly(A) content; that released in the presence of ATP includes the 5S peak and otherwise comprises material sedimenting at 8–18S, with virtual absence of higher-molecular-weight species; about 50% of this RNA contains poly(A). Moreover, reports from other laboratories have indicated that

the material eluted by ATP in such experiments is mainly messenger (Ishikawa *et al.*, 1969, 1970a; Schumm & Webb, 1972; Raskas, 1971; Racevskis & Webb, 1974; Sato *et al.*, 1977a,b). Studies on the permeability of the nuclear envelope (Paine *et al.*, 1975) suggest that tRNA and material of similar molecular size could leave the nuclei by passive diffusion, but mRNA species could not. This seems to be consistent with the RNA efflux observed in the presence and absence of ATP. The claim that messenger material is eluted in these experiments cannot,

on the evidence available, be extended to a claim that the messenger is intact.

Trypsin treatment (Fig. 2) also destabilizes the nuclei to some extent, but it abolishes the stimulation of RNA efflux by ATP. In view of the 95% inactivation of the nuclear-envelope NTPase by this treatment (Agutter *et al.*, 1979), this result suggests that the stimulation of RNA efflux by ATP is essentially linked with ATP hydrolysis at the pore complex. It is noteworthy that other nuclear-envelope enzymes are not affected so markedly by the brief trypsin treatment.

The relationship is further suggested by the effect of increasing the ionic strength of the medium by addition of KCl (Fig. 3) and by the substrate specificity of the effect (Table 1), except for the marked stimulation of RNA efflux when ATP and GTP are simultaneously included in the medium; under these conditions, however, there is a substantial loss of nuclear integrity as revealed by light-microscopy. The results shown in Table 1 are consistent with those previously described (Agutter *et al.*, 1976). Glucose 6-phosphate, hydrolysis of which is catalysed at the nuclear envelope (Milne *et al.*, 1978), gives no significant stimulation of RNA efflux. Moreover, the dependence of efflux on ATP concentration parallels the kinetic behaviour of the NTPase to a remarkable extent (Figs. 4 and 5). First, a similar deviation from

Fig. 4. Kinetics of RNA release and NTPase activity in rat liver nuclei

The Wolfe plots show the dependence of RNA release over a 10min incubation (○) and of NTPase activity (●) on MgATP^{2+} concentration. The values for RNA efflux are corrected for background release (in the absence of ATP). Values are means $\pm$ S.E.M. for three determinations for RNA release, and for 11 determinations for NTPase activity.

Fig. 5. Kinetics of RNA release and NTPase in SV40-3T3-cell nuclei

Symbols and ordinates of the Wolfe plots are as described for Fig. 4. Values are means $\pm$ S.E.M. for six determinations for RNA release, and for three determinations for NTPase activity.

Fig. 6. Effect of increased ionic strength on the kinetics of RNA release and NTPase activity in SV40-3T3-cell nuclei

The Wolfe plots show the dependence of RNA release over a 10min incubation (○) and of NTPase activity (●) in SV40-3T3-cell nuclei in the presence of 150 mM-KCl. Values are means $\pm$ S.E.M. for three determinations.

Michaelis-Menten kinetics is apparent (Agutter *et al.*, 1977, 1979): concave-downwards Wolfe plots, corresponding to concave-upwards Eadie-Hofstee plots, are generated under the conditions used.

Fig. 7. pH-dependence of RNA release and NTPase activity in SV40-3T3-cell nuclei

RNA efflux rate over a 10 min incubation of the nuclei was measured in the presence (■) and absence (□) of 2.5 mM-ATP at various pH values. NTPase activity (▲) in isolated nuclear envelopes was also measured. Experimental details are given in the text. Mean values $\pm$ S.E.M. of three determinations are shown.

Secondly, both the NTPase and RNA efflux curves for each cell type extrapolate to the same point on the negative substrate-concentration axis. This observation constitutes very strong evidence in favour of the relationship under discussion, since the two pairs of intercepts differ significantly between the two cell types examined: 0.09 ± 0.02 mM for rat liver (Fig. 4) and -0.25 ± 0.04 mM for SV40-3T3 cells (Fig. 5). Thirdly, the kinetic plots become linear when the ionic strength of the medium is increased to 160 mM (Fig. 6). Under these conditions, the K_m for $MgATP^{2-}$ is 0.39 ± 0.03 mM for both NTPase and RNA efflux from SV40-3T3-cell nuclei.

The pH-dependence of the process (Fig. 7) is also consistent with a functional relationship between the NTPase and RNA efflux. RNA efflux rate increases as the pH of the medium is increased to 8.0, the optimum for the enzyme, although ATP-independent leakage of nuclear material into the medium at higher pH values makes accurate determination of the efflux rate impossible.

