Nucleocytoplasmic transport of RNA

The effect of 3'-deoxyadenosine triphosphate on RNA release from isolated nuclei

Rolf F. KLETZIEN*

Division of Cell Growth and Regulation, Sidney Farber Cancer Institute, Boston, MA 02655, U.S.A.

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The addition of 3'-deoxyadenosine (cordycepin) to cells in culture results in the inhibition of the appearance of mRNA in the cytoplasm through a mechanism thought to involve the inhibition of polyadenylate synthesis. I studied the effect of 3'-deoxyadenosine triphosphate, the physiologically active form of 3'-deoxyadenosine, on RNA release from isolated nuclei. Nuclei were isolated from baby-hamster kidney (BHK) fibroblasts that had been given a short pulse of radioactive uridine or adenosine in the presence of a low concentration of actinomycin D before harvest. RNA release from the isolated nuclei under the appropriate incubation conditions was time-, temperature- and ATP-dependent. 3'-Deoxyadenosine triphosphate inhibited RNA release from the isolated nuclei. However, RNA that was restricted to the nuclei during incubation with the drug could be chased out of the nuclei if the incubation medium was replaced with medium containing only ATP. The chased poly(A)⁺ (polyadenylated) RNA had shortened poly(A) tracts, indicating that poly(A)⁺ RNA with shortened poly(A) tracts can be transported out of the nucleus. An experiment was designed to test the effect of 3'-deoxyadenosine triphosphate on the release of poly(A)⁺ RNA at drug concentrations which caused 33 or 64% inhibition of RNA release. The release of poly(A)⁺ RNA and poly(A)⁻ RNA (not polyadenylated) was equally inhibited by the drug. Thus, although 3'-deoxyadenosine triphosphate does inhibit release of RNA from the nucleus, it would appear that the drug does so through a mechanism independent of the inhibition of polyadenylation. The process that is inhibited must be one that is common to both poly(A)⁺ and poly(A)⁻ RNA. The possibility that 3'-deoxyadenosine triphosphate inhibits a reaction at the nuclear membrane or nuclear pore complex is considered.

Most eukaryotic mRNA contains a covalently linked poly(A) segment at the 3'-end of the molecule. Although the biological function of the poly(A) segment is not understood (Lewin, 1974; Perry, 1976), it has been suggested that it may play some role in the nucleocytoplasmic transport of mRNA (Darnell *et al.*, 1973). Perry (1976) has commented that the fact that the drug cordycepin (3'dA) concomitantly blocks polyadenylation and the appearance of newly synthesized mRNA in the cytoplasmic

polyribosomes led to the idea that polyadenylation is an obligatory step in the processing of the poly(A)⁺ mRNA species. However, 3'dA is a potent inhibitor of RNA synthesis (Beach & Ross, 1978), and in intact cells it is difficult to sort out the effects of the drug on transcription from those on post-transcriptional processing of mRNA. Indeed, past work does not allow a distinction between an effect of 3'dA on synthesis or on transport (Adesnik *et al.*, 1972). I have studied the effect of the physiologically active form of 3'dA, 3'dATP, in a subcellular system in which only post-transcriptional aspects of mRNA biogenesis are occurring. The results of this study establish that 3'dATP does indeed block nucleocytoplasmic transport of RNA, but does so through a mechanism that is independent of the inhibition of polyadenylation.

Abbreviations used: 3'dA, 3'-deoxyadenosine; 3'dATP, 3'-deoxyadenosine triphosphate; poly(A)⁺ RNA, poly(A)-containing RNA; poly(A)⁻ RNA, RNA lacking poly(A); SDS, sodium dodecyl sulphate.

* Present address: Department of Biochemistry, West Virginia University Medical School, Morgantown, WV 26506, U.S.A.

Materials and methods

Materials

Serum and culture medium were obtained from Gibco. Sucrose (ribonuclease-free) and supplies for electrophoresis were from Bio-Rad. Oligo(dT)-cellulose and tRNA were from Calbiochem. RNA standards and 3'dATP were purchased from Miles. Protosol, all radiochemicals, except labelled RNA standards (Schwartz/Mann), and scintillation fluid (Biofluor) were from New England Nuclear. All other chemicals were purchased from Sigma Chemical Co.

