Lateral Diffusion in Nuclear Membranes

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ABSTRACT   Chemical modification of rat liver nuclei with citraconic anhydride selectively removed outer nuclear membrane. This conclusion was based on (a) transmission electron microscopy, (b) lipid analysis, (c) lamin B as an inner membrane–associated marker, and (d) the demonstration of phospholipid lateral mobility on outer membrane–depleted nuclei as a criteria for inner membrane integrity. Addition of urea or N-ethylmaleimide resulted in the additional disruption of inner membrane. Fluorescence photobleaching was used to determine the long range (>4 μm) lateral transport of lectin receptors and a phospholipid analog in both membranes. The diffusion coefficient for wheat germ agglutinin on whole nuclei was 3.9×10^{-10} cm^2/s whereas the diffusion coefficient for wheat germ agglutinin in outer membrane–depleted nuclei was $\leq 10^{-12}$ cm^2/s. Phospholipid mobilities were the same in whole and outer membrane–depleted nuclei (3.8×10^{-9} cm^2/s). The protein diffusion differences observed between whole and outer membrane–depleted nuclei may be interpreted in the context of two functionally different membrane systems that compose the double bilayer of the nucleus.

The cell nucleus is encapsulated by two bilayer membranes that appear to converge at the periphery of pore complexes (1–3). The outer membrane (cytoplasmic) has been demonstrated to merge with endoplasmic reticulum (ER),[1] while biochemical evidence suggests that a number of ER enzymes may be present in the outer nuclear membrane (1, 2). The composition and nature of the inner nuclear membrane (nucleoplasmic) has for the most part remained a mystery. Morphologically, the inner nuclear membrane appears to be precipitated on a dense lamella or nucleo-cytoskeleton (2, 3). This superstructure is predominantly composed of a series of polypeptides, termed lamins, that form a well-organized polymeric network (4, 5). Evidence from Gerace and Blobel (5) suggest that a subgroup of lamins, lamin B, forms a tight association with the inner nuclear membrane that can be abrogated with Triton X-100. Although these two membranes would appear to be very different structurally, i.e., inner nuclear membrane attaches to a cytoskeletal structure, their separation and characterization has proven difficult. The two most widely used methods for nuclear membrane separation and isolation have been demonstrated to be ineffective (Triton X-100; see reference 6) or chemically disruptive to membrane enzymes through acid denaturation (2.5% citric acid; see references 7 and 8). The evidence used to validate each

procedure has been (*a*) electron microscopic analysis of inner and outer membrane, and (*b*) the presence of phospholipid in pelleting nuclear structures after separation treatment. By these previously used criteria and two others, the requirement of lamin B for inner nuclear membrane integrity (5) and phospholipid lateral mobility on outer membrane–depleted nuclei, it will be shown that citraconic anhydride, a reversible modification reagent capable of selectively solubilizing certain nuclear components (9), simply and rapidly removes outer nuclear membrane under mild reaction conditions that do not result in the proteolysis of nuclear proteins (9). These outer membrane–depleted nuclei have been used in a comparative study to elucidate the lateral mobility of wheat germ agglutinin (WGA), concanavalin A (Con A) receptors, and a phospholipid analog in the outer and inner nuclear membrane. The mobility differences observed suggest two dynamically different membrane systems that may provide some clues to different functional roles.

MATERIALS AND METHODS

Nuclear Isolation and Modification: Nuclei were isolated and prepared essentially as previously described (10) with minor modifications (9). Citraconylation was carried out as described in Schindler (9). Pellet and supernatant fractions were saved for analysis. 1–20 μl of stock reagent (0.89 M) was used to produce the range of modifications reported in this paper. The nuclei had to be present in suspension before the addition of the reagent because of the speed of anhydride hydrolysis.

Transmission Electron Microscopy (TEM):   TEM was performed on nuclear pellets resuspended with 2% glutaraldehyde freshly diluted from 50% stock with pH 7.4 buffer (10 mM HEPES, 1 mM Mg), and fixation was

allowed to proceed for 30 min at 4°C. The nuclei were subsequently washed twice in the same buffer and postfixed in 2% OsO_4 for 1 h. Samples were then pelleted, taken through dehydration, and embedded in Epon/Araldite. Sectioned grids were stained with 2% uranyl acetate for 30 min. Sample size is indicated by the bar which represents 1.0 μm in Fig. 1, A, B, and B', and 0.2 μm in Fig. 1A'.

