

# Chemical Factors That Influence Nucleocytoplasmic Transport: A Fluorescence Photobleaching Study

Lian-Wei Jiang and Melvin Schindler

Department of Biochemistry, Michigan State University, East Lansing, Michigan 48824. Dr. Jiang's permanent address is Department of Medical Physics, Beijing Medical University, Beijing, China.

**Abstract.** The technique of fluorescence redistribution after photobleaching was used to measure the translocation rate of fluorescein-labeled dextrans across the nuclear pore complex in isolated rat liver nuclei. A transport assay system was established that could monitor the effect of biologically active molecules, e.g., ATP, GTP, cAMP on the translocation process. The results show that ATP, phosphoinositides, RNA, and insulin can enhance transport rates from 195 to

432%. It was further demonstrated that concanavalin A, but not wheat germ or soybean agglutinin, can block dextran transport completely. The effectors of dextran transport are similar to substances demonstrated to effect the efflux of RNA from isolated nuclei. A model for translocation through the nuclear pore is now presented that incorporates data from protein influx and RNA efflux experiments into a single pathway controlled by ATP.

THE nuclear pore complex provides a communication channel between the nuclear and cytoplasmic compartments of the cell (8, 12, 18, 21). This complex, which spans two membranes and a perinuclear space, is composed of an arrangement of globular and strand-like subassemblies forming an octagonally symmetrical structure (14, 35). Electron microscopic techniques reveal a central channel or annulus in the pore complexes that is found to vary in cross-section from 0 to ~400 Å in diameter (14). Early studies by DuPraw provided evidence that the channel or annular diameter may significantly vary after physical stretching of the nuclear envelope (11). This suggested a flexible transport channel that could expand and contract in the manner of a muscle sphincter (11).

Although studies that examine the transport rate of non-nuclear proteins suggest an exclusion limit of 90–120 Å in diameter (21, 23), transport of beads (50–200 Å in diameter) coated by a nuclear protein, nucleoplasmin, was found to occur unhindered (13). In addition, 18 S and 28 S ribonucleoprotein particles (RNP)<sup>1</sup> (34), and 300–400 Å RNP granules from Balbiani rings were found to squeeze through the complex as a rod-like form of ~200 Å in diameter (33). Any explanation of transport would either need to reconcile the large variations in calculated pore diameter observed for non-nuclear proteins, nuclear proteins, synthetic globular particles, and ribonucleoprotein particles into one mechanism, or assume different transport modes for each class of components through the same channel.

In an attempt to dissect the nuclear transport system, we have used the nuclear microphotolysis technique of Peters

(23) in an investigation of chemical factors that may control the functional pore diameter. Using spot scanning fluorescence photobleaching on isolated rat liver nuclei (29), we have attempted to examine the role of nuclear ATPases and phosphoinositides on the nuclear transport of fluorescently derivatized dextrans, compounds not found in cell nuclei. This study also examines the effect of insulin on dextran transport and the effects of a variety of lectins. Although these measurements are performed on isolated rat liver nuclei, sufficient electron microscopic investigations have demonstrated that in gross morphology and number, nuclear pore complexes in isolated nuclei resemble pore complexes observed in the nuclei of whole cells (18, 20, 35). The results to be reported are interpreted in terms of a dynamic pore structure whose diameter may be controlled by alterations in ATPase activity. Such a mechanism appears to reconcile most observations on RNA export (2) and protein import (8).

## Materials and Methods

### Nuclear Isolation

Rat liver nuclei were isolated as described (17) with minor modifications (28). Complete characterization of these nuclei was performed in Schindler (28). Nuclei stored at 4°C in 0.25 M sucrose-10 mM HEPES-1 mM Mg<sup>2+</sup>-pH 7.4 buffer were used within 3 d of preparation.

### Reagents Used in Dextran Influx Assays

Phosphatidylinositol (PI), phosphatidylinositol 4-phosphate (PIP), ADP, ATP, AMP-PCP, RNA, spermidine, spermine, glucose, and fluorescein-labeled dextrans were all purchased from Sigma Chemical Co. (St. Louis, MO). Insulin was obtained from Gibco (Grand Island, NY). Soybean agglutinin and wheat germ agglutinin are from Vector Laboratories, Inc. (Burlingame, CA), whereas concanavalin A (Con A), anti-Con A antibodies, and succinyl-Con A (s-Con A), were gifts of Dr. J. Wang from this department.

<sup>1</sup> Abbreviations used in this paper: Con A, concanavalin A; PI, phosphatidylinositol; PIP, phosphatidylinositol 4-phosphate; PIP<sub>2</sub>, phosphatidylinositol 4,5-bisphosphate; RNP, ribonucleoprotein; s-Con A, succinyl-Con A.

