Chamberlin, M., McGrath, J., & Waskell, L. (1970) *Nature (London) 228*, 227–231.
Chapman, K. A., & Wells, R. D. (1982) *Nucleic Acids Res. 10*, 6331–6340.
Davanloo, P., Rosenberg, A. H., Dunn, J. J., & Studier, F. W. (1984) *Proc. Natl. Acad. Sci. U.S.A. 81*, 2035–2039.
Dunn, J. J., & Studier, F. W. (1983) *J. Mol. Biol. 166*, 477–535.
Galas, D. J., & Schmitz, A. (1978) *Nucleic Acids Res. 5*, 3157–3170.
Hertzberg, R. P., & Dervan, P. B. (1982) *J. Am. Chem. Soc. 104*, 313–315.
Hertzberg, R. P., & Dervan, P. B. (1984) *Biochemistry 23*, 3934–3945.
Ikeda, R. A., & Richardson, C. C. (1986) *Proc. Natl. Acad. Sci. U.S.A. 83*, 3614–3618.
Jollife, L. K., Carter, A. D., & McAllister, W. T. (1982) *Nature (London) 299*, 653–656.
Kastrup, R. V., Young, M. A., & Krugh, T. R. (1978) *Biochemistry 17*, 4855–4865.
Kornberg, A. (1980) in *DNA Replication*, pp 139–142, Freeman, San Francisco.
Maxam, A. M., & Gilbert, W. (1980) *Methods Enzymol. 65*, 520–523.
McAllister, W. T., Morris, C., Rosenberg, A. H., & Studier, F. W. (1981) *J. Mol. Biol. 153*, 527–544.
Record, M. T., deHaseth, P. L., & Lohman, T. M. (1977) *Biochemistry 16*, 4791–4796.
Schneider, T. D., Stormo, G. D., Gold, L., & Ehrenfeucht, A. (1986) *J. Mol. Biol. 188*, 415–431.
Strauss, H. S., Burgess, R. R., & Record, M. T. (1980) *Biochemistry 19*, 3504–3515.
Studier, F. W., & Rosenberg, A. H. (1981) *J. Mol. Biol. 153*, 503–525.
Tabor, S., & Richardson, C. C. (1985) *Proc. Natl. Acad. Sci. U.S.A. 82*, 1074–1078.

Fluorescence Photobleaching Analysis of Nuclear Transport: Dynamic Evidence for Auxiliary Channels in Detergent-Treated Nuclei†

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Received April 29, 1986; Revised Manuscript Received October 28, 1986

ABSTRACT: Nuclear transport experiments were performed on isolated rat liver nuclei to examine the permeability of membrane and detergent-free peripheral nuclear lamina. The transport of 64K molecular weight fluorescent-derivatized dextrans was measured by using the technique of fluorescence redistribution after photobleaching. Results of these experiments provide evidence for transport pathways that appear to be functionally distinct from nuclear pore complex channels. The suggestion is made that these supplemental pathways are embedded in the peripheral nuclear lamina and are normally masked by the inner nuclear membrane.

Nucleocytoplasmic communication appears to be mediated by a multicomponent octagonally symmetric cylindrical structure spanning the double membrane of the nucleus (deRobertis, 1983; Gall, 1967; Kessel, 1973). These nuclear pore complexes have been investigated by a number of microscopic techniques which, for the most part, suggest a hollow cylinder containing strandlike structures. The cytoplasmic face of the nuclear pore complex is decorated with spherical components (Kessel, 1973; Unwin & Milligan, 1982). In all cases, these pore complexes have been visualized in nuclei that have retained their membranes following isolation (Kessel, 1973; Unwin & Milligan, 1982) or nuclei that have been treated with the detergent Triton X-100 (Kirschner et al., 1977; Schindler, 1984). Recently, Schindler and Hogan (1985) succeeded in preparing nuclei that had neither membranes nor detergent bound to their surfaces. In addition, the content of lamins (A, B, and C) was significantly reduced. The exposed nuclear surface showed a highly pebbled surface consisting of an interlocking array of circular particles with diameters of ~80–100 nm. Many of these particles had holes in their center and suggested the structure of pore complexes without the octagonal bonnet of annular subunits. Such structures have also been observed by Kuzmina et al. (1981), Schatten and Thoman (1978), and Kirschner et al. (1977), who considered them incomplete pore complexes. The surface density of these structures was 2–3 times that observed for "classic" pore complexes in whole nuclei or nuclei following Triton X-100 treatment (Kirschner et al., 1977). The suggestion was made that such structures may serve as preformed pore templates capable of activation to form complete nuclear pore complexes (Schindler & Hogan, 1985). This was an appealing hypothesis considering the dynamic nature of pore assembly and disassembly which appears to vary with cell metabolism and stimulation (Maul, 1977a,b). The pore precursor suggestion also correlated with observations that pore synthesis was found to occur without concomitant protein biosynthesis (Maul, 1977a,b) or nuclear surface expansion (Maul & Deaven, 1977). To investigate the possibility that the holes observed in detergent and membrane-free nuclei may indeed serve as potential transport routes, we have examined the transport rate of 64K molecular weight fluorescent-labeled dextrans into nuclei treated with a number of membrane-active agents. Using the technique of fluorescence redistribution after photobleaching (FRAP), we observe transport rates that suggest the holes, observed following detergent treatment, have some of the transport properties of nuclear pore complexes as previously demonstrated by Jiang and Schindler (1986), Schindler and Jiang (1986), and Peters (1983, 1984).