Phospholipid Labeling and Analysis: The initial approach (endogenous) used the centrifugal separation of the reaction mixture after citraconylation (9) into supernatant and pellet fractions which were extracted with chloroform/methanol (2:1). Phosphate analysis was performed (11) and the percent chloroform-soluble phosphate to total phosphate in pellet and supernatant fractions was plotted. In another approach (exogenous), radioactive lipid extracted from the membranes of *Salmonella typhimurium* G30 (12) was solubilized in ethanol and added to whole nuclei. By assaying for the release of exogenously added phospholipid from nuclei and endogenous phospholipid release, and demonstrating that response to chemical modification is identical, it is presumed that release is a bulk phenomenon. After 15 min of incubation at room temperature and extensive washing, the amount of phospholipid transferred was equivalent to ~5–12% of the initial nuclear phospholipid. These nuclei were then treated with modification reagent and analyzed by scintillation counting of pellet and supernatant fractions. A third approach was to incorporate fluorescently labeled N-4-nitrobenzo-2-oxa-1,3-diazole derivatized phosphatidylethanolamine (NBD-PE) into nuclei (exogenous) by addition of NBD-PE in ethanolic solution to nuclei (13). After reaction, supernatant and pellet were solubilized in 2% Triton X-100 and the absolute fluorescence was determined in a Perkin-Elmer spectrofluorimeter (650-40).

Analysis of Phospholipid Composition and Distribution: Control and modified (20 mM citraconic anhydride) nuclear pellet and super-

natant fractions were chromatographed as described (14).

Fluorescence Redistribution after Photobleaching: NBD-PE purchased from Avanti Polar Lipids (Birmingham, AL) was used as a probe for phospholipid mobility. Incorporation of this labeled lipid into modified and unmodified nuclei was done in the following manner: NBD-PE in ethanol (3.75 μg/ml) was added to 20 OD_{260} units of nuclei (6) in 1 ml of labeling buffer (50 mM Hepes, 1 mM Mg^{++}, [pH 7.5]). The suspension was incubated with shaking for 20 min at 25°C. The nuclei were then pelleted in an Eppendorf centrifuge for 3 s at 15,000 g (25°C). Supernatant was removed and 1 ml fresh labeling buffer was added, followed by resuspension of the nuclei with a glass rod. This washing procedure was performed three times. Fluorescein-labeled WGA, Con A, and succinylated Con A (Vector Laboratories, Inc., Burlingame, CA) were used to measure glycoprotein mobility. Fluorescein-labeled lectins (10–30 μg/ml) were added to 20 OD_{260} units of nuclei suspended in 1 ml of labeling buffer. The reaction was allowed to proceed at 25°C for 10 min. The washing procedure used was the same as that used for NBD-PE labeling. 10 μl of the labeled nuclear suspension was placed on a slide and a coverslip was applied. The coverslip edges were then sealed with wax. The data obtained from fluorescence redistribution after photobleaching consists of a series of discrete points composing a fluorescence excitation scan produced by the controlled movement of a focused laser beam ($\leq$1 μm) across the nucleus. The fluorescence excited by the beam as it traverses the nucleus appears as a symmetrical double-peak profile characteristic of surface labeling on a spherically shaped structure (15). At a predetermined time, one edge is bleached by an increase in excitation energy (~5,000 times more intense than monitoring intensity); this results in photobleaching and a destruction of the fluorescence signal at the point of bleaching (see Fig. 4a, arrow). The entire structure is then repeatedly scanned at monitoring intensities (an intensity at which bleaching is limited). The
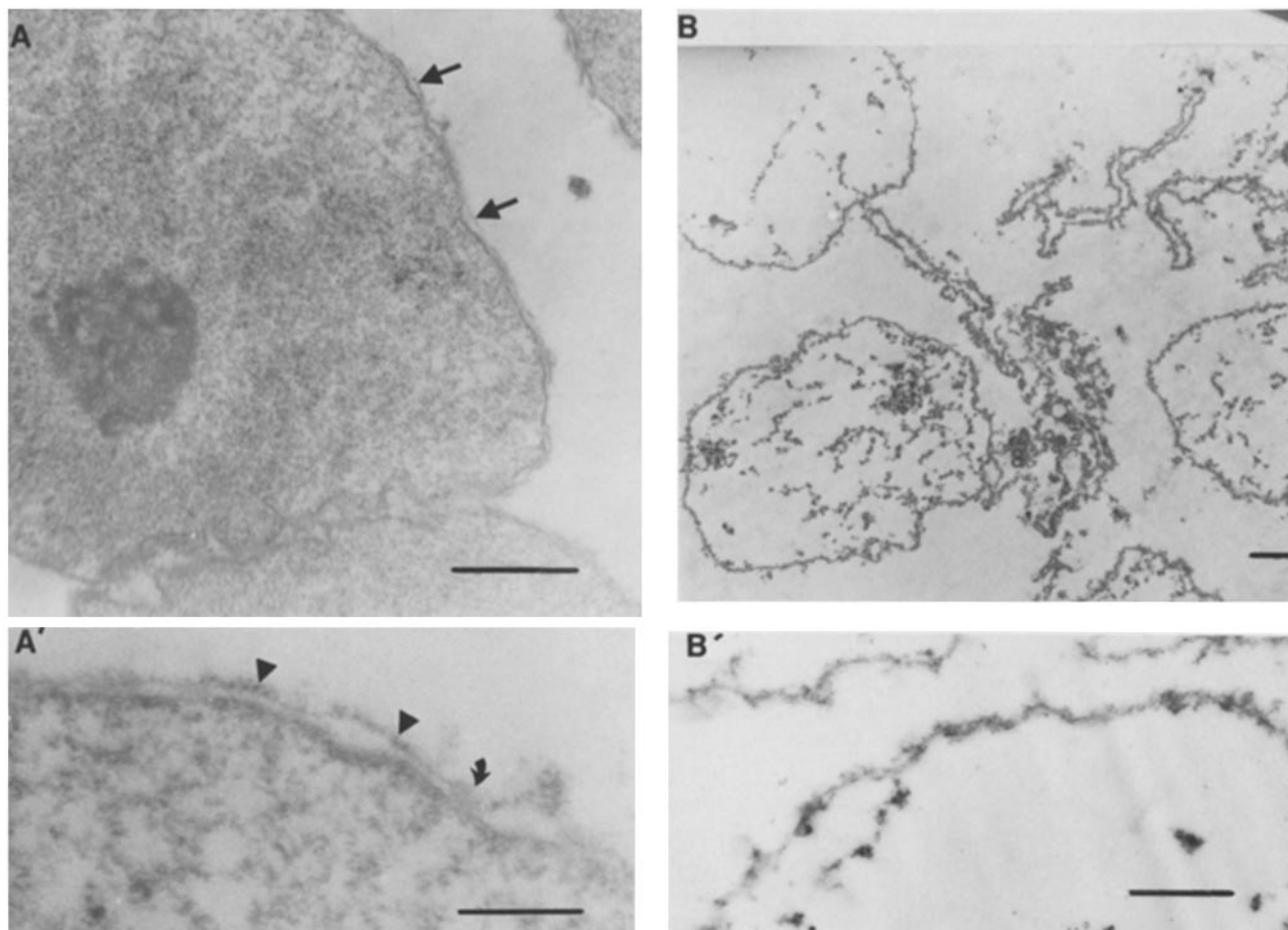