Cell culture

Baby-hamster kidney cells (BHK₂₁C₁₃) were grown in Dulbecco's modified Eagle's medium containing 10% calf serum on 100mm-diam. Falcon plastic dishes. Cells were brought to quiescence by placing the cells in medium containing 0.1% calf serum.

Isolation of nuclei

BHK cells were harvested by rinsing each plate of cells with 10ml of phosphate-buffered saline (0.14M-NaCl, 2.7mM-KCl, 8.1mM-Na₂HPO₄, 1.5mM-KH₂PO₄) and then scraping the cells off the plate with a rubber policeman. The cells were washed once in phosphate-buffered saline and then resuspended in 2vol. of homogenization buffer, consisting of 25mM-KCl, 10mM-Hepes [4-(2-hydroxyethyl)-1-piperazine-ethanesulphonic acid], pH 7.4, and 2.5mM-MgCl₂. The cells were broken with 50 strokes of a Potter-Elvehjem homogenizer run at 200rev./min. The homogenate was adjusted to 0.25M-sucrose and centrifuged at 500g for 5min to pellet the nuclei. The nuclei were resuspended in 0.25M-sucrose/homogenization buffer and were purified by the procedure of Blobel & Potter (1966) with the following modifications. A 1.9M-sucrose cushion was used rather than 2.2M. The sucrose solutions all contained 10mM-Hepes, pH 7.4, rather than Tris. All solutions contained 5mM-MgCl₂. After centrifugation through the sucrose 'cushion', the nuclei were resuspended in medium used for the RNA-transport studies (containing 10mM-KCl, 10mM-Hepes, pH 7.4, 2.5mM-MgCl₂, 2mM-dithiothreitol, 0.25mM-sucrose, and 1mg of tRNA/ml).

The purity of the nuclei was assessed by both electron-microscopic and marker-enzyme analysis. The electron micrographs revealed the virtual absence of any plasma membrane, smooth or rough endoplasmic reticulum and mitochondria. Cytoplasmic tabs were attached to the nuclei, as reported for rat liver by Blobel & Potter (1966). Although the tabs could be removed by 0.5% Triton X-100, this was not routinely done, since this procedure resulted in a large loss of the ATP-dependent

RNA-transport activity. Marker-enzyme analysis showed that the nuclear preparation was substantially free of mitochondrial and lysosomal enzymes.

Assay of RNA release from nuclei

Highly purified nuclei ($5 \times 10^5/0.2$ ml) suspended in RNA-release medium (see above) were treated as indicated in the Figure legends. At the end of the incubation period, nuclei were rapidly spun down at 1000g for 5min at 4°C and the supernatant was removed. In experiments in which only radioactivity (c.p.m.) released was to be measured, the RNA in the supernatant was precipitated with 10% (w/v) trichloroacetic acid and the precipitate dissolved in 0.2M-NaOH, and 100μl samples were counted for radioactivity in Biofluor with an Inter-technique liquid-scintillation counter. The data are expressed as RNA released (c.p.m.) per μg of nuclear DNA. DNA was determined by the Burton (1956) method. In experiments in which the RNA was to be further characterized, the supernatant was immediately made 1% in SDS and applied to oligo(dT)-cellulose columns or extracted in phenol.

Various modifications of the above assay system were tried before choosing the above system for the experiments described in this paper. For example, I examined the effect of inclusion of 0.5mM-MnCl₂ or 0.5mM-CaCl₂ in the incubation mixture and found that these ions had no effect on the basic characteristics of RNA release. A variety of ribonuclease inhibitors were examined, and it was found that none protected as well as having an excess of tRNA present. An ATP-generating system (pyruvate kinase and phosphoenolpyruvate) was found to maintain RNA release for a longer period of time, but did not affect RNA release over 40min at the amount of added nuclei used in these experiments. Therefore I have used 40min or shorter time periods in the experiments in which I examined the effect of 3'dATP, since I did not want the complication of rephosphorylation of 3'dADP compromising the experiments.