† This work was supported by NIH Grant GM 30158.
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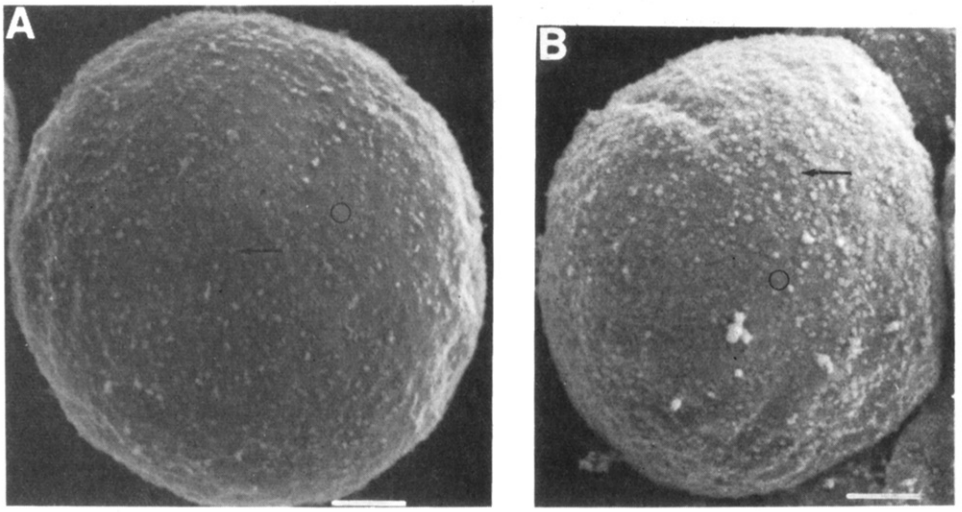

FIGURE 1: Scanning electron micrographs (SEM) of nuclear surfaces following detergent treatment. Nuclei treated (as described under Materials and Methods) with 2% Triton X-100 (A) and octyl-POE (B). The bars represent 1 μm.

Materials and Methods

Nuclear Isolation and Detergent Treatment. Rat liver nuclei [100 OD_{260} units (Schindler, 1984)] prepared as described (Schindler, 1984) were suspended in 50 mM *N*-(2-hydroxyethyl)piperazine-*N'*-2-ethanesulfonic acid (Hepes)–1 mM Mg^{2+}–1% octylpoly(oxyethylene) (octyl-POE)–0.1 mM dithiothreitol, pH 7.4, and incubated on ice for 0.5 h. The suspension (1 mL) was washed 3 times with 10 mM Hepes–1 mM Mg^{2+}–0.25 M sucrose, pH 7.4, by suspension and pelleting in an Eppendorf centrifuge (15000*g* for 5 s). For Triton X-100 experiments, nuclei [100 OD_{260} units (Schindler, 1984)] were suspended in 2% Triton X-100 (1 mL) as described in Kirschner et al. (1977). Incubation proceeded for 0.5 h on ice and was followed by three washes with 10 mM Hepes–1 mM Mg^{2+}–0.25 M sucrose, pH 7.4. Antibodies to lamins A and C were kindly supplied by Dr. Larry Gerace, Department of Cell Biology, Johns Hopkins University.

Scanning Electron Microscopy. Samples for scanning electron microscopy (SEM) were fixed in 4% glutaraldehyde for 1 h, followed by postfixation in 2% O_SO_4 for 30 min. The nuclei were then added to slides coated with 1% poly(L-lysine) (220 K molecular weight). Adherence occurred following 5-min settling on the still-damp slide. Samples were gently washed and dehydrated through graded ethanols and then critically point dried, gold coated (coat routinely less than 15 nm), and viewed.

Polyacrylamide Gel Electrophoresis. Samples were electrophoresed with an 11% sodium dodecyl sulfate–polyacrylamide gel system as described (Schindler, 1984).