FIGURE 1 Transmission electron micrographs of the nuclear periphery after citraconylation. Nuclei were isolated and prepared essentially as described (18) with minor modifications (19). These micrographs are representative of samples modified with 0 mM (A, A') and 11 mM (B, B') citraconic anhydride. Arrowheads in A' are pointing to black spherical objects attached to the outer membrane that have the size and the distribution of ribosomes. These structures have been characterized as ribosomes by Aaronson and Blobel (6), Schattan and Thoman (17), and Kirschner et al. (32). Similar staining structures in the interior of the nucleus have been identified as HNRNA-containing structures and preribosomal subunits (33). Stringy structures in the middle and upper right corner of B are collapsed nuclei. Bars, 1 μm in A, B, and B', and 0.2 μm in A'.

redistribution of labeled molecules after a localized photobleaching pulse was analyzed with a normal mode analysis, following the approach of Koppel et al. (15). NBD-PE and fluorescein-labeled lectin fluorescence was monitored with an incident wavelength of 4,765 Å and a combination of Leitz dichroic mirror TK510 and barrier filter K510.

RESULTS

Preparation and Characterization of Outer Membrane–depleted Nuclei

TEM of isolated rat liver nuclei showed the characteristic double bilayer with ribosomes (17) located exclusively on the outer membrane (arrows) (Fig. 1A). An enlarged view of the outer membrane is presented in Fig. 1A'. The arrowheads point to black dots which are ribosomes, while the curved arrow shows a pore complex. Previous studies have shown that when nuclei are treated with citraconic anhydride (10–18 mM), a reagent specific for primary amines (16), certain components (DNA, RNA, histones) are selectively removed (9). We found that citraconic anhydride also has specific effects on the two membranes of nuclei. TEM analysis demonstrated that with increasing concentration of citraconic anhydride, a progressive loss of outer membrane (data not shown) occurs, until no outer membrane or ribosomes are observed (Fig. 1B). A closer view of the nuclear surface after citraconylation is presented in Fig. 1B'. The ultrastructural results suggest that treatment with citraconic anhydride selectively removed the outer membrane of nuclei.

