Identification of a Major Polypeptide of the Nuclear Pore Complex

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ABSTRACT The nuclear pore complex is a prominent structural component of the nuclear envelope that appears to regulate nucleoplasmic molecular movement. Up to now, none of its polypeptides have been defined. To identify possible pore complex proteins, we fractionated rat liver nuclear envelopes and microsomal membranes with strong protein perturbants into peripheral and intrinsic membrane proteins, and compared these fractions on SDS gels. From this analysis, we identified a prominent 190-kilodalton intrinsic membrane polypeptide that occurs specifically in nuclear envelopes. Lectin binding studies indicate that this polypeptide (gp 190) is the major nuclear envelope glycoprotein. Upon treatment of nuclear envelopes with Triton X-100, gp 190 remains associated with a protein substructure of the nuclear envelope consisting of pore complexes and nuclear lamina. We prepared monospecific antibodies to gp 190 for immunocytochemical localization. Immunofluorescence staining of tissue culture cells suggests that gp 190 occurs exclusively in the nucleus during interphase. This polypeptide becomes dispersed throughout the cell in mitotic prophase when the nuclear envelope is disassembled, and subsequently returns to the nuclear surfaces during telophase when the nuclear envelope is reconstructed. Immunoferritin labeling of Triton-treated rat liver nuclei demonstrates that gp 190 occurs exclusively in the nuclear pore complex, in the regions of the cytoplasmic (and possibly nucleoplasmic) pore complex annuli. A polypeptide that cross-reacts with gp 190 is present in diverse vertebrate species, as shown by antibody labeling of nitrocellulose SDS gel transfers. On the basis of its biochemical characteristics, we suggest that gp 190 may be involved in anchoring the pore complex to nuclear envelope membranes.

The nuclear envelope is a complex membrane structure that defines the boundary of the nuclear compartment in eukaryotic cells (for reviews see references 7, 8, 10, 20). Important architectural features of this organelle include its double membrane, the nuclear lamina (a protein meshwork that is associated with the nucleoplasmic surface of the inner nuclear membrane), and pore complexes (elaborate protein structures that occur at regions where the inner and outer nuclear membranes are joined to form pores). Nuclear pore complexes provide channels through the nuclear envelope for nucleocytoplasmic molecular movement. Although many proteins and all smaller molecules appear to traverse the pore complex by passive diffusion, it is likely that the majority of RNA species are translocated through this structure by discriminatory energy-requiring mechanisms (8, 24). Hence, to understand the process of RNA export from the nucleus, it will be important to define the basic molecular features of the pore complex.

A considerable amount of ultrastructural information has been obtained on the pore complex (7, 8), but much less is presently known about its biochemistry. Procedures have been devised to mass-isolate nuclear envelopes with morphologically well-preserved pore complexes (e.g. references 5, 14, 21), providing an important basis for biochemical analysis of pore complex structure. Furthermore, it is possible to subfractionate preparations of nuclear envelopes by treatment with Triton X-100 and high concentrations of monovalent salt to obtain a protein-rich nuclear envelope substructure comprised of pore complexes attached to the nuclear lamina (the "pore complex-lamina" fraction; references 2, 5, 17, 27). However, isolation of nuclear pore complexes separate from lamina or other nuclear envelope constituents has not been achieved. The pore complex is composed at least mainly of protein (7, 8, 20), but none of its polypeptides have previously been defined.

In this study, we have identified a prominent intrinsic mem-

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brane glycoprotein of nuclear envelopes that is not present in endoplasmic reticulum membranes, and that remains associated with the pore complex-lamina material following Triton treatment of nuclear envelopes. We prepared monospecific antibodies to this polypeptide, and demonstrated by immunocytochemical techniques that it is specifically localized in the nuclear pore complex. The possible role of this polypeptide in pore complex structure is discussed.