FRAP Measurement and Analysis. Incubation of dextrans with nuclei and photobleaching were performed as described (Jiang & Schindler, 1986; Peters, 1983). Briefly, a laser beam with a Gaussian profile (~6 μm in diameter) is scanned across a spherical nucleus suspended in a fluorescent dextran solution. The beam in the focal plane covers a major part of the nuclear cross section, ensuring that a photobleach will maximally destroy the fluorescence in the nucleus. After a bleaching cycle, redistribution of fluorescence between the nucleoplasm and solution was calculated by the method of Peters (1983, 1984). As demonstrated by Peters (1983), the data are satisfactorily represented by the equation:

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

or the sum of two exponentials (Peters, 1983, 1984). $F(-)$ is the fluorescence signal before (prebleach), $F(0)$ immediately after, and $F(t)$ at time t after photobleaching. In all instances, multiple bleaches on the same sample demonstrated equivalent recovery profiles, which suggests no major photochemical damage.

Results

Examination of Octyl-POE and Triton X-100 Extraction on the Surface Morphology and Nuclear Polypeptide Profile of Isolated Rat Liver Nuclei. Rat liver nuclei isolated as described (Schindler, 1984) were used as the starting material for detergent treatment. Figure 1 presents scanning electron micrographs of the nuclear surface treated with Triton X-100 (Figure 1A) and octyl-POE (Figure 1B). Although characteristic pore complexes are present in Figure 1A,B (arrows), the surface in Figure 1B appears coarser with more observable holes (circle). The Triton X-100 surface also had some holes but to a lesser extent (circle). The white puffs represent chromatin extruding through the surface, presumably through the hole (as determined by TEM; Schindler & Hogan, 1985). These images serve to show that no major gaps or breaks occur in the nuclear surface as a consequence of detergent treatment and washing. Sodium dodecyl sulfate (SDS)–polyacrylamide gels demonstrate that the polypeptide profiles are similar, particularly in the region of the lamins (Figure 2; see arrowhead), for detergent-treated nuclei when compared to non-detergent-treated nuclei. A careful measurement of cross-sectional diameters suggests that detergent treatment does not significantly alter nuclear size; if anything, the nuclei expand mildly. Thin-layer chromatography demonstrated that detergent treatment followed by washing (2–3 times with nuclear buffer) removed both nuclear membrane phospholipids (Schindler & Hogan, 1985). A significant difference, however, was observed in the amount of detergent remaining following washes; Triton X-100 bound to the surface following the washes, while octyl-POE binding was considerably less after washes (Schindler & Hogan, 1985).

Measurement of Fluorescent Dextran Transport in Nuclei. Nuclei, with and without detergent treatment, were subjected to the fluorescent dextran transport assay developed by Peters (1983, 1984) and employed by Jiang and Schindler (1986). Figure 3 presents the recovery patterns for 64K molecular weight fluorescent dextrans in control, Triton X-100 treated, and octyl-POE-treated nuclei. Octyl-POE treatment of nuclei results in an enhanced recovery rate of dextrans. This also

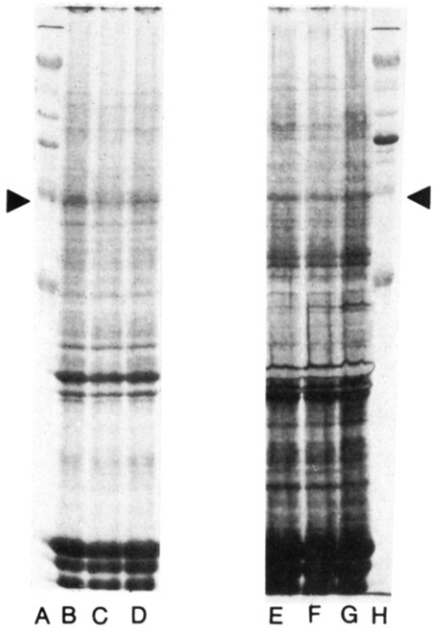

FIGURE 2: SDS–polyacrylamide gel electrophoretogram of Triton X-100 and octyl-POE-treated nuclei. The lanes represent the following: standard proteins (A); control nuclei (no detergent treatment) (B); 2% Triton X-100 nuclear pellet fraction (C); 2% octyl-POE nuclear pellet fraction (D). Lanes A–D are the Coomassie-stained pattern, while lanes E–H are the same samples, respectively, but visualized with silver stain. The standards from the top of the gel to the bottom are as follows: myosin [200 kilodaltons (kDa)]; β-galactosidase (166.5 kDa); phosphorylase B (92.5 kDa); bovine serum albumin (66.2 kDa); and ovalbumin (45 kDa), respectively. Arrows indicates the molecular weight region containing the lamins.