Analysis of RNA

The ribonucleoprotein particles released from the isolated nuclei were analysed on neutral sucrose gradients as follows. The supernatant was adjusted to 50mM-EDTA and then layered on to a linear 10–30% (w/v) sucrose gradient (14ml). Centrifugation was carried out for 16h at 25000rev./min in a Beckman SW27 rotor. Fractions (0.6ml) were collected and assayed for radioactivity. The gradient buffer contained 20mM-Hepes (pH 7.4), 50mM-EDTA and 25mM-KCl. Sedimentation values were determined by running purified ribosomal 40S and 60S subunits.

The RNA was extracted from the ribonucleo-

protein particles with phenol/chloroform. The supernatant containing the RNA was adjusted to 0.2% SDS, and 2 vol. of ethanol was added. After 3 h at -20°C , the precipitate was spun down (10000 *g* for 20 min). The precipitate was dissolved in 0.9 ml of 0.1 M-Tris/HCl (pH 9) and 0.1 ml of 5% SDS; 1 ml of redistilled phenol was added and mixed. Then 1 ml of chloroform/3-methylbutan-1-ol (25:1, v/v) was added and mixed. After 10 min at 4°C with gentle mixing, the phases were broken by centrifugation (500 *g* for 10 min). The aqueous phase was removed and the phenol layer was re-extracted. The aqueous layers were combined and again extracted with phenol/chloroform. The aqueous phase was then treated with 2 vol. of ethanol and the mixture left overnight to precipitate. The precipitate was collected by centrifugation as above, and washed once with ethanol.

The purified RNA was analysed on sucrose gradients as follows. Ethanol-precipitated RNA was dissolved in gradient buffer (100 mM-NaCl, 20 mM-sodium acetate, 1.0 mM-EDTA, 0.2% SDS, pH 5) and centrifuged through a 10–30% sucrose gradient for 20 h at 25000 rev./min in an SW27 rotor. Fractions (0.6 ml) were collected and analysed for radioactivity. Sedimentation values were determined by running purified 28S, 18S and 4S RNA.

Electrophoresis of the poly(A) tracts was performed in 12.5% polyacrylamide gels as described by Morrison *et al.* (1974), with 0.2% SDS present. Poly(A)⁺ RNA [from the oligo(dT)–cellulose column] was dissolved in 0.3 M-NaCl/0.01 M-Tris/HCl (pH 7.5)/2 M-EDTA. Ribonucleases A and T₁ (2 units each) were added. Digestion was carried out for 30 min at 37°C . The poly(A) fragments were isolated from the total digest by oligo(dT)–cellulose chromatography. The poly(A) fragments were eluted in water and the solution was freeze-dried. The samples were dissolved in 1% SDS/1 mM-EDTA/30 mM-Na₂HPO₄/36 mM-Tris (pH 7.8)/10% sucrose. The samples were run initially for 15 min at 1 mA/gel and then for 5 h at 5 mA/gel. Gels were sliced in 1 mm sections, dissolved in Protosol and counted for radioactivity in Biofluor. Parallel gels containing ³H-labelled 4S and 5S RNA were run as markers.

Affinity chromatography of RNA on oligo(dT)–cellulose columns was performed as described by Pemberton & Baglioni (1972). All solutions contained 0.5% SDS and binding of RNA was carried out in 0.5 M-NaCl/10 mM-Hepes, pH 7.4. Poly(A)⁺ RNA was eluted in 10 mM-Hepes/0.5% SDS.

Results

The following experiment was carried out to establish conditions in which RNA could be selectively released from isolated nuclei while maintaining the integrity of the organelle during the incubation

Fig. 1. Comparison of RNA and DNA release from isolated nuclei

Cells were pulsed with [³H]thymidine and [¹⁴C]-adenosine as indicated in the text. Nuclei were isolated as described in the Materials and methods section. The nuclei were suspended in RNA-release medium and the release of RNA (a) and DNA (b) was monitored. Nuclei were incubated at 30°C ; ATP was added at $t = 10$ min as indicated by the arrow and samples were taken at the appropriate times and analysed for ³H (released DNA) or ¹⁴C (released RNA) as indicated in the Materials and methods section (□). Other samples were incubated with ATP but at 4°C (○), or at 30°C but without ATP (●). Each point represents the mean of four determinations from two separate experiments.