Quantitation of Nuclear Membrane Release after Citraconylation

Since phospholipids represent 80–90% of the lipid found in isolated nuclear envelope fractions (6), its presence in nuclear structures may be directly correlated to the existence of membrane material. Fig. 2a demonstrates the effect of

citraconylation on removal of nuclear phospholipid. Because mobility measurements to be performed depend on a phospholipid analog NBD-PE sampling the same membrane environments, experiments comparing retention of endogenous and exogenously incorporated phospholipid as a function of citraconylation were performed. Exogenously incorporated ^{3}H-labeled phospholipid and NBD-PE were used as described in Materials and Methods. The results using the three probes for phospholipid distribution were remarkably similar (see Fig. 2a), which suggested that (a) ~40–50% of the nuclear phospholipid can be removed by citraconylation, (b) the other 50–60% is relatively refractile to very high concentrations of modification reagent, (c) 94–96% of the phospholipid can be released by 1–2% Triton X-100 (Table I), and (d) chemical modification of lipid head group does not play a major role in phospholipid release, since NBD-PE can not react with the reagent. These results further suggested that exogenously incorporated phospholipids sample both membrane compartments because of their nearly identical response to citraconylation when compared with endogenous phosphate analysis for phospholipid retention. The loss of phospholipid (40–50%) with increasing citraconylation coincided with the observed depletion of outer membrane (Fig. 1); this supported the notion that membrane phospholipid release can be used as a biochemical probe for membrane presence.

Phospholipid Analysis and Distribution after Modification

To rule out the possibility of preferential release of phospholipid from outer or inner membrane, we examined the phospholipid composition of pellet and supernatant fractions after citraconylation. Table II demonstrates that a completely representative phospholipid profile is obtained from the released membrane vesicles in the supernatant fraction when compared with whole nuclei–characterized phospholipid.
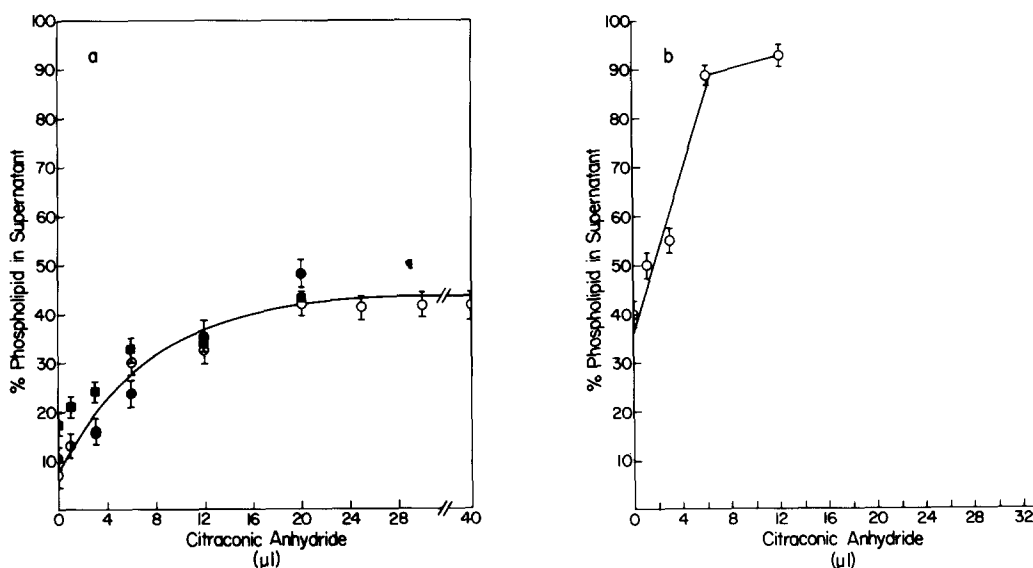

FIGURE 2   Effect of citraconylation on nuclear phospholipids. (a) Radioactivity was measured in supernatant and pellet fractions of exogenously incorporated ^{3}H phospholipid (●). NBD-PE fluorescence was determined in pellet and supernatant fractions (■). Phosphate analysis was performed (20) on endogenous phospholipid (○). (b) Citraconylation was performed in the presence of 3 M urea. The analysis of phospholipid distribution in b, and Table I, was performed using exogenously incorporated ^{3}H-labeled phospholipids (described in Materials and Methods). All phospholipid analyses were corrected to the amount of lamins A–C or lamin B in the case of urea treatment calculated by densitometry of an aliquot of each sample electrophoresed on reduced SDS PAGE gels.