MATERIALS AND METHODS

Preparation of Subcellular Fractions

Unless otherwise noted, all manipulations related to preparation of subcellular fractions and membrane fractionation were performed at 0–4°C. Rat liver subcellular fractions were prepared from male 200–250 g Sprague Dawley rats starved 16 h before sacrifice. Nuclei (3) and nuclear envelopes (5) were purified as described, except that all buffers contained 0.0005 M phenylmethylsulfonyl fluoride (PMSF) (freshly added from a 0.1 M stock solution in ethanol) and 0.001 M dithiothreitol (DTT). Also, during preparation of nuclear envelopes, protease-free pancreatic RNAse A (Sigma Chemical Co., St. Louis, MO) was included in the nuclear suspensions at both DNAse digestion steps at a concentration of 1 μg/ml. Nuclei were prepared from chicken liver, dog kidney, and bovine thymus according to the procedure described for rat liver (3). For preparation of rat liver microsomal membranes, 1 volume of the postmitochondrial supernatant of a rat liver homogenate in NB1 (0.25 M sucrose, 0.05 M triethanolamine-HCl pH 7.4, 0.025 M KCl, 0.005 M $MgCl_2$, 0.0005 M PMSF, 0.001 M DTT) was mixed with 5 volumes of a solution containing 2.3 M sucrose, 0.05 M triethanolamine-HCl pH 7.4, 0.025 M KCl, 0.005 M $MgCl_2$, 0.0005 M PMSF, 0.001 M DTT. In appropriate centrifuge tubes, 27 ml of this solution was overlaid with 7 ml of a solution containing 1.9 M sucrose, 0.05 M triethanolamine-HCl pH 7.4, 0.025 M KCl, 0.005 M $MgCl_2$, 0.0005 M PMSF, 0.001 M DTT; followed by 2 ml of NB1. The gradients were then centrifuged in a Beckman SW28 rotor (Beckman Instruments Inc., Palo Alto, CA) at 27,000 rpm for 5 h to float the microsomal membranes. The well-defined microsomal membrane band occurring between the 0.25 M sucrose and 1.9 M sucrose layers was harvested with a syringe and cannulus. The harvested samples were then diluted with 3 volumes of NB1, and aliquots were pelleted for 1 h at 48,000 rpm in a Beckman 50Ti rotor to obtain purified microsomal membranes. Following preparation, both microsomal membranes and nuclear envelopes were frozen as pellets in liquid nitrogen, and stored at −80°C until use.

Membrane Fractionation

For all biochemical experiments described in this paper, nuclear envelopes were first washed with a buffer containing 0.5 M NaCl as described (5) to solubilize the bulk of contaminating chromatin that copurifies with nuclear envelopes. Some degree of chromatin contamination remains after this condition of salt treatment, as indicated by the presence of histones in SDS gels of nuclear envelopes (e.g. Fig. 1).

To accomplish chemical fractionation of nuclear envelopes or microsomal membranes (Fig. 1), membrane pellets were suspended at a protein concentration of 0.25–0.5 mg/ml in solutions containing either (*a*) 0.1 M NaOH, 0.01 M DTT, (*b*) 6 M urea, 0.05 M triethanolamine-HCl, pH 7.4, 0.01 M DTT, (*c*) 6 M guanidine-HCl, 0.05 M triethanolamine-HCl, pH 7.4, 0.01 M DTT, or (*d*) 4 M $MgCl_2$, 0.05 M triethanolamine-HCl, pH 7.4, 0.01 M DTT. Subsequently, material in solutions (*b*) and (*d*) was incubated for 30 min (material in solutions (*a*) and (*c*) received no incubation), and samples were centrifuged for 60 min at 48,000 rpm in a Beckman 50Ti rotor. Only the results for solutions (*a*) and (*b*) are shown in Fig. 1, because similar fractionation patterns were obtained for the other chemical conditions. Extraction of nuclear envelopes with Triton X-100 (see Fig. 3) involved resuspension of membrane pellets in either (*a*) 10% sucrose, 2% (wt/vol) Triton X-100, 0.02 M triethanolamine-HCl, pH 7.4, 0.02 M KCl, 0.005 M $MgCl_2$, 0.001 M DTT or (*b*) 10% sucrose, 2% Triton X-100, 0.02 M triethanolamine-HCl, pH 7.4, 0.15 M KCl, 0.005 M $MgCl_2$, 0.001 M DTT. Following an incubation for 30 min, samples were centrifuged for 60 min at 48,000 rpm in a Beckman 50Ti rotor.