in vitro. Proliferating BHK cells were pulsed for 24 h with [³H]thymidine (0.01 $\mu\text{Ci/ml}$). The medium containing [³H]thymidine was then replaced with medium containing 0.1% calf serum. The cells doubled once in the low-serum medium, and more than 95% of the cells were arrested in G₁ phase of the cell cycle after 36 h of incubation. Actinomycin D (0.05 $\mu\text{g/ml}$) was added to the cultures, and 30 min later the cells were pulsed with [¹⁴C]adenosine (1.0 $\mu\text{Ci/ml}$) for 15 min and harvested. The nuclei were then isolated as described in the Materials and methods section. Purified nuclei were suspended in RNA-release medium. After 10 min at 30°C , ATP was added to the appropriate samples (Fig. 1a). RNA was released from the nuclei in response to ATP addition at 30°C , but not at 4°C , and accounted for about 14% of the total labelled nuclear RNA. Release of RNA was not inhibited by the addition of actinomycin, nor did cycloheximide or puromycin alter the amount of RNA released. RNA release was optimal at 2.5 mM-ATP and 5 M-Mg²⁺. In contrast with this result, DNA released from the same nuclei was less than 0.2%

of the total labelled nuclear DNA, and DNA release was not increased by ATP or increasing temperature (Fig. 1b). In similar experiments [^3H]tRNA was added to the incubation medium containing the purified nuclei. After 40 min of incubation, more than 99% of the RNA was precipitable with trichloroacetic acid. The [^3H]tRNA was isolated and run on 15% polyacrylamide gels. No change was noted in the electrophoretic pattern after incubation. Inclusion of other nuclease inhibitors such as polyvinyl sulphate had no effect. Thus we have established a system *in vitro* in which the selective release of RNA from isolated nuclei can be studied.

Characterization of released RNA

Fig. 2 depicts the profile in neutral sucrose gradients of the product released from isolated

Fig. 2. Analysis of RNA released from isolated nuclei in response to ATP

Cells were pulsed with [^3H]uridine and nuclei isolated as in Fig. 1. The nuclei were resuspended in RNA-release medium and ATP was added. Incubation was carried out at 30°C for 30 min. At that point nuclei were spun down and the released RNA was either subjected directly to gradient analysis or extracted with phenol as described in the Materials and methods section. O, Released RNA before phenol extraction; ●, released RNA after phenol extraction. Positions of standards of known *s* values are indicated at the top for the respective traces.

nuclei in response to ATP addition. The material ran at about 40–60S, indicating that the product was present as a ribonucleoprotein particle. If the released RNA was subjected to phenol/chloroform extraction, most of the labelled material ran as a heterogeneous peak between 4 and 18S (Fig. 2). The gradient profiles in Fig. 2 are representative of the RNA released by nuclei isolated from resting cells and pretreated with actinomycin before a short pulse of radioactive uridine or adenosine. Different profiles of released RNA were obtained if the cells were growing and pulsed with uridine or adenosine for longer periods of time in the absence of actinomycin. However, in the experiments carried out to examine the effects of 3'dATP on RNA release, cells were cultured in low serum concentrations for 36–40 h before harvest. This greatly decreases the synthesis of rRNA.

The percentage of poly(A)⁺ RNA released from isolated nuclei was determined by oligo(dT)-cellulose affinity chromatography. As indicated in Table 1, the percentage of poly(A)⁺ RNA released was dependent on the pulse time and the presence or absence of actinomycin D. Under the conditions used in the subsequent experiments on the effect of 3'dATP on RNA release, 23–28% of the released RNA was poly(A)⁺. Use of higher concentrations of actinomycin resulted in a higher percentage of poly(A)⁺ RNA being released, but the overall amount of RNA labelled was decreased. Similarly, use of a pulse time shorter than 15 min resulted in a higher percentage of poly(A)⁺ RNA, but the low overall amount of labelled RNA was difficult to work with.