Table I: 64K Dextran Transport in Rat Liver Nuclei

treatment of nuclei	flux rate ($\times 10^3$ s)	% change from control
(A) Controls		
buffer	2.2 ± 0.8[a] (14)[b]	
anti-lamins (A, C) (20 μL)[c]	3.2 ● 1.5 (6)	+45
AMPPCP (1 mM)	2.1 ± 1.1 (16)	−5
150K dextrans	NT[f]	
Con A (0.1 mg/mL)	≤0.1 (10)	
ATP (1 mM)	7.4 ● 1.6 (4)	+236
(B) Membrane Agents		
octyl-POE (1%)	7.0 ± 1.6 (15)	+218
octyl-POE (1%) + ATP (1 mM)	11.0 ± 3.0 (9)	+400
octyl-POE (1%) + AMPPCP (1 mM)	6.4 ± 1.4 (4)	+191
octyl-POE (1%) + Con A (0.1 mg/mL)	≤0.1 (5)	
Con A (0.1 mg/mL) + octyl-POE (1%)[d]	5.1 ± 1.3 (5)	+132
Con A (0.1 mg/mL) + octyl-POE (1%) + ATP[d]	5.2 ± 1.7 (3)	+136
Con A (0.1 mg/mL) + octyl-POE (1%) ± ATP[e]	≤0.1 (7)	
octyl-POE (1%) + 150K dextrans	NT[f]	
Triton X-100 (2%)	4.4 + 0.7 (7)	+100
citraconic anhydride (6 mM)	3.5 ± 1.0 (8)	+59

[a] Mean ● SD. [b] Number of experiments. [c] 20 μL of anti-lamin (A, C) solution of 650 μg/mL total protein. [d] Pretreatment with Con A followed by octyl-POE extraction. [e] Simultaneous incubation of Con A and octyl-POE followed by washing as described under Materials and Methods. [f] NT, no transport.

occurs to a more limited extent following Triton X-100 extraction. Table I is a compilation of the transport results. The transport rate obtained for dextrans in isolated rat liver nuclei without any treatment is the same as reported by Peters (1983)

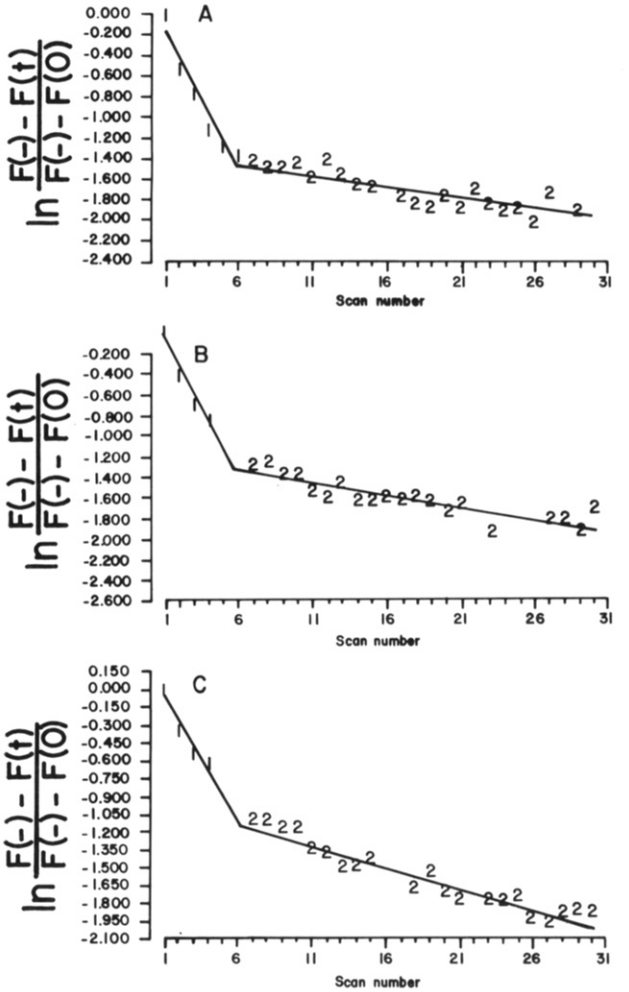

FIGURE 3: Fluorescence recovery of labeled dextrans in detergent-treated nuclei after photobleaching. A semilogarithmic plot of $\ln\{[F(-) - F(t)]/[F(-) - F(0)]\}$ as a function of scan number, where $F(-)$, $F(0)$, and $F(t)$ are the fluorescence signals before the photobleach, after, and at time t following the bleach (Jiang & Schindler, 1986). Recovery of fluorescence can best be fitted by least-squares fits to the sum of two first-order terms. Component 1 is sufficiently fast to be ascribed to surface adsorption, while component 2 corresponds to the recovery term used by Peters (1983) to calculate dextran flux. (A) represents fluorescence recovery of dextrans in control nuclei, (B) shows recovery in nuclei extracted with Triton X-100, while (C) shows recovery in octyl-POE-extracted nuclei. Each scan with delay is 5 s.