Production and Purification of Antibodies

To obtain antisera to gp 190, a 0.1 N NaOH pellet of nuclear membranes (Fig. 1) was electrophoresed on preparative SDS gels (see below) and the 190-kilodalton band was excised and electrophoretically eluted following visualization by Coomassie Blue staining. The eluted protein was then used to immunize chickens as described (11). A column containing lectin-purified nuclear envelope intrinsic membrane glycoproteins conjugated to Sepharose 4B was used for affinity purification of specific anti-gp 190 antibodies. To prepare this affinity column, a 0.1 N NaOH pellet fraction of nuclear envelopes (Fig. 1) was precipitated in 90% acetone for 60 min at 0°C and pelleted for 4,000 g_{max} for 10 min. The pellet was then extracted with 95% methanol for an additional 30 min at 0°C and centrifuged as before. The pellet from the last step was then solubilized at a protein concentration of ~0.4 mg/ml by boiling for 3 min in a solution containing 0.4% SDS, 0.05 M triethanolamine-HCl pH 7.4, 0.1 M NaCl, 0.001 M DTT. This solution was cooled, and made 2% in Triton X-100 by appropriate addition from a 20% (wt/vol) Triton solution. Subsequently, at 4°C the solution was passed over a column of *Lens culinaris* agglutinin (lentil lectin) conjugated to agarose (Sigma Chemical Co.) at a flow rate of ~2 ml/h. A 5-ml column (~4.5 mg lentil lectin/ml gel beads) was used for 25 mg of solubilized nuclear envelope intrinsic membrane proteins. The column was subsequently washed at 4°C with 5 column volumes of 2% Triton X-100, 0.4% SDS, 0.05 M triethanolamine-HCl pH 7.4, 0.1 M NaCl, 0.001 M DTT before elution of glycoproteins from the column at room temperature with 3 column volumes of a buffer containing 1.0 M α-methyl-mannoside, 0.2% Triton X-100, 0.04% SDS, 0.1 M triethanolamine-HCl, pH 7.4, 0.05 M NaCl, and 0.001 M DTT. Eluted proteins were then dialyzed against 0.005 M triethanolamine-HCl, pH 7.4, and lyophilized. The sample was then reconstituted with H_2O to a volume of ~4 ml (for 25 mg of initial nuclear envelope intrinsic proteins), precipitated with 20% trichloroacetic acid for 5 h at 0°C and pelleted at 4,000 g_{max} for 10 min. The pellet was next extracted for 30 min at 0°C with 90% acetone, and pelleted as before. Finally, the pellet was solubilized by boiling for 2 min in 1% SDS, 0.2 M sodium phosphate, pH 8.0, and was conjugated to cyanogen bromide-activated Sepharose 4B (Pharmacia Fine Chemicals, Piscataway, NJ) at a concentration of 500 μg protein/ml beads. The protein profile of material conjugated to this column was identical to that shown in Fig. 4 *a* (*Coomassie Blue, lentil lectin: mm* lane), and consists mostly of gp 190.

To affinity-purify anti-gp 190 antibodies on this column, antisera were made 2% in Triton X-100, 0.01 M in EDTA, and 0.001 M PMSF by appropriate additions from 20% Triton, 0.2 M EDTA, and 0.1 M PMSF stock solutions, and were centrifuged for 15 min at 48,000 rpm in a Beckman 50Ti rotor. The sera were then passed over the affinity column at 4°C at a rate of 0.5 ml/h. The column was then washed with 15 column volumes of 0.01 M sodium phosphate pH 7.4, 0.14 M NaCl, 0.02% NaN_3 (PBS) containing 2% Triton, followed by 10 column volumes of PBS, 10 column volumes of 0.010 M sodium phosphate, 0.5 M NaCl, and finally 5 column volumes of PBS. Antibodies were eluted from the column at 4°C with 0.1 M glycine HCl, pH 3.0, and immediately neutralized by collection in a tube containing 0.4 M sodium phosphate, pH 7.4.