## Fluorescent Dextran Influx Assay

Nuclei (3–5 optical density [OD]<sub>260</sub> units [28]) were suspended in 1 ml 0.25 M sucrose-10 mM Hepes-1 mM Mg<sup>++</sup>-pH 7.4 buffer that contained 1 μM fluorescein-labeled 64-kD dextrans (Mw/Mn < 1.25). The nuclei were incubated and equilibrated in the dextran solution for 30 min at room temperature. All reagents described in Table I were incubated with nuclei during the equilibration step and maintained in solution throughout each experiment. A 5-μl aliquot of this nuclear suspension was placed on a slide, and a coverslip was placed on top of the sample. Melted paraffin was used to seal the coverslip to the slide. Photobleaching experiments were essentially done as described by Peters (23, 25) (a detailed description of the technique can be found in Peters [24, 25]) with the following technical modifications. Instead of a stationary beam, we scanned a defocused Gaussian profile laser beam (~6 μm in diameter) across the spherical nuclei. The scanning procedure is as described in Schindler et al. (29). Experiments by Peters (25) demonstrate that neither the size (although it should have a diameter greater than that of the nucleus) nor the intensity profile of the laser beam is critical. The beam in the focal plane covers a major part of the cross-section of the nucleus, which ensures that a photobleach will maximally deplete the fluorescence in the nucleus (25). After a nuclear photobleach, redistribution of fluorescence between the nuclear compartment and solution was calculated by the method of Peters (23, 25). Considering that diffusion across the membrane is the rate-limiting step for dextran influx, kinetics then follow the equation

$$\frac{C_e - C(t)}{C_e - C(0)} = e^{-kt}, \quad (1)$$

in which  $C_e$ ,  $C(0)$ , and  $C(t)$  are tracer concentrations in the nucleus at equilibrium, zero time, and time  $t$ , respectively. Rate constant  $k$  is related to permeability coefficient  $P$  by  $P = (V/A)k$ , in which  $V$  and  $A$  are the volume and area, respectively of the nucleus. As with all photobleaching experiments fluorescence intensities measured are representative of the fluorescent-derivatized solute concentration at any particular time ( $t$ ). Accordingly, Eq. 1 may be represented as

$$\frac{F(-) - F(t)}{F(-) - F(0)} = e^{-kt} \text{ with } F(-), F(0), \text{ and } F(t) \quad (2)$$

fluorescence signals before (pre-bleach), after, and at time  $t$  after photobleaching. As demonstrated by Peters (23) the data is satisfactorily plotted as

$$\frac{F(-) - F(t)}{F(-) - F(0)} = a_1 e^{-k_1 t} + a_2 e^{-k_2 t} \quad (3)$$

or the sum of two exponentials (23, 25). Although Peters introduces another series of assumptions for calculating pore diameter in one paper (24), we have used the assumptions and equations presented in another paper by Peters (23). Further, a careful analysis of the equations presented in reference 24 lead to the same relationships presented in reference 23. In all instances, multiple bleaches demonstrated the same recovery profiles, which suggests no major photochemical damage.

## Results

Rat liver nuclei (3–5 OD<sub>260</sub> units [28]) were suspended in 1 ml of 0.25 M sucrose-10 mM Hepes-1 mM MgCl<sub>2</sub>-1 μM fluorescently labeled dextran at pH 7.4 and incubated at room temperature for 30 min. Fluorescence photobleaching measurements performed on these samples followed the basic approaches of Peters (23–25) (see Materials and Methods). An example of a typical laser beam scan is presented in Fig. 1. Fig. 1A is a fluorescence intensity scan across a nucleus equilibrated with a 64-kD fluorescently labeled dextran. Note the fairly constant intensity profile as the laser beam excites fluorescence in the surrounding fluorescent media and the nuclear interior. Using a defocused laser beam with an essentially Gaussian profile, the entire nucleus is photobleached, which results in a large loss in fluorescence intensity centered about the bleach point (Fig. 1B). The fluorescence recovery was analyzed as described by Peters (23–25). Our results are consistent with the analysis of fluorescence recovery demonstrated by Peters (23) for isolated rat liver nuclei. Two com-