TABLE I

Effect of Sulphydryl Reagents and Citric Acid on Nuclear Phospholipids

Treatment	% Phospholipid in supernatant
8 mM citraconic anhydride	42 ± 4[§]
8 mM citraconic anhydride + 5 mM N-ethylmaleimide[*]	60 ± 5
1% citric acid[‡]	30 ± 3
2.5% citric acid[‡]	30 ± 3
2.0% Triton X-100	94 ± 2

[*] Nuclei were pretreated with 5 mM β-mercaptoethanol followed by two washes in pH 7.5 buffer (50 mM HEPES, 1 mM Mg^{++}). These nuclei were then reacted with 10 mM N-ethylmaleimide and analyzed for phospholipid release.
[‡] Citric acid in water.
[§] Average value of five determinations $\pm$ SD.

TABLE II

Phospholipid Composition of Pellet and Supernatant Fraction[*]

Phospholipid	Whole nuclei (control) (%)	Pellet (%)	Supernatant (%)
Phosphatidylcholine	64.3[‡]	66.7	66.9
Phosphatidylethanolamine	18.1	16.0	17.3
Phosphatidylinositol	7.2	6.5	6.0
Phosphatidylglycerol	3.1	3.3	2.1
Phosphatidic acid	2.8	2.1	2.2
Phosphatidylserine	4.5	5.4	4.0
	$n = 7$	$n = 6$	$n = 6$

[*] Nuclei were treated with 20 mM citraconic anhydride as described in Materials and Methods.
[‡] Values are all $\pm$ 2-3% SD.

Role of Lamins in Inner Membrane Stability

When 3 M urea was used in conjunction with low levels of citraconic anhydride, a much higher percentage of phospholipid was released from the matrix (Fig. 2b). Phospholipid loss was linear (Fig. 2b) which suggested that two different phospholipid compartments observed in Fig. 2a have been normalized. The loss of an additional 50-60% phospholipid (compare Fig. 2, a and b) or complete phospholipid removal parallelled the disassembly of the nuclear matrix. A careful analysis of the gels in Fig. 3 demonstrates that proteolysis occurred on a number of nuclear polypeptides, particularly the lamins. This proteolysis occurred as a consequence of protein denaturation induced by 3 M urea, because it does not occur after citraconylation of nuclei without denaturants (9). Since lamin A and C have been demonstrated to be sensitive to salt-induced solubilization and urea, whereas lamin B is resistant (5), the gel band visible at 66-68 kD after 3 M urea treatment can be assigned lamin B. With increasing citraconylation, this lamin and the whole remaining nuclear structure was solubilized. These results in the context of work done by Gerace and Blobel (5) may provide further evidence that lamin B is an inner membrane associated-matrix component and may serve as an inner membrane marker. The loss of the remaining phospholipid (50-60%) with release of lamin B would consequently mirror inner membrane release (Fig. 1, B and B').

Effects of Sulphydryl Reagents and Citric Acid on Membrane Stability

The nuclear matrix proteins, lamins A-C, have been dem-

onstrated to be rich in sulphydryl groups that artifactually form disulfide cross-links after nuclear isolation (18, 19). We investigated the possible involvement of these sulphydryl groups in stabilizing inner membrane attachment to nuclear matrix by reducing whole nuclei with mercaptoethanol (5%), and treating them with N-ethylmaleimide (5 mM), followed by citraconylation. The data in Table I provides evidence that
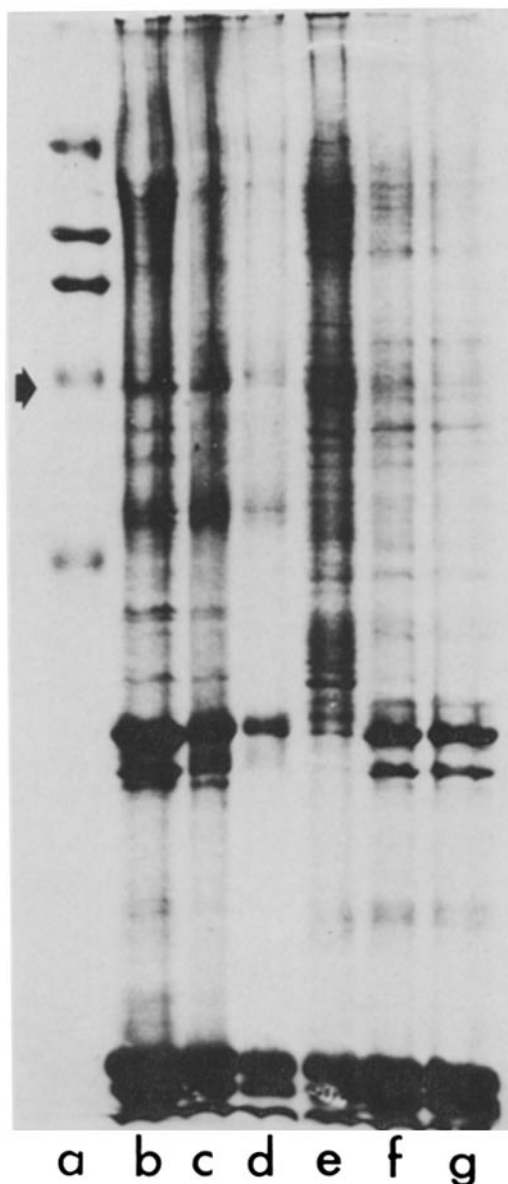