The temperature-dependence of the processes, however, appears to be inconsistent with the claim that they are functionally related. The curvature of the Arrhenius plot of RNA efflux (Fig. 8) is highly significant ($P < 0.01$ by regression analysis and chi-

square), whereas the Arrhenius plot for the NTPase is linear. The curvature may be attributable to a change in fluidity of the lipid in one or both nuclear membranes in the immediate environment of the pore complex. Wunderlich (1972) and Nägel & Wunderlich (1977) have already shown a correspondence between nuclear-envelope lipid fluidity and discontinuities in the temperature-dependence of RNA transport *in situ* with *Tetrahymena*. Stuart *et al.* (1977) have indicated that the Arrhenius plot is linear for cultured myeloma cells, but this may reflect leakage of nuclear contents, since these authors report elution of 20% of the nuclear DNA in their experiments; it is notable that ATP-independent RNA efflux gives linear Arrhenius plots in our experience (Fig. 8b).

The effects of various NTPase inhibitors and thiol-blocking reagents are summarized in Tables 2 and 3 respectively. In general, the effects of the inhibitors on the enzyme parallel their effects on efflux of RNA. More-lipid-soluble inhibitors, however, affect the efflux of RNA more markedly than would be predicted on the basis of their action on the enzyme. This observation may again be interpreted with reference to the membrane lipid close to the pore complex, if the local increase in membrane micro-

viscosity resulting from uptake of the lipid-soluble inhibitor by the envelope has an effect similar to that observed when the system is cooled (Fig. 8b). Despite this unexpectedly marked effect of 2,4-dinitrophenol, colchicine and phenyl arsenoxide, however, the correlations between the NTPase activity values and the ATP-stimulated RNA efflux rates given in Tables 2 and 3 are significant. Although proflavin inhibits both the enzyme and the efflux process, actinomycin D inhibits neither; this is consistent with the finding by Egyhazi (1974) that the rate of nucleocytoplasmic RNA transport is independent of the rate of RNA synthesis. The inhibitory effect of colchicine was also noted by Schumm & Webb (1974), who suggested on this basis that the pore complex might contain microtubule proteins. In our experience, nuclear envelopes do not react with anti-tubulin, and there is no detectable band on sodium dodecyl sulphate/polyacrylamide-gel electrophoresis

Fig. 8. Arrhenius plots of NTPase activity and RNA release in SV40-3T3-cell nuclei

(a) Arrhenius plot of NTPase activity in isolated nuclear envelopes with MgATP^{2-} as substrate. (b) Arrhenius plots of RNA release over a 10 min incubation in the presence (■) and absence (□) of 2.5 mM-ATP.

Table 2. Effects of inhibitors on NTPase activity and RNA efflux rate in SV40-3T3-cell nuclei

Nuclear-envelope NTPase activity and RNA efflux rate over a 10 min incubation of the nuclei were measured in the presence of 2.5 mM-ATP or of 2.5 mM-EDTA. Experimental details are given in the text. Results are means $\pm$ s.e.m. for four triplicate determinations. Significant inhibition (significance was determined by analysis of variance, and accepted at the 5% level) is indicated by an asterisk. The correlation between NTPase activity and ATP-stimulated RNA efflux is significant by multiple linear regression ($r = +0.546$; $P < 0.05$).

Inhibitor	Concn.	Sp. activity of NTPase ($\mu\text{mol of P}_i/\text{h per mg of protein}$)		RNA efflux rate (d.p.m. released/10 min per 10^6 nuclei)		
		+2.5 mM-ATP	+2.5 mM-EDTA	+2.5 mM-ATP	+2.5 mM-EDTA	Difference
None (control)	—	7.1 ± 1.1	—	232 ± 25	92 ± 10	140
Ethanol	7% (v/v)	7.1 ± 1.3	—	250 ± 30	104 ± 10	146
Actinomycin D	10 $\mu\text{g}/\text{ml}$	6.8 ± 1.0	—	218 ± 25	84 ± 10	134
Atractyloside	50 $\mu\text{g}/\text{ml}$	7.2 ± 1.2	—	255 ± 30	100 ± 15	155
Colchicine	0.5 mM	6.8 ± 1.1	—	162 ± 20	97 ± 10	65*
	2.0 mM	$5.3 \pm 0.8^*$	—	90 ± 15	89 ± 10	1*
2,4-Dinitrophenol	1.0 mM	7.5 ± 1.3	—	187 ± 20	90 ± 10	97
	2.0 mM	7.7 ± 1.2	—	118 ± 15	80 ± 10	38*
Gramicidin A	50 $\mu\text{g}/\text{ml}$	7.8 ± 1.0	—	264 ± 30	122 ± 30	142
Oligomycin	5 $\mu\text{g}/\text{ml}$	$5.0 \pm 0.8^*$	—	180 ± 30	108 ± 10	72*
	20 $\mu\text{g}/\text{ml}$	$3.7 \pm 0.8^*$	—	145 ± 20	95 ± 10	50*
Ouabain	1 mM	7.3 ± 1.1	—	248 ± 25	102 ± 15	146
Proflavin	0.1 mg/ml	$5.4 \pm 0.9^*$	—	156 ± 20	76 ± 10	80
	0.5 mg/ml	$3.8 \pm 0.7^*$	—	111 ± 15	76 ± 10	35*
Quercetin	5 $\mu\text{g}/\text{ml}$	$3.6 \pm 0.5^*$	—	134 ± 15	72 ± 10	62*
	20 $\mu\text{g}/\text{ml}$	Zero*	—	82 ± 10	71 ± 10	11*
[γ -Thio]ATP	1.3 mM	$4.5 \pm 0.9^*$	1.1 ± 0.6	187 ± 20	109 ± 15	78*
	2.5 mM	$3.3 \pm 1.4^*$	2.6 ± 1.2	119 ± 15	116 ± 15	3*
ADP	5.0 mM	6.6 ± 1.3	0.4 ± 0.3	218 ± 20	97 ± 10	121
AMP	5.0 mM	8.2 ± 1.9	2.1 ± 0.8	228 ± 20	105 ± 15	123
Cyclic AMP	5.0 mM	6.1 ± 1.2	0.5 ± 0.2	203 ± 20	93 ± 10	110