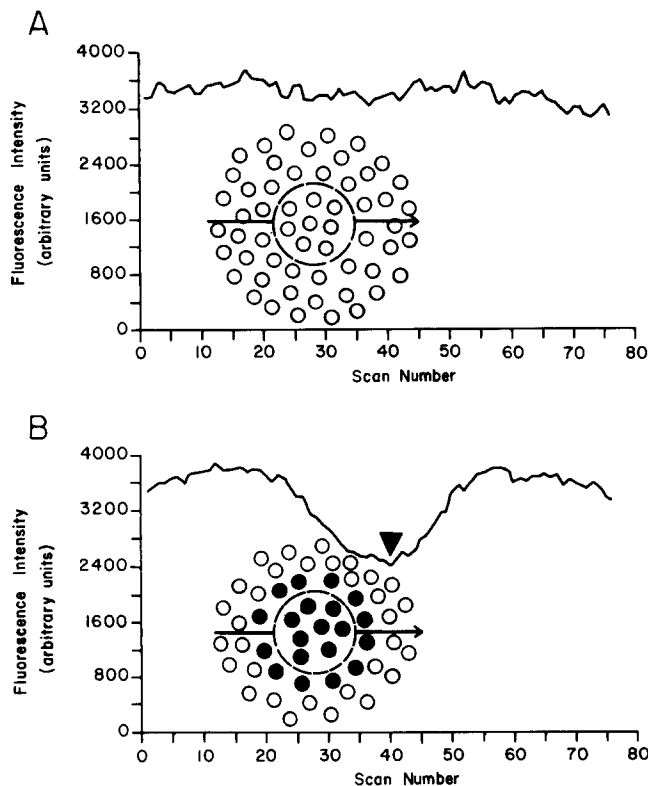
ponent recovery is observed in Fig 2A. The fast rate of recovery for component 1 suggests surface adsorption. A number of scans showed slightly higher levels of fluorescence as the laser beam excited the nuclear surfaces (data not shown). This would be suggestive of some mild preferential surface adsorption. The calculated rate constant for this component varied by less than a factor of 1.5 for all transport conditions examined. Component 2, on the other hand, gave results very similar to the rate constant of influx for 62-kD dextrans obtained by Peters (23). The control value for dextran (64-kD in apparent molecular weight) transport in our system was  $2.2 (\pm 0.8) \times 10^{-3} \text{ s}^{-1}$  (Table I), whereas Peters (23) reports  $2.6 (\pm 0.6) \times 10^{-3} \text{ s}^{-1}$  for dextrans (62 kD) in similar isolated rat liver nuclei. Using equations derived by Pappenheimer et al. (22) and Renkin (27) and cited by Peters (24), this transport number yields a calculated functional pore diameter of ~117 Å. This calculation used  $D \sim 3\text{--}4 \times 10^{-7} \text{ cm}^2/\text{s}$  and Stokes radius of ~56 Å for the 64-kD dextran (23). All subsequent rate constants presented in Table I are based on component 2 recovery in the same manner as the control (Table I, A1). Further evidence that component 2 reflects pore transport is provided by Con A inhibition of component 2 fluorescence recovery. No such effect was observed for

Table I. Chemical Factors Affecting Nuclear Transport

Treatment	Dextran transport rate coefficient ( $\times 10^3 \text{ s}^{-1}$ )	% Change from control
	<i>mean ± SD</i>	
<b>A Nucleotides</b>		
1 Control	2.2 ± 0.8 (14)*	—
2 ADP (1 mM)	2.5 ± 1.2 (5)	+14
3 cAMP (1 mM)	2.0 ± 0.8 (5)	-9
4 GTP (1 mM)	2.8 ± 1.2 (5)	+27
5 AMP-PCP (1 mM)	2.1 ± 1.1 (16)	-5
6 ATP (1 mM)	7.4 ± 1.0 (4)	+236
7 ATP (5 mM)	8.5 ± 1.2 (5)	+286
<b>B PI metabolism effectors</b>		
1 PI (15 μM)	6.4 ± 0.6 (5)	+191
2 PI (50 μM)	8.6 ± 1.6 (8)	+291
3 PI (1,000 μM)	5.5 ± 1.6 (8)	+150
4 RNA (50 μg/ml)	6.5 ± 1.2 (8)	+195
5 PIP (50 μM)	9.4 ± 1.1 (8)	+327
6 PIP (50 μM) + RNA (50 μg/ml)	11.7 ± 2.1 (10)	+432
7 Spermidine (1 mM)	3.8 ± 1.0 (10)	+73
8 Spermine (1 mM)	5.2 ± 0.9 (6)	+136
<b>C Lectins</b>		
1 SBA (0.1 mg/ml)	3.5 ± 0.4 (3)	+59
2 WGA (0.1 mg/ml)	4.6 ± 0.9 (15)	+109
3 Con A (0.1 mg/ml)	≤0.1 (10)	≥-95
4 Con A (0.1 mg/ml) + α-methyl-mannoside (0.1 M)	3.7 ± 1.2 (4)	+68
5 Con A (0.1 mg/ml + 1 mM ATP)	0.1 to 1.0 (5)	-95 to -54
6 s-Con A (0.1 mg/ml)	3.2 ± 1.2 (5)	+45
7 s-Con A (0.1 mg/ml) + anti-Con A (0.5 mg/ml)	0.7 ± 0.3 (3)	-68
<b>D Other reagents</b>		
1 Insulin (0.5 μg/ml)	7.7 ± 1.8 (8)	+250
2 Insulin (5 μg/ml)	9.1 ± 3.1 (8)	+314
3 Glucose (1 mM)	2.4 ± 1.2 (8)	+9

SBA, soybean agglutinin; WGA, wheat germ agglutinin.