and Jiang and Schindler (1986). It is important to note that in whole nuclei, Con A blocks transport as previously reported (Jiang & Schindler, 1986). Dextrans larger than the calculated pore exclusion size are excluded (no transport observed for the 150K dextrans). The lamin antibodies and adenosine 5′-(β-γ-methylenetriphosphate) (AMPPCP) have no effect on transport. Extraction with octyl-POE (1%) increases transport by ~218%. Addition of AMPPCP (1 mM), an ATPase competitive inhibitor, to octyl-POE-extracted nuclei did not decrease the rate enhancement, while addition of ATP (1 mM) enhanced dextran flux approximately +57% when compared to octyl-POE-treated nuclei. An ATP-mediated increase is also observed in untreated nuclei, resulting in a +236% rate enhancement over whole untreated nuclei (Jiang & Schindler, 1986). Addition of Con A to octyl-POE-extracted nuclei essentially blocked transport as was demonstrated in untreated controls. This result, in conjunction with the demonstration that 150-kilodalton dextrans do not enter untreated or octyl-POE-extracted nuclei, and the scanning electron micrographs (Figure 1) provide evidence that the enhanced transport

Table II: 64K Dextran Transport in the Presence of Anti-lamins and Anti-actin

treatment of nuclei	flux rate ($\times 10^3$ s)	% change from octyl-POE-extracted sample
octyl-POE (1%)	7.0 ± 1.6[a] (15)[b]	
octyl-POE (1%) + anti-lamins A and C (20 μL)[c]	2.9 ± 1.0 (3)	−59
	~0.1 (3)	−99
octyl-POE (1%) + anti-actin (20 μL)[d]	4.1 ± 1.5 (4)	−41

[a] Mean ± SD. [b] Number of experiments. [c] 20 μL of an anti-lamin (A and C) solution of 650 μg/mL total protein. [d] 20 μL of an anti-actin solution of 640 μg/mL total protein.

rate of dextrans following detergent treatment is not a consequence of major nuclear surface disruptions but occurs across structurally preserved peripheral lamina.

In an additional attempt to demonstrate that nuclear transport can occur through submembranous channels that are exposed by detergent treatment, transport studies were performed on nuclei pretreated with Con A and then washed with octyl-POE. The transport rate, when compared with whole untreated nuclei, was enhanced by +132% rather than the +218% for octyl-POE-treated nuclei, suggesting that POE extraction exposes the available submembranous channels allowing transport through those channels, while Con A blocks the pore complexes. That Con A indeed remains attached in the presence of octyl-POE is also demonstrated by incubating Con A and octyl-POE together and then washing the nuclei to remove detergent. Under these conditions, transport is once again blocked completely (compare parts A and B of Table I). To examine whether the alternate transport channels are affected by ATP, nuclei were again pretreated with Con A and washed with octyl-POE. These nuclei were then treated with ATP and resulted in no transport enhancement when compared to those without adding ATP. Similarly, nuclei coincubated with Con A and octyl-POE, washed, and then assayed for transport in the presence of ATP were still incapable of transport. This is quite different from the observation of ATP-mediated rate enhancement for octyl-POE (1%) treated nuclei without Con A (+57% when compared to control octyl-POE nuclei).

Inhibitory Effects of Anti-lamins and Anti-actin Antibodies. Addition of anti-lamin A and C antibodies to whole nuclei had no inhibitory effect on dextran transport (Table I). When these antibodies are added to octyl-POE-extracted nuclei, however, transport was inhibited between −59 and −99% (Table II). Anti-actin antibody also decreased the dextran flux in octyl-POE-extracted nuclei by −41% (Table II).

Other Membrane Reagents and Effects on Flux. Citraconic anhydride (6 mM) was demonstrated to specifically remove the phospholipids of the outer membrane (Schindler et al., 1985). Nuclei treated with 6 mM citraconic anhydride were assayed for dextran transport. A small increase was observed, approximately +59%, but this rate increase was still considerably less than that observed upon removal of both membranes, +218%. In fact, the transport value obtained following treatment with 6 mM citraconic anhydride was closer to the increase noted following Triton X-100 treatment, ~100% (Table I).