Effect of 3'dATP on RNA synthesis

The addition of 3'dA to cells inhibits RNA synthesis (Beach & Ross, 1978). I have examined the effect of 3'dATP on RNA synthesis in isolated nuclei under conditions similar to those used to study RNA release, except that GTP, CTP and UTP were also present. RNA synthesis as measured by UTP incorporation into the trichloroacetic acid-precipitable fraction was not inhibited by 3'dA

Table 1. Effect of pulse time and actinomycin D on percentage of poly(A)⁺ RNA released from isolated nuclei
Cells were pulsed with 5.0 μCi of [^3H]adenosine/ml in the presence or absence of actinomycin D for the indicated periods of time. Nuclei were isolated and incubated in the RNA-release medium as described in the Materials and methods section. The percentage of released RNA that also contained poly(A) was determined. Each value represents the mean $\pm$ S.E.M. for three experiments.

Pulse time (min)	Actinomycin D (0.05 $\mu\text{g}/\text{ml}$)	Poly(A) ⁺ RNA released (%)
5	—	13 $\pm$ 1.1
5	+	36 $\pm$ 3.1
15	—	12 $\pm$ 1.5
15	+	26 $\pm$ 1.9
60	—	7 $\pm$ 1.3
60	+	21 $\pm$ 2.2

Fig. 3. *Effect of 3'dA and 3'dATP on RNA synthesis* Nuclei were isolated and suspended in RNA-release medium as indicated in Fig. 1 and the Materials and methods section. At $t = 10$ min GTP, CTP and [5,6- ^3H]UTP (final concns. 1 mM) were added to the nuclei. The following additions were made and incubation was continued at 30°C: 2.5 mM-ATP (○), 2.5 mM-ATP and 2.5 mM-3'dA (▲), 2.5 mM-ATP and 0.25 mM-3'dATP (△), 2.5 mM-ATP and 2.4 mM-3'dATP (■), 2.5 mM-3'dATP (□); and 2.5 mM-ATP with incubation at 4°C (●). At the appropriate time-points, 0.1 ml samples were taken, treated with 2 ml of 5% trichloroacetic acid and the precipitate was spun down and washed twice more in 5% trichloroacetic acid. The washed pellet was dissolved in 0.2 M-NaOH and samples were taken for liquid-scintillation counting. Each value represents the mean for three determinations.

(Fig. 3). However, the presence of 0.25 mM- or 2.5 mM-3'dATP caused a 29–36% or 60–64% (10–30 min) inhibition of RNA synthesis respectively. Total replacement of ATP by 3'dATP permitted only a small amount of UTP incorporation. The experiment depicted in Fig. 3 thus establishes that 3'dATP is the active form of the drug with respect to RNA synthesis.

Effect of 3'dATP on RNA release

The effect of 3'dATP on the release of RNA from isolated nuclei under conditions in which only post-transcriptional events were occurring was studied in the experiment depicted in Fig. 4. ATP, 3'dATP or combinations thereof were added at 10 min to nuclei isolated from cells that had been pulsed with [^3H]uridine for 15 min in the presence of actinomycin D. Total replacement of ATP with 3'dATP caused complete inhibition of RNA release. Equimolar amounts of ATP and 3'dATP caused 75% inhibition of RNA release, whereas 3'dATP

Fig. 4. *Effect of 3'dATP on RNA release from isolated nuclei*

Cells were pulsed for 15 min with 5 μCi of [^3H]uridine/ml as in Fig. 1. The nuclei were isolated and suspended in RNA-release medium. At $t = 10$ min, nuclei at 30°C in separate tubes were treated with 2.5 mM-ATP (□), 2.5 mM-ATP and 0.5 mM-3'dATP (■), 2.5 mM-ATP and 2.5 mM-3'dATP (△), 2.5 mM-3'dATP (▲), or no ATP (●). Another tube of nuclei received ATP at $t = 10$ min and incubation was carried out at 4°C (○). At the appropriate time points, samples were taken to measure RNA release as described in the Materials and methods section. Each value represents the mean from three separate experiments.

at one-fifth the concentration of ATP caused a 37% inhibition. As noted above, this system was set up such that 23–28% of the released RNA was poly(A) $^+$. Therefore 3'dATP appeared to have a greater effect on RNA release than what would be predicted if 3'dATP was inhibiting poly(A) $^+$ RNA release by blocking polyadenylation.