\* Number of experiments (in parentheses).



**Figure 1.** Fluorescent dextran transport as measured by fluorescence redistribution after photobleaching. A laser beam excited pre-bleach emission pattern of a nucleus in equilibrium with a solution of fluorescently labeled dextran (64 kD) (*A*). The ordinate is an arbitrary scale of emitted fluorescence intensity, whereas the abscissa is the scan number. A scan is the time required by the laser beam to traverse the sample field (5–10 s). After the pre-bleach scan the laser intensity is increased by  $\sim 5,000$ , which results in the photobleaching of the entire nucleus. This is represented by the intensity dip observed in *B*. The recovery of this dip to pre-bleach levels is proportional to the dextran flux rate (23–25). The diagrams in *A* and *B* represent the bleaching process. The open circles in *A* are exaggerated fluorescent dextran molecules within and surrounding the nucleus. The arrow represents the path of the expanded 6- $\mu\text{m}$  beam. The black circles in *B* represent the bleached dextran.

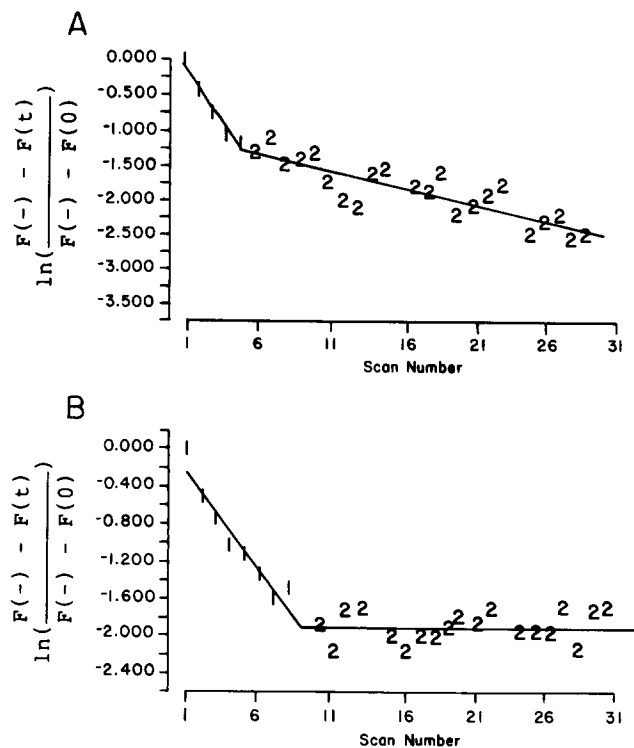
component 1 (Fig. 2*B*). This shows that the two components reflect two distinctly different physical processes.

#### Nucleotide Dependence of Dextran Influx

Nucleotides, particularly ATP, have been implicated as requirements for RNA transport from the nucleus (1, 2). We therefore examined the possibility that ATP, GTP, or cAMP might have a role in a more general control of nuclear pore transport. An analysis of nucleotide dependence demonstrates that ATP (5 mM) uniquely stimulates transport by  $\sim 300\%$ . Hydrolysis of a high energy  $\gamma$  phosphate bond is clearly required (compare AMP-PCP with ADP), and there is a specificity for an adenine-ribose in the high energy nucleotide moiety (compare GTP with ATP). The cyclic nucleotide cAMP, involved in multiple cellular enzymatic events, is not stimulatory at 1 mM concentration.

#### Effect of Phosphoinositides, RNA, and Organic Cations on Dextran Flux

Because of recent observations by Smith and Wells (31), which



**Figure 2.** Fluorescence recovery of labeled dextrans in isolated rat liver nuclei after photobleaching. A semilogarithmic plot of  $\ln [F(-)/F(t) - F(0)]$  as a function of scan number, where  $F(-)$ ,  $F(0)$ , and  $F(t)$  are the fluorescence before the photobleach, after, and at time  $t$  after the bleach (23). The fluorescence values used for these calculations are determined at the bleach point (arrowhead in Fig. 1). Recovery of fluorescence can best be fitted by least squares fits to the sum of two first order terms as described in Peters (23). Component 1 is sufficiently fast to be ascribed to surface adsorption, whereas component 2 corresponds to the recovery term used by Peters (23) to calculate dextran flux. *A* represents fluorescence recovery in the presence of 50  $\mu\text{g/ml}$  RNA, whereas *B* presents a series of scans in the presence of 0.1 mg/ml Con A. Note component 1 is not affected. Each scan is 5 s.