Table 3. *Effects of thiol-blocking agents on NTPase activity and RNA efflux rate in SV40-3T3-cell nuclei*
Nuclear-envelope NTPase activity and RNA efflux rate over a 10 min incubation of the nuclei were measured in the presence of various thiol-blocking agents. The RNA efflux experiments were carried out in the medium described in the Materials and Methods section, without 2-mercaptoethanol. Other experimental details are given in the text. The concentrations of thiol-blocking agents used were those required for maximal and half-maximal inhibition of the enzyme (Agutter *et al.*, 1979). Values are means $\pm$ s.e.m. of four triplicate determinations. Asterisks indicate inhibition significant by analysis of variance at the 5% level.

Reagent	Concn. (mM)	Sp. activity of NTPase (μ mol of P _i /h per mg of protein)	RNA efflux rate (d.p.m./10 min per 10 ⁶ nuclei)		
			+2.5 mM-ATP	Without ATP	Difference
None (control)	—	7.1 $\pm$ 1.1	378 $\pm$ 85	151 $\pm$ 30	227
Iodoacetamide	5.0	7.2 $\pm$ 1.4	436 $\pm$ 90	186 $\pm$ 30	250
N-Ethylmaleimide	5.0	6.2 $\pm$ 1.3	352 $\pm$ 70	169 $\pm$ 25	183
p-Chloromercuribenzenesulphonate	0.3	4.4 $\pm$ 0.9*	296 $\pm$ 60	182 $\pm$ 35	114*
p-Hydroxymercuribenzoate	0.7	0.9 $\pm$ 0.2*	184 $\pm$ 60	147 $\pm$ 35	37*
	0.6	5.5 $\pm$ 1.2*	307 $\pm$ 65	132 $\pm$ 30	175
	0.8	4.2 $\pm$ 1.0*	259 $\pm$ 55	110 $\pm$ 30	149*
Phenyl arsenoxide	0.5	5.3 $\pm$ 1.1*	207 $\pm$ 55	124 $\pm$ 25	83*
	1.0	3.5 $\pm$ 0.8*	142 $\pm$ 30	109 $\pm$ 25	33*
Atoxyl	0.2	5.9 $\pm$ 1.2*	303 $\pm$ 65	194 $\pm$ 40	109*
	0.6	4.7 $\pm$ 1.2*	221 $\pm$ 35	168 $\pm$ 40	53*

that co-migrates with tubulin monomers; we therefore regard the inhibition of RNA efflux by colchicine, which is also found with luminocolchicine, as attributable to a non-specific effect on lipid microviscosity.

The evidence presented in this paper strongly suggests that ATP hydrolysis by the pore-complex NTPase is a prerequisite for RNA efflux from isolated nuclei and hence, given the well-preserved integrity of the nuclei in the incubation medium used and the normal nuclear restriction exhibited, that it is a prerequisite for nucleocytoplasmic RNA transport *in vivo*. The evidence of Fig. 8(b) and of Tables 2 and 3 also supports the finding of Nägel & Wunderlich (1977) that the fluidity of the envelope lipid also regulates this process.

We are indebted to Dr. M. Kadlubowski, Dr. R. W. Old and Ms. A. J. L. Shorthose for helpful discussions of this work and criticisms of the manuscript, and to Principal J. Dunning, C. B. E., and Dr. K. J. Anderson for placing the facilities of the College at our disposal.

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