Anti-lamins A and C antibodies were affinity-purified from chicken antisera raised to electrophoretically purified lamin A (11). Purification was accomplished as described above, using a column containing electrophoretically purified lamin A conjugated to Sepharose 4B. Similarly, monospecific antilamin B antibodies were affinity-purified from chicken antiserum raised to electrophoretically purified lamin B (11), using a column containing electrophoretically purified lamin B conjugated to Sepharose 4B.

Electrophoresis Procedures

SDS PAGE was accomplished as described previously (19). For gel sample preparation, proteins were precipitated with 10% or 20% TCA for 60 min at 0°C, and samples were centrifuged at 14,000 g_{max} for 5 min. The protein pellets were then suspended in SDS sample buffer, boiled for 3 min, and alkylated with iodoacetamide as described (5). In cases where detergents (e.g. Triton X-100, see Fig. 3) were present in protein solutions precipitated with TCA, the resulting pellets were extracted with 90% acetone for 15 min at 0°C to remove the precipitated detergents, and repelleted at 14,000 g_{max} for 5 min before solubilization in SDS sample buffer. Unfractionated nuclei (see Fig. 9) were suspended at a protein concentration of ~10 mg/ml in 0.05 M triethanolamine, pH 7.4, 0.005 M $MgCl_2$, 0.0005 M PMSF, and were digested with 25 μg/ml pancreatic DNAse and 25 μg/ml pancreatic RNAse for 30 min at 0°C before gel sample preparation.

Apparent molecular weights (e.g. of gp 190) were estimated from a log mol wt vs. polypeptide mobility plot, following electrophoresis of samples on a straight 7.5% SDS gel with a 4% stacker. For molecular weight standards, we used a Bio-Rad (Bio-Rad Laboratories, Richmond, CA) high molecular weight mix (including rabbit skeletal muscle myosin, 200,000; β-galactosidase, 116,000; phosphorylase A, 93,000; bovine serum albumin, 68,000; ovalbumin, 43,000) and DNAse I, 31,000; chymotrypsinogen, 23,000; cytochrome *c*, 12,000.

Usually, for gels with dimensions 30 cm × 19 cm × 0.1 cm (slot width 1.0 cm), gel lanes contained the nuclear envelope protein obtained from 10 A_{260} units of nuclei (equivalent to ~75 μg of protein), or the protein subfractions derived from this quantity of nuclear envelopes. For electrophoretic transfer experiments, we used gels with dimensions 20 cm × 15 cm × 0.075 cm (slot width 0.6 cm) and protein loads of ~15 μg. Electrophoretic transfer of the polypeptide profile of SDS gels to nitrocellulose paper (Schleicher and Scheull Inc., Keene, NH) was

accomplished by the procedure of Towbin et al. (31) except that the transfer buffer contained 0.025 M Tris, 0.190 M glycine at pH 8.6 with no methanol. Transfer was accomplished with a Bio-Rad Trans Blot cell (Bio-Rad Laboratories, Richmond, CA) run at 400 mA constant current for 4 h. In certain instances, transfers were stained with 0.2% Amido black in 50% methanol, 10% acetic acid.

Labeling of Nitrocellulose Gel Transfers

Before labeling of nitrocellulose SDS gel transfers with ^{125}I concanavalin A (Con A), excess binding sites on the nitrocellulose paper were saturated by incubation of the transfers for 1 h at 37°C in a solution containing 1 mg/ml denatured hemoglobin in WB (0.5% Triton X-