show that phosphoinositides and RNA enhance the activity of a delipidated nuclear envelope ATPase, we chose to examine their role in nuclear transport. When PI was assayed at three concentrations, 15  $\mu\text{M}$ , 50  $\mu\text{M}$ , and 1 mM, maximal transport enhancing activity was observed at 50  $\mu\text{M}$ . Dextran influx was enhanced by  $\sim 290\%$ . Transport at 15  $\mu\text{M}$  and 1 mM was less than that observed at 50  $\mu\text{M}$  PI (Table I). This concentration response was also reported by Smith and Wells (31) for reconstitution of ATPase activity in the presence of PIP and phosphatidylinositol 4,5-bisphosphate (PIP<sub>2</sub>).

Because of the documented response of the RNA transport system to RNA and polycations, dextran influx studies were performed in the presence of RNA, spermine, and spermidine. These last two compounds have also been shown to stabilize PI and PIP in membranes (32). The results in Table I show that RNA and spermine were particularly stimulatory for transport. When PIP and RNA (50  $\mu\text{g/ml}$ ) were used together, the highest rate enhancement of dextran influx occurred (equivalent to a 10 Å increase in functional pore diameter). This rate enhancement was greater than the rate increase caused by either alone, but less than the summation of their individual activities. The reconstituted nuclear envelope

ATPase was also found to provide the maximal response in the presence of PIP and RNA (50  $\mu\text{g/ml}$ ) (31).

### Effect of Lectins on Dextran Flux

A high molecular weight protein, which is the major Con A-binding glycoprotein in the nuclear envelope, was shown to be a pore complex protein (15), predominantly localized at the cytoplasmic face of the pore complex. Considering the location of the Con A-binding glycoprotein in the pore complex, it seemed plausible that the addition of Con A to isolated nuclei that contained fluorescent dextran might either sterically block dextran transport, or indirectly effect structural rearrangements in the pore required for influx after photobleaching. Dextran influx after Con A (0.1 mg/ml) addition was  $\leq 10^{-4} \text{ s}^{-1}$  or essentially blocked, whereas Con A in the presence of 0.1 M  $\alpha$ -methyl-mannoside demonstrated no inhibitory effect (Table I). Soybean and wheat germ agglutinin did not inhibit dextran flux. S-Con A was also without effect, which suggests that binding is not enough, but aggregation of the glycoprotein receptor is necessary. This was further demonstrated when the addition of anti-Con A antibody to nuclei that had been incubated in s-Con A decreased dextran transport considerably (Table I). In the presence of ATP (1 mM), the blocking effect of Con A is partially reversed. The possibility that Con A blocks transport by binding the dextrans rather than the pore glycoprotein is ruled out by the following two experiments: (a) 1  $\mu\text{M}$  dextran in the elution buffer does not alter the elution pattern of Con A in a Sephadex G150 column (Fig. 3), and (b) s-Con A has virtually no effect on transport, whereas s-Con A in the presence of anti-Con A suppresses transport (Table I).

### Other Agents Effecting Transport

A body of literature has evolved on the direct role of insulin on nuclear activity, particularly RNA transport (26, 30). This question was also probed in our dextran transport assay. Insulin at 0.5  $\mu\text{g/ml}$  was found to stimulate influx by 250%. A 10-fold higher concentration stimulated influx approxi-

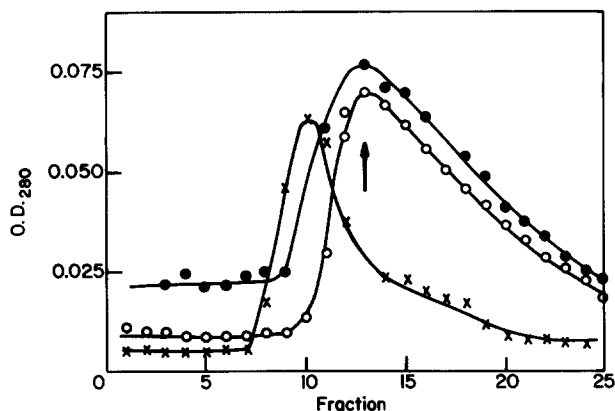


Figure 3. Con A-dextran gel filtration binding assay. Sephadex G-150 gel filtration chromatography was performed to examine Con A-fluorescent dextran interactions. Con A (0.1 mg/ml) was eluted with phosphate-buffered saline (PBS), pH 7.4 (●); Con A eluted with PBS + 1  $\mu\text{M}$  64-kD dextran (○); Con A eluted with PBS + 0.1 M  $\alpha$ -methyl mannoside (X). The column was (15  $\times$  3 cm), and 3-ml fractions were collected. Con A-containing fractions were detected using OD<sub>280</sub>. Arrow indicates common elution maxima for Con A, and Con A eluted with dextrans.

mately the same amount. Glucose, however, was found to be virtually ineffective in stimulating influx.