Poly(A) $^+$ RNA and poly(A) $^-$ RNA were completely restricted to the nucleus during incubation with 3'dATP in the absence of ATP (Fig. 4). This RNA could be chased out of the nuclei if they were washed free of 3'dATP and resuspended in medium containing ATP (Fig. 5). Resuspension of the nuclei in medium containing 3'dATP resulted in restriction of the RNA to the nucleus. The amount of RNA released from nuclei resuspended with ATP was comparable with that released by nuclei that had been incubated in nucleoside triphosphate-free medium for 30 min and then washed and resuspended in medium containing ATP. Thus 3'dATP did not irreversibly trap RNA within the nucleus, which might occur if 3'dATP was restricting RNA to the nucleus by causing permanent damage to RNA sequences such as causing premature shortening of poly(A) tracts.

The poly(A) segments of the released RNA from

Table 2. Inhibition of release of poly(A)⁺ and poly(A)⁻ RNA by 3'-deoxyadenosine triphosphate

The experiment was designed as described in Fig. 4 and the percentage of RNA that contained poly(A) was determined by oligo(dT)-cellulose chromatography. The values represent the means for two experiments.

Additions	RNA released [c.p.m. (% of total)]	
	Poly(A) ⁻	Poly(A) ⁺
ATP	3400 (74%)	1190 (26%)
ATP + 0.25 mM-3'-dATP	2360 (77%)	705 (23%)
ATP + 2.5 mM-3'-dATP	1210 (73%)	450 (27%)

Fig. 5. Release of RNA from nuclei pretreated with 3'dATP

Cells were pulsed with [¹⁴C]adenosine, nuclei isolated and prepared for the RNA-release assay as described in the Materials and methods section. The nuclei were incubated with 3'dATP for 30 min and then spun down at 600g for 10 min. The supernatant containing 3'ATP was aspirated and the nuclei were resuspended in fresh RNA-release medium. The nuclei were divided into separate groups to which 2.5 mM-ATP was added and incubated at 30°C (□) or 4°C (○), no ATP was added and incubated at 30°C (●) or to which 2.5 mM-3'dATP was added again and incubation carried out at 30°C (Δ). At appropriate time points, 0.1 ml of the suspension was assayed for RNA release as described in the Materials and methods section. Each value represents the average from two separate experiments.

the experiment in Fig. 5 were sized on polyacrylamide gels (Fig. 6). The gel profile in Fig. 6 shows that the prior incubation of nuclei in medium containing 3'dATP resulted in the release of poly(A)⁺ RNA in the presence of ATP that contained shortened poly(A) tracts relative to RNA released from nuclei that had never been treated with 3'dATP. In the absence of 3'dATP, the mean size of the poly(A) segment was about 150 nucleotides. Incubation of nuclei with 3'dATP followed by incubation with ATP resulted in poly(A) segments with a mean size at about 55–65 nucleotides and a more heterogeneous distribution. Therefore a shortened poly(A) segment does not preclude transport of RNA from the nucleus.

Fig. 6. Size of poly(A) segments of released RNA. Cells were pulsed with either [³H]adenosine (○) or [¹⁴C]adenosine (●). Nuclei were isolated from each set of cells. The nuclei containing [¹⁴C]adenosine were incubated with 3'dATP as described in Fig. 5. Then the nuclei were spun down and resuspended in medium containing ATP. The RNA released in 20 min was mixed with the RNA released from the nuclei containing [³H]-adenosine in 20 min after ATP addition. The RNA was processed for poly(A) analysis as described in the Materials and methods section. The gels were cut into 1 mm fractions and processed for scintillation counting.

If a poly(A) segment of a certain length was required for nucleocytoplasmic transport of poly(A)⁺ RNA, then one might expect 3'dATP preferentially to inhibit the release of poly(A)⁺ RNA from the nucleus. However, at concentrations of 3'dATP that produced 64 or 33% inhibition of RNA release, I found that poly(A)⁺ RNA release was inhibited to the same extent as was poly(A)⁻ RNA release (Table 2). Thus 3'dATP does not selectively inhibit poly(A)⁺ RNA release.