Discussion

In a careful analysis of the surface of Triton X-100 treated rat liver nuclei, Kirschner et al. (1977) presented scanning and transmission electron micrographs showing nuclear pore complexes and additional structures resembling interlocking doughnuts which were reminiscent of nuclear pore complexes, but without the presence of additional capping elements, e.g., annular subunits. These structures were suggested to be incomplete pore complexes which Kirschner et al. (1977) suggested "reflect the dynamic state of the nuclear pore complexes and their formation and dissolution in response to structural or functional alterations in the underlying chromatin". Such "doughnuts" had also been previously reported by Kuzmina et al. (1981) and Schatten and Thoman (1978). Arechago and Bahr (1985), using a number of electron microscopic techniques, presented evidence for "nuclear holes" integrated into the nuclear lamina and masked by the inner nuclear membrane. The diameter of these holes, as calculated by them, was similar to the channel diameter observed for nuclear pore complexes (Arechago & Bahr, 1985). Employing scanning electron microscopy, Schindler and Hogan (1985) provided evidence that treatment of isolated rat liver nuclei with octyl-POE, a detergent easily removable by washing, exposed 2–3 times the number of potential doughnut structures with holes when compared to untreated, isolated nuclei containing inner and outer membranes. Considering the limitations of electron microscopy in examining dynamic events and the requirement for sample preparation and fixation, this study attempted a dynamic approach based on the use of classic diffusion equations to define the diameter and density of transport channels. Since the rate of transport is proportional to the physical properties of the transport channel and may, therefore, be used to make statements about diffusive competency, it could provide information not obtainable by electron microscopy, namely, a transport rate and not a colocalization as is observed for examining colloidal gold transport through the nuclear pore (Feldherr, 1984). Previous work by us (Jiang & Schindler, 1986) and Peters (1983, 1984) has demonstrated that nuclear transport rates may be obtained by using fluorescent-derivatized dextrans and the technique of fluorescence redistribution after photobleaching (FRAP). The use of dextrans as a transport probe is significant, since they do not contain putative nuclear targeting, transport, or sequestering sequences (Kalderon et al., 1984). Modification of transport rate, therefore, may be interpreted as changes in the diameter of the channel or alterations in the number of channels. Using this transport assay, we now compared whole nuclei to octyl-POE-extracted nuclei, finding a 218% increase in dextran flux from 2.2 (±0.1) × 10^{-3} to 7.0 (±1.6) × 10^{-3} s^{-1}. The enhancement of transport appears to be related to an increase in the number of channels, since (a) detergent treatment does not introduce large gaps in the nuclear surface as demonstrated by scanning electron microscopy (Figure 1) or result in major changes in the nuclear polypeptide profile (Figure 2), (b) a major change in pore diameter is unlikely, considering that 150K molecular weight dextrans were still excluded from nuclear entry and Con A was still capable of blocking transport, and (c) octyl-POE does not activate a nuclear pore ATPase which may alter channel diameter (Schindler & Jiang, 1986), since enhancement still occurs in the presence of AMPPCP, an ATPase competitive inhibitor. If one assumes that the transport enhancement is predominantly related to the increase in patent channels following detergent-mediated unmasking, then a comparison of rates suggests an increase of ~2.2-fold in channel density (obtained from the observed +218% rate enhancement over untreated nuclei). This, in fact, fits extremely well with our scanning electron microscopic evidence showing a 2–3-fold density increase in channellike structures following octyl-POE extraction. Another dynamic approach to ascertaining whether octyl-POE exposes previously

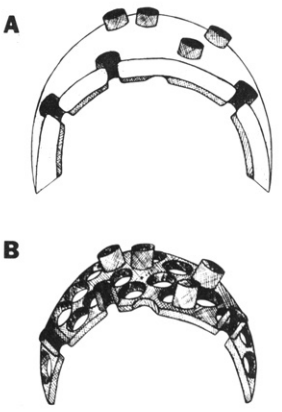

FIGURE 4: Schematic representation of nuclear surface. Scheme A represents the nuclear surface composed of nuclear pore complexes (cylinders) and the double bilayer/lamina envelope. Scheme B represents a postulated membrane-free nuclear surface containing unmasked "holes" and nuclear pore complexes.

masked transport competent channels involves the use of Con A to block transport. Treatment of nuclei with octyl-POE exposes the nuclear surface. If Con A is added either to octyl-POE-extracted nuclei or in the presence of octyl-POE, transport is blocked. This provides evidence that all channels, both nuclear pore complexes and previously masked "holes", have glycoproteins capable of binding Con A. If, on the other hand, nuclei are pretreated with Con A, washed, and then extracted with octyl-POE, nuclear transport occurs, but only +132% rate enhancement over untreated nuclei. Theoretically, if nuclear pore complexes are completely blocked, and octyl-POE only unmasks ~2.2-fold more transit channels, then one would expect a transport rate enhancement of +120% for Con A pretreatment followed by octyl-POE extraction of nuclei. The experimental and theoretical values differ by ~10%. Additional evidence that these membrane-masked channels are in the nuclear lamina may be inferred from the blocking effect of anti-lamin A and C antibodies on transport.

Lamin Interactions with Nuclear Holes. Anti-lamin A and C antibodies were found to inhibit transport of dextrans in octyl-POE-extracted nuclei but not in whole nuclei. Two possibilities for this effect are that the anti-lamin antibodies react with lamins that either are in intimate contact with the hole structures or actually are elements of the holes. In this case, antibodies could sterically block access to the channels. Another possibility is that binding of antibodies to lamins can indirectly influence transport through the holes. Our present evidence cannot discriminate between these two possibilities.