### Discussion

A reasonable division of the process of nucleocytoplasmic transport for RNA was recently provided by Agutter (2). Transport was suggested to require: (a) detachment of RNP from the nuclear matrix (release), (b) passage of RNP through the nuclear envelope (translocation), and (c) binding of RNP to intracytoplasmic structures (cytoskeletal binding). In the context of our results, we would like to focus on the translocation step and attempt to draw some general conclusions about translocation for proteins and RNA. Previous attempts at dissecting elements of the nuclear translocation process have been limited by the particular choice of nucleus used in the experiments. For example, rat liver nuclei have provided the dominant source of information on RNA transport (2), whereas amphibian oocyte nuclei offered the best experimental system for examining nuclear protein transport (7, 8, 21). The specialized and different biochemical properties of each type of nucleus make it difficult to compare the events for possibly common mechanisms involved in RNA efflux and protein influx. Recent technological advances that permit the measurement of macromolecular transport in rat liver nuclei (23–25) now provide the opportunity to quantitatively compare, in the same nuclear type, the translocation process of macromolecular influx with a large body of results on the control of RNA efflux.

A general summary of published results that emanate from work on *Xenopus* oocyte and rat liver nuclei suggests that (a) an Mg-dependent ATPase and/or nucleoside triphosphatase is vital for transport of RNA (2, 6); (b) non-nuclear macromolecules of  $\leq 64$  kD can diffuse across the pore complex with transit rates inversely proportional to size, which suggests molecular sieve properties for the pore (21, 23–25); (c) nuclear proteins that accumulate in the nucleus can co-transport globular beads of  $\sim 200$  Å in diameter across the pore complex (13); (d) RNA transport is stimulated by RNA, poly A, poly G, and insulin (2, 30); (e) proteins  $\geq 95$  kD have a specific amino acid sequence that facilitates their nuclear transport (9, 10, 16); (f) agents that inhibit nucleoside triphosphate activity also limit efflux of prelabeled RNA from isolated nuclei (1, 2).

We believe the results reported in this paper provide a means to incorporate most of the transport data mentioned above into a single hypothesis for RNA and protein translocation through the nuclear pore. The key observation of our study is that the transport of a non-nuclear, non-cytoplasmic macromolecule in isolated rat liver nuclei can be modulated and controlled by the type of effector agents and energy-dependent mechanisms required for RNA export. Additionally, the pore displays the molecular sieve properties observed in the past for protein import (neither 150-kD dextran nor IgG diffuse into the nucleoplasm), and the flux of dextrans is greatly stimulated by ATP hydrolysis, RNA, and insulin. All these latter chemical factors have been shown to enhance RNA efflux. Not previously shown has been the effect of phosphoinositides in stimulating a nuclear translocation process, and the capability of insulin to enhance nuclear macromolecular uptake. There is general agreement that a nucleoside triphosphatase is involved in the RNA efflux, however,

no previous evidence has suggested that protein influx may also be controlled by this enzyme(s). Of particular relevance to these results are observations that the nuclear matrix-pore complex-lamina fraction isolated from *Drosophila melanogaster* embryos contains a 188-kD ATPase/dATPase (5), and that rat liver nuclear envelopes contain a protein (~200 kD) with ATPase activity that is stimulated by phosphoinositides, RNA, and polycations (31). Whether these are the same or similar is not known, but in the context of our results it would appear that an ATPase that can be modulated by a number of effector molecules is a central element in the translocation process of not only RNA, but now may be suggested to control protein/macromolecular translocation as well. The results presented suggest that PI, PIP, and/or PIP<sub>2</sub> may act as direct effectors of the ATPase activity that controls translocation. The observed insulin effect on dextran influx may now be considered a component of a general phosphoinositide-stimulated mechanism of translocation considering that insulin has been demonstrated to effect PI metabolism (19). Whether insulin actually binds to a unique nuclear insulin receptor, or binds to enzymes involved in PI metabolism, or to the ATPase itself is not known. It also appears unlikely that transport is effected by cAMP-mediated mechanisms, or "second messenger" pathways.