Discussion

A major effect of 3'dA on cellular RNA metabolism is to decrease the amount of mRNA that enters the polyribosomes (Darnell *et al.*, 1973; Perry, 1976). After it was recognized that most eukaryotic mRNA contains a poly(A) segment at

the 3'-end of the molecule, the effect of 3'dA was interpreted as follows. The 3'dA caused premature termination of the synthesis of the poly(A) segment, which resulted in the presence in the nucleus of heterogeneous nRNA and mRNA that contained shortened poly(A) tracts. This defective RNA did not undergo the correct processing events and thus was not selected for transport to the cytoplasm.

Our work with a subcellular system supports this interpretation in part in that RNA is restricted to the nucleus in the presence of the physiologically active form of 3'dA, 3'dATP. However, 3'dATP inhibits the release of both poly(A)⁺ and poly(A)⁻ RNA from isolated nuclei (Table 2). Furthermore, poly(A)⁺ RNA that contains shortened poly(A) tracts can be transported out of isolated nuclei if ATP is supplied (Fig. 5). Thus 3'dATP must inhibit a process that is common to the nucleocytoplasmic transport of both poly(A)⁺ and poly(A)⁻ RNA, since the ATP analogue does not selectively inhibit poly(A)⁺ RNA release. The nature of this 3'dATP-inhibitable process is unknown, but it may involve some aspect of the passage of RNA through the nuclear pore. It has been suggested that an ATPase activity is located at the nuclear pore (Franke, 1974) and may be involved in the passage of macromolecules through the nuclear pore. Inhibition of this activity by 3'dATP could result in the inhibition of the nucleocytoplasmic transport of both poly(A)⁺ and poly(A)⁻ RNA.

Previous work with intact cells has also established that shortened poly(A) tracts are not a sufficient condition to restrict poly(A)⁺ RNA to the nucleus. Adesnik *et al.* (1973) have shown that about 20% of the poly(A)⁺ RNA does reach the polyribosomes even in the presence of a high concentration of 3'dA, and this RNA does contain shortened poly(A) tracts. Schumm & Webb (1974) examined RNA release from isolated rat liver nuclei in which the intact animal was treated for 30 min with 3'dA before injection of radiolabelled RNA precursor. The 3'dA-treated nuclei released about 40% less RNA when incubated with ATP than did nuclei from control animals. However, the percentage of poly(A)⁺ RNA released was the same in each case [as measured by poly(U)-cellulose affinity chromatography], although the released poly(A)⁺ RNA contained shortened poly(A) tracts.

Agutter *et al.* (1976) have provided evidence which suggests that a nucleoside triphosphatase in the nuclear membrane is an essential component of the system responsible for nucleocytoplasmic RNA transport. Agutter & McCaldin (1979) have more recently shown that neither 3'dA nor 3'dATP inhibits this triphosphatase, yet 3'dATP inhibited RNA transport as did 3'dA in the presence of ATP. My results also show that 3'dATP inhibited RNA transport, but that 3'dA did not. The reason for

this difference is not immediately clear, but may be due to the use of different cell types. In addition, I found that the onset of 3'dATP inhibition was more rapid than that reported by Agutter & McCaldin (1979). This suggests that 3'dATP may be inhibiting a reaction on the surface of the nuclei other than the triphosphatase. In this regard, it has been found that highly purified nuclear membrane contains endogenous protein kinase activity (Lam & Kasper, 1979), which phosphorylates intrinsic proteins of the nuclear membrane. One of the phosphoproteins has a mol.wt. of 68 000, which is similar to that reported for the proteins associated with the nuclear pore isolated from the rat liver nuclear membrane (Dwyer & Blobel, 1976). Others have observed that protein phosphorylation is inhibited by 3'dATP (Legraverend & Glazer, 1978). Regulation at the level of the nuclear pore by a phosphorylation-dephosphorylation mechanism may be partially responsible for some aspects of post-transcriptional regulation of gene expression, and this may be one site of 3'dATP action, which could explain the equal inhibitory effect of 3'dATP on both poly(A)⁺ and poly(A)⁻ RNA transport.

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