Membrane Masked Transport Channels: A Model. Figure 4 presents a model explaining the observed flux increase in terms of more available size-limited transport channels. Scheme A presents the classic view of pores embedded in the double nuclear membrane. The circles beneath the inner membrane are masked transport channels or incomplete pores (Kirschner et al., 1977). Following removal of the membranes, the channels become patent and should theoretically increase transport rates by approximately a factor of 3–4 or (6.6–8.8) $\times 10^{-3}$ s^{-1} from 2.2×10^{-3} s^{-1}. The actual number obtained is 7.0×10^{-3} s^{-1}. Additional evidence is provided by the ATP enhancement effect. If we assume that the nascent transport channels are precursors and do not have the ATPase activity demonstrated for whole nuclear pore transport (Jiang & Schindler, 1986), then the addition of ATP should only enhance the full-formed pore transport and not affect the nascent channel transport. The resulting theoretical rate for ATP enhancement based on scheme B would be 12.2×10^{-3} s^{-1} $[2.2(2.2 \times 10^{-3}\ s^{-1}) + 7.4 \times 10^{-3}\ s^{-1}]$. The actual rate for ATP (1 mM) enhancement was 11×10^{-3} s^{-1} or 10% below the theoretical value. These results suggest that scheme B may be appropriate and that the holes or pore precursors may be missing the pore diameter controlling elements. This is further supported by experiments with anti-actin antibodies. Actin was demonstrated to be an ATP-responsive controlling element in dextran transport (Schindler & Jiang, 1986). Addition of anti-actin to octyl-POE-extracted nuclei should theoretically decrease the flux to approximately 4.8×10^{-3} s^{-1} considering its strong inhibitory effect on pore transport. The measured flux in the presence of anti-actin was 4.1×10^{-3} (Table II) or within 15% of the theoretical value based on the assumption of no ATP-dependent controlling elements in the holes. Although the control elements appear to be missing from the detergent-unmasked holes, Con A receptors apparently are associated with these structures since Con A essentially blocks dextran transport in the octyl-POE-treated nuclei. Since the dominant Con A binding glycoprotein in the nuclear lamina has been implicated as an element in anchoring nuclear pore complexes (Davis & Blobel, 1986), it could exist as a component of incomplete pores.

Detergent-Treated Nuclei and Inner Membrane Attached Nuclei. Further support for our contention that inner membrane masks pore precursors or transit holes and that detergents that bind to the nuclear surface may similarly block or mask these structures is obtained by a comparison of flux rates for nuclei treated with Triton X-100 and 6 mM citraconic anhydride. Nuclei treated with 1–2% Triton X-100 show the classic pore complexes interspersed by smooth surfaces (Figure 1A)(Jiang & Schindler, 1986). Treatment of nuclei with 6 mM citraconic anhydride selectively removes outer nuclear membrane (Schindler et al., 1985). Transport rates observed for nuclei subjected to these two treatments which remove the outer membrane but maintain a phospholipid or detergent layer on the nuclear lamina (defined here as an integrated lamin/pore precursor surface) are similar (3.5×10^{-3} s^{-1} for citraconic treatment compared to 4.4×10^{-3} s^{-1} for Triton X-100).

Functional Implications. A key metabolic observation shows that neither protein biosynthesis (Maul, 1977a,b) nor nuclear surface expansion (Maul & Deaven, 1977) is required when the pore complex number is doubled. This would suggest that a pool of pore precursors exists in the cell that can be recruited during cell division or mitogenic stimulation. Further, the pore density in the most transcriptionally and metabolically active nucleus, *Xenopus laevis* oocyte, is ~48.0 ± 5.3 pores/μm^2 (Maul, 1977a,b), while that for rat hepatocyte has been reported to be 14–16 pores/μm^2 (Maul, 1977a,b). A comparison of these numbers might suggest that the nucleus may, in fact, have a 2–3-fold reserve capacity with regard to channels that can be transformed into regulatable nuclear pore complexes by the addition of appropriate polypeptides. Our data would suggest that an ATPase would be one of those elements. In the context of gene expression, it would be interesting to establish whether nuclear pore complexes may be formed in response to gene activation; this would imply that the putative membrane masked pore precursors represent transport channels not in use because of association with nontranscribing genes.

ACKNOWLEDGMENTS

We appreciate the technical assistance of M. Hogan in preparing and photographing SEM samples and S. Meiners in drafting Figure 4.