Inhibition of dextran uptake by Con A but not by s-Con A provides further support that these transport phenomena are occurring through the pore. Gerace et al. (15) demonstrated that the major Con A binding site in the nuclear envelope is on the cytoplasmic face of the nuclear pore complex. Consequently, multiple Con A attachment to binding sites could block transport either sterically or by binding around the periphery of the channel at its waist. This binding could squeeze the channel shut, or prevent protein conformational changes. The latter case may be more likely considering the probable location of the carbohydrate side chain in the perinuclear space (15, 36). The lack of inhibition observed for s-Con A and the inhibition of Con A blocking by  $\alpha$ -methylmannoside suggests a blocking that results from Con A binding and aggregation. This is supported by the s-Con A/anti-Con A results (Table I). Baglia and Maul (4) demonstrated a similar Con A inhibition of RNA release from isolated nuclei. These studies thus provide a further demonstration of a common feature in RNA and macromolecular transport.

Early observations by DuPraw (11) on stretched honeybee embryonic nuclei suggested that nuclear annuli (pores) may operate by relaxing and contracting in the manner of tiny sphincter muscles. Additional work by others (14) provided evidence that annular diameters may be found to vary from 0 to 400 Å, whereas Wunderlich and Franke (37) have referred to the "open" or "closed" configuration of the pore in *Tetrahymena* nuclei. In light of these results, we believe that a nuclear pore may be viewed as a variable polyfunctional entry and exit port whose opening is controlled by an ATPase "doorman". Whether this ATPase controls the opening of a single channel, or varies a distribution between "residual" and "transport" channels as suggested by Feldherr et al. (13), remains to be determined. Molecules  $\leq$ 64 kD can freely diffuse through the pore with rates dependent on size; their cytoplasmic or nuclear residency is defined by the specificity of interaction with insoluble nuclear or cytoplasmic components. Larger molecules, e.g., nucleoplasmin, SV40 large T antigen, or more complex structures, such as RNPs, may

require activation of the pore complex ATPase to enlarge the functional pore channel. Insulin (26), RNA, poly A, poly G (2), possibly mRNA-bound proteins (3), and perhaps a stretch of charged amino acid sequences in nuclear bound proteins (10, 16) could possibly activate an ATPase providing a "passport for transport". The observation that spermine and spermidine cause transport stimulation (Table I) may be a reflection of this latter possibility. A prediction of this model is that the nuclear pore complex has contractile machinery to "open and close" the pore. Such physical alterations may involve actin and myosin, or conformational changes in diaphragm proteins.

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## References

1. Agutter, P. S. 1980. Influence of nucleotides, cations and nucleoside triphosphatase inhibitors on the release of ribonucleic acid from isolated rat liver nuclei. *Biochem. J.* 188:91-97.
2. Agutter, P. S. 1984. Nucleocytoplasmic RNA transport. *Subcell. Biochem.* 10:281-357.
3. Baer, B. W., and R. D. Kornberg. 1983. The protein responsible for the repeating structure of cytoplasmic poly(A)-ribonucleoprotein. *J. Cell Biol.* 96:717-721.
4. Baglia, F. A., and G. G. Maul. 1983. Nuclear ribonucleoprotein release and nucleoside triphosphatase activity are inhibited by antibodies directed against one nuclear matrix glycoprotein. *Proc. Natl. Acad. Sci. USA.* 80:2285-2289.
5. Berrios, M., B. Blobel, and P. A. Fisher. 1983. Characterization of an ATPase/dATPase activity associated with the *Drosophila* nuclear matrix-pore complex-lamina fraction. *J. Biol. Chem.* 258:4548-4555.
6. Clawson, G. A., J. James, C. H. Woo, D. S. Friend, D. Moody, and E. A. Smuckler. 1980. Pertinence of nuclear envelope nucleoside triphosphatase activity to ribonucleic acid transport. *Biochemistry.* 19:2748-2756.
7. Dabauvalle, M. C., and W. W. Franke. 1982. Karyophilic proteins: polypeptides synthesized *in vitro* accumulate in the nucleus on microinjection into the cytoplasm of amphibian oocytes. *Proc. Natl. Acad. Sci. USA.* 79:5302-5306.
8. deRobertis, E. M. 1983. Nucleocytoplasmic segregation of proteins and RNA's. *Cell.* 32:1021-1025.
9. Dingwall, C., S. V. Sharnick, and R. A. Laskey. 1982. A polypeptide domain that specifies migration of nucleoplasmin into the nucleus. *Cell.* 30:449-458.
10. Dingwall, C., and J. Allan. 1984. Accumulation of the isolated carboxy-terminal domain of histone H1 in the *Xenopus* oocyte nucleus. *EMBO (Eur. Mol. Biol. Organ.) J.* 3:1933-1937.
11. DuPraw, E. J. 1970. DNA and Chromosomes. Holt, Rinehart, and Winston, New York. 170-180.
12. Feldherr, C. M. 1965. The effect of the electron-opaque pore material on exchanges through the nuclear annuli. *J. Cell Biol.* 25:43-53.
13. Feldherr, C. M., E. Kallenbach, and N. Schultz. 1984. Movement of a karyophilic protein through the nuclear pore of oocytes. *J. Cell Biol.* 99:2216-2222.
14. Gall, J. G. 1967. Octagonal nuclear pores. *J. Cell Biol.* 32:391-399.
15. Gerace, L., Y. Ottaviano, and C. Kondor-Koch. 1982. Identification of a major polypeptide of the nuclear pore complex. *J. Cell Biol.* 95:826-837.
16. Kalderon, D., W. D. Richardson, A. F. Markham, and A. E. Smith. 1984. Sequence requirements for nuclear location of Simian virus 40 large-T antigen. *Nature (Lond.)* 311:33-38.
17. Kay, R. R., D. Fraser, and I. R. Johnston. 1972. Method for the rapid isolation of nuclear membranes from rat liver. Characterization of the membrane preparation and its associated DNA polymerase. *Eur. J. Biochem.* 30:145-154.
18. Kessel, R. G. 1973. Structure and function of the nuclear envelope and related cytomembranes. *Prog. Surf. Membr. Sci.* 6:243-329.
19. Machicao, E., and O. H. Wieland. 1984. Evidence that insulin receptor-associated protein kinase acts as a phosphatidylinositol kinase. *FEBS (Fed. Eur. Biochem. Soc.) Lett.* 175:113-116.
20. Maul, G. G. 1977. The nuclear and the cytoplasmic pore complex structure, dynamics, distribution, and evolution. *Int. Rev. Cytol.* 6:75-186.
21. Paine, P. L., L. C. Moore, and S. B. Horowitz. 1975. Nuclear envelope permeability. *Nature (Lond.)* 254:109-114.
22. Pappenheimer, J. R., E. M. Renkin, and L. Borrero. 1951. Filtration, diffusion, and molecular sieving through peripheral capillary membranes. *Am. J. Physiol.* 167:12-31.