FIGURE 3 SDS PAGE of nuclei treated with 3 M urea and then citraconylated. SDS PAGE was performed as previously described (9). (a) Standards; (b) urea-treated nuclei; (c) urea-treated nuclei reacted with 0.9 mM citraconic anhydride; (d) urea-treated nuclei reacted with 3.0 mM citraconic anhydride. All of the above samples are nuclear pellets obtained as described in Materials and Methods. e-g are the respective supernatant fractions. A decrease in the soluble lamins bands is observed following citraconylation after urea treatment (f and g). Enhanced proteolysis of these bands after citraconylation in the presence of urea is probably due to the effect of a phenylmethylsulfonylfluoride-insensitive protease on urea-denatured nuclear proteins (Schindler, M., unpublished results). Arrow indicates migration area of lamins. Standards are: myosin (200 kD), β-galactosidase (116.5 kD), phosphorylase B (92.5 kD), bovine serum albumin (66.2 kD), and ovalbumin (45 kD), respectively.

a percentage of phospholipid species that was refractile to high levels of modification is now available for release from matrix. In a related series of investigations, a number of other possible membrane–active conditions was used. Treatment with 1.0–2.5% citric acid has been suggested to release outer membrane (7, 8) or approximately half the total phospholipid (7, 20) as judged by electron microscopy and phospholipid analysis. The results presented in Table I suggest that only 30% of the ^{3}H phospholipid of rat liver nuclei is released over a range of concentrations.

Diffusion of Lectins and Phospholipid in the Outer and Inner Nuclear Membrane

Fluorescence redistribution after photobleaching was performed on whole and modified rat liver nuclei to measure the lateral mobility of a fluorescently derivatized phospholipid analog NBD-PE and a number of fluorescein derivatized lectins, namely WGA, Con A, and succinylated Con A. These lectins readily bound to isolated rat liver nuclei while the phospholipid incorporated into the bilayers. Lectin binding appeared to be specific in that it was prevented when the incubation mixture contained the appropriate inhibitory sugar for each lectin (0.2 M α-methylmannoside for Con A and 0.2 M N-acetyl-glucosamine for WGA). Fig. 4 represents a typical bleaching experiment used to determine lipid diffusion. The NBD-PE analysis results in a phospholipid diffusion coefficient of 3.7 ± 1.1 × 10^{-9} cm^2/s (Table III). When analyzed as detailed by Koppel et al. (15) for diffusion on spherical structures, recovery varied between 75–100%. Re-bleaching the initial bleach spot resulted in complete recovery
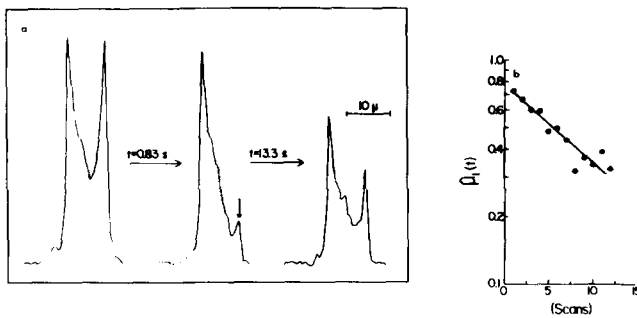

FIGURE 4 (a) FRAP of NBD-PE in nuclear membranes. Arrow indicates bleached point. (b) Quantitative analysis of recovery for NBD-PE in the membranes of whole rat liver nuclei. μ_1 (t) is the experimental estimate of the normalized first moment of the fluorophore concentration distribution. Each scan is equivalent to 0.85 s.

with the same calculated diffusion coefficient. When nuclei were treated with 5 mM citraconic anhydride, lipid diffusion was again observed (Table III) with the same diffusion coefficient. Since the lipid probe apparently distributed itself between the inner and outer membrane approximately equally (see Fig. 2a), this diffusion coefficient apparently reflected mobility, predominantly on the inner membrane. At higher modification reagent concentrations (11 and 18 mM), recovery was still observed (Table III); this suggests that the segmented lipid areas observed on the nuclear periphery (data not shown) maintained continuity with each other. Because the pore complex lamina generally masks the inner membrane, the observation of phospholipid mobility in nuclei depleted of outer membrane as judged by phospholipid release, TEM, and scanning electron microscopy, provided strong evidence that a continuous membrane surface exists in the absence of outer membrane. The inner nuclear membrane surface is observed as following the contour of the nuclear matrix (Fig. 1, B and B').

The WGA receptors, which are proteins (the nucleus apparently does not contain glycolipids [21]), diffused ~10 times slower than the phospholipid. A significant difference is that fluorescence recovery is quite a bit less than phospholipid (40–60%) and would suggest immobile lectin receptors. The data suggest that these mobile receptors are more likely to be found on the outer nuclear membrane (Table III), since no mobility was observed after major depletion of outer nuclear membrane (at 11 and 18 mM citraconic anhydride). When nuclei were treated with 2% glutaraldehyde for 0.5 h, no recovery was observed for WGA.

Con A and succinylated Con A, on the other hand, demonstrated no diffusion under any circumstances (Table III). The membrane receptor for this lectin has been localized to a high molecular weight protein suggested to anchor pore complexes to the nuclear matrix (22). The lack of mobility for this protein was consistent with its role as a structural component of the nuclear pore complex or matrix.

DISCUSSION

Outer and Inner Membrane Separation

Two methods exist in the literature for the separation of outer and inner nuclear membrane. The treatment of nuclei with low concentrations of Triton X-100 was suggested to remove outer membrane preferentially. Electron micrographs were used as evidence to support these claims. When these detergent-treated nuclei were subjected to a phospholipid analysis (6), no obvious concentration range was observed in which only 50% of the phospholipid was released as could be expected for differential solubilization. Citric acid, when used

TABLE III
Diffusion Coefficients for Phospholipid and WGA Receptors in Nuclear Membrane

Treatment	% Components/Modified nuclear pellet				Diffusion coefficient*		
mM citraconic anhydride	Phospho-lipid	DNA	RNA	Protein	WGA	Con A	NBD-PE
0	93	100	100	100	3.9 ± 1.3‡ × 10^{-10}	≤10^{-12}	3.7 ± 1.1 × 10^{-9}
5	70	30	60	35	5.5 ± 1.5 × 10^{-10}	≤10^{-12}	3.8 ± 1.3 × 10^{-9}
11	62	15	35	27	≤10^{-12}	≤10^{-12}	3.8 ± 1.3 × 10^{-9}
18	58	8	20	27	—	—	—
2% Triton X-100	5	100	100	—	—	—	—

* Units of cm^2/s.
‡ Mean ± SD (generally 10–15 determinations).

to prepare outer membrane–depleted nuclei (as judged by electron microscopy), apparently releases only 30% of nuclear phospholipid (Table I) and can inactivate membrane enzymes because of acid denaturation effects. The results reported here for citraconic anhydride–mediated release of outer nuclear membrane use the dual criteria of morphological analysis (TEM) and phospholipid retention used in evaluating the other membrane separation methods. In addition, the lamin B–inner membrane association reported by Gerace and Blobel (5) is used as a third biochemical marker for membrane separation whereas perhaps, most uniquely, long range lateral mobility of phospholipids in outer membrane–depleted nuclei can be used to provide evidence for inner membrane integrity after citraconylation. The use of enzymes for specific membrane markers that has proven successful in other membrane systems has been difficult in nuclear membrane analysis. A nuclear membrane–specific marker enzyme or antigen, for either the outer or inner membrane has not been unambiguously demonstrated by a variety of biochemical criteria. Although citraconylation has been demonstrated to release a large number of nuclear proteins (9), we believe that inner membrane proteins would be resistant to this solubilization for the following reasons. In a classic study on membrane protein solubilization, Steck and Yu (23) demonstrated that 0.1 M NaOH and citraconic anhydride liberate the same set of polar polypeptides from erythrocyte membranes while the other more hydrophobic species invariably remained associated with the membrane residue. This was the chemical basis for classifying peripheral and integral membrane proteins. Another such example is in work presented by Moldow et al. (24), who demonstrate succinylation of erythrocytes results in release of soluble proteins but does not release integral membrane proteins from their association with membrane. In retrospect, this result would seem intuitive since membrane proteins mask most of their charged amino acid side-chains through salt-linkages or unusual pK values. Thus, modification of integral membrane proteins by citraconic anhydride would not be expected to alter their interactions, which are hydrophobic not ionic, with the bilayer. This was also found when porin, an integral membrane protein, was modified by citraconic anhydride (25) and demonstrated no alteration in its detergent-binding properties. The separation of membranes by citraconylation may provide an opportunity to isolate and characterize specific outer and inner membrane antigens for use as membrane markers. In fact, Smith and Wells (26) have demonstrated that 80–90% of the activity of phosphatidylinositol kinase, an enzyme suggested to tightly associate with inner nuclear membrane, is retained in outer membrane–depleted nuclei after treatment with 20–25 mM citraconic anhydride.

Lateral Mobility in Nuclear Membranes

The ability of plasma membrane components to laterally and rotationally diffuse with subsequent redistribution in the plane of the membrane has been a central element in present theories on transmembrane signaling, endocytosis, virus assembly, and the development of specific cell surface distributions of membrane-bound molecules (27, 28). Although a great deal of evidence has been generated to support the role of altered mobility in the plasma membrane as a vehicle for signal transduction in the cell's chemical dialogue with its environment, no body of data is available with regard to the role of lateral transport in the intracellular movement of proteins, lipids, and glycolipids. This process may be of par-

amount importance in the vectorial movement of membrane components between adjacent compartments under conditions in which the membranes are contiguous (29).

The mobility data show that nuclei containing outer membrane have mobile WGA receptors (D [diffusion coefficient] = $[3.9 \pm 1.3] \times 10^{-10}$ cm^2/s) and mobile phospholipid (D = $[3.7 \pm 1.1] \times 10^{-9}$ cm^2/s), whereas outer membrane depletion either immobilizes the inner membrane WGA receptors or provides evidence that immobile WGA receptors are localized at the inner membrane. Because phospholipid mobility remains essentially unchanged in outer membrane–depleted nuclei, and the citraconyl groups bound to inner membrane proteins have been removed by exposure of nuclei to pH 6.8 at 4°C for 2–4 h (a condition that does not affect whole nuclei mobility), it is proposed that the latter interpretation (i.e., immobile WGA receptors are localized at the inner membrane) is more likely. This implies that the outer membrane connected to ER is dynamic and may thus serve as a communication pathway between ER and nuclei. Inner nuclear membrane, on the other hand, may represent a static mosaic surface for the attachment of nuclear matrix, chromatin, and ribonucleoprotein structures. The phospholipid, matrix, heterochromatin interface may affect mobility in a manner analogous to submembranous membrane structures in erythrocytes (13) and other cells (30). In the case of the nucleus, the submembranous elements would be far more densely configured, enhancing the matrix-mediated immobilization of membrane proteins completely. In the only other report on nuclear membrane dynamics, a viral protein most presumably located on the inner nuclear membrane is found to be immobile (31).

Both the dynamic and chemical results reported in this paper provide support for the view of two membrane systems surrounding the nucleus with very different physical properties. The outer membrane studded with ribosomes is extremely sensitive to chemical modification by a reagent that alters the net charge of lysyl residues by −2. During modification, the outer membrane vesiculates and its physical continuity with the nucleus is broken. The inner nuclear membrane, on the other hand, maintains a tight association with the submembranous array of lamins and pore complexes. These attachments appear to be abrogated with the progressive loss of lamins after citraconylation in the presence of urea. The possibility that lamin B provides a protein connection for the membrane to nuclear matrix in a manner reminiscent of ankyrin in erythrocytes has been suggested (5) and is consistent with our results. The sensitivity of inner membrane release to sulphydryl reagents may suggest that the lamins stabilize the membrane by the formation of disulphide linkages with inner membrane integral membrane proteins.

The dynamic and physical differences in the two membranes may be representative of the fact that they may serve two very different functions. The outer membrane may function as a communications link with the ER and other intracellular membranes for biosynthetic purposes, while the inner membrane may (a) offer a two-dimensional catalytic surface for replicating enzymes and DNA attachment, (b) offer a microenvironment for specific enzymatic events and DNA/ RNA packaging, (c) offer domains for viral assembly, and (d) provide additional recognition information for correct nuclear reassembly.

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