REFERENCES
Arechago, J., & Bahr, G. F. (1985) *J. Cell. Biochem., Suppl. 9A*, 23–50.
Davis, L. I., & Blobel, G. (1986) *Cell (Cambridge, Mass.) 45*, 699–709.
deRobertis, E. M. (1983) *Cell (Cambridge, Mass.) 32*, 1021–1025.
Gall, J. G. (1967) *J. Cell Biol. 32*, 391–399.
Gerace, L., & Blobel, G. (1982) *Cold Spring Harbor Symp. Quant. Biol. 16*, 967–978.
Jiang, L.-W. & Schindler, M. (1986) *J. Cell Biol. 102*, 853–858.
Kalderon, D., Roberts, B. L., Richardson, W. D., & Smith, A. E. (1984) *Cell (Cambridge, Mass.) 39*, 499–509.
Kessel, R. G. (1973) *Prog. Surf. Membr. Sci. 6*, 243–329.
Kirschner, R. H., Rusli, M., & Marten, T. E. (1977) *J. Cell Biol. 72*, 118–132.
Kuzmina, S., Buldyeva, T., Troitkaya, L., & Zborsky, J. (1981) *J. Cell Biol. 25*, 225–232.
Maul, G. G. (1977a) *Int. Rev. Cytol. 6*, 75–186.
Maul, G. G. (1977b) *J. Cell Biol. 74*, 492–500.
Maul, G. G., & Deavan, L. (1977) *J. Cell Biol. 73*, 748–760.
Peters, R. (1983) *J. Biol. Chem. 258*, 11427–11429.
Peters, R. (1984) *EMBO J. 3*, 1831–1836.
Schatten, G., & Thoman, M. (1978) *J. Cell Biol. 77*, 517–535.
Schindler, M. (1984) *Exp. Cell Res. 150*, 84–96.
Schindler, M., & Hogan, M. (1985) *J. Cell. Biochem., Suppl. 9A*, 51–61.
Schindler, M., & Jiang, L.-W. (1986) *J. Cell Biol. 102*, 859–862.
Schindler, M., Holland, J. F., & Hogan, M., (1985) *J. Cell Biol. 100*, 1408–1415.
Unwin, P. N. T., & Milligan, R. A. (1982) *J. Cell Biol. 93*, 63–75.

A Fluorescence Study of the Binding of Eucaryotic Initiation Factors to Messenger RNA and Messenger RNA Analogues†

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Received July 30, 1986; Revised Manuscript Received November 24, 1986

ABSTRACT: The binding of the eucaryotic polypeptide chain initiation factors (eIFs) 4A, 4B, and 4F to poly(1,N^6-ethenoadenylic acid) [poly(εA)] was investigated by fluorescence spectroscopy. Competition experiments allowed us to determine the relative affinity of these proteins for mRNA cap analogues and the triplets AUG, GUG, UUU, UAA, and UGA. The salt dependence of eIF-4A binding to poly(εA) and mRNA suggested that the binding was largely electrostatic and was enhanced in the presence of Mg^{2+} and ATP. The size of the binding site of eIF-4A, eIF-4B, and eIF-4F on poly(εA) was approximately 13, 25, and 35 nucleotides, respectively. Fluorescence studies with the cap analogue 7-methylguanosine triphosphate as well as competition studies with poly(εA) provide further evidence for a direct interaction of eIF-4F with the cap region. There was no evidence that either eIF-4B or eIF-4A bound the mRNA cap directly. In contrast to the other two factors, eIF-4B was found to bind preferentially to AUG, and of all the triplets tested, AUG was the most effective competitor for poly(εA) binding.

The interaction of the polypeptide chain initiation factors with mRNA is an important step in the initiation of protein synthesis. This interaction involves the recognition of various structural features of the message including the 7-methylguanosine cap at the 5′ mRNA terminus, the secondary structure, and the AUG initiation codon. Several proteins have been implicated in this process, including polypeptide chain initiation factor 4A (eIF-4A),[1] eIF-4B, and eIF-4F (Grifo et al., 1983; Benne & Hershey, 1978). Recent work has described the activity of these proteins with respect to recognition of the structural features of the mRNA (Shatkin, 1976; Kozak, 1982; Boss et al., 1981; Lomedico & Andrew, 1982; Butler & Clark, 1984; Goss et al., 1985) and changes in the secondary structure of the message induced by these factors (Ray et al., 1985). Butler and Clark (1984) reported that eIF-4B from wheat germ binds to the AUG initiation codon region of satellite tobacco necrosis viral (STNV) messenger RNA. In addition, Ray et al. (1985) reported that eIF-4A has an ATP-dependent mRNA unwinding activity and this activity was more efficient when eIF-4A was part of the eIF-4F complex. It was also suggested that eIF-4F may be a RNA unwinding enzyme which catalyzes the melting of mRNA secondary structure (Sonenberg et al., 1982; Lee et al., 1983;

† Supported in part by grants from the Research Corp., by a PSC-CUNY Faculty Research Award, by an American Heart Association, New York City affiliate, Grant-in-Aid and Investigator Award (to D.J.G.), and by NIH Grant GM 25451.
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[1] Abbreviations: poly(εA), poly(1,N^6-ethenoadenylic acid); eIF, eucaryotic polypeptide chain initiation factor; CD, circular dichroism; HEPES, *N*-(2-hydroxyethyl)piperazine-*N*′-2-ethanesulfonic acid; IF-3, procaryotic polypeptide chain initiation factor 3; STNV, satellite tobacco necrosis virus; Tris, tris(hydroxymethyl)aminomethane; AMPPNP, 5′-adenylyl imidodiphosphate.

0006-2960/87/0426-1551$01.50/0