23. Peters, R. 1983. Nuclear envelope permeability measured by fluorescence microphotolysis of single liver cell nuclei. *J. Biol. Chem.* 258:11427-11429.
24. Peters, R. 1984. Nucleo-cytoplasmic flux and intracellular mobility in single hepatocytes measured by fluorescence microphotolysis. *EMBO (Eur. Mol. Biol. Organ.) J.* 3:1831-1836.
25. Peters, R. 1984. Flux measurement in single cells by fluorescence microphotolysis. *Eur. Biophys. J.* 11:43-50.
26. Purrello, F., R. Vigneri, G. A. Clawson, and I. D. Goldine. 1982. Insulin stimulation of nucleoside triphosphatase activity in isolated nuclear envelopes. *Science (Wash. DC)*. 216:1005-1007.
27. Renkin, E. M. 1954. Filtration, diffusion, and molecular sieving through porous cellulose membranes. *J. Gen. Physiol.* 38:225-243.
28. Schindler, M. 1984. Alterations in nuclear anatomy by chemical modification of proteins in isolated rat liver nuclei. *Exp. Cell Res.* 150:84-96.
29. Schindler, M., J. F. Holland, and M. Hogan. 1985. Lateral diffusion in nuclear membranes. *J. Cell Biol.* 100:1408-1415.
30. Schumm, D. E., and T. E. Webb. 1981. Insulin-modulated transport of RNA from isolated liver nuclei. *Arch. Biochem. Biophys.* 210:275-279.
31. Smith, C. D., and W. W. Wells. 1984. Solubilization and reconstitution of a nuclear envelope-associated ATP-ase. *J. Biol. Chem.* 259:11890-11894.
32. Smith, C. D. 1984. Polyphosphoinositide metabolism in rat liver nuclear envelopes and its role in regulating nucleoside triphosphatase activity. Ph.D. thesis. Michigan State University, East Lansing, MI. 1-233.
33. Stevens, B. J., and H. Swift. 1966. RNA transport from nucleus to cytoplasm in *Chironomus* salivary glands. *J. Cell Biol.* 31:55-77.
34. Stevens, B. J., and J. Andre. 1969. The nuclear envelope. *Handb. Mol. Cytol.* 15:837-871.
35. Unwin, P. N. T., and R. A. Milligan. 1982. A large particle associated with the perimeter of the nuclear pore complex. *J. Cell Biol.* 93:3-75.
36. Virtanen, I. 1977. Identification of concanavalin A binding glycoproteins of rat liver cell nuclear membranes. *Biochem. Biophys. Res. Commun.* 78:1411-1417.
37. Wunderlich, F., and W. W. Franke. 1968. Structure of macronuclear envelopes of *Tetrahymena pyriformis* in the stationary phase of growth. *J. Cell Biol.* 38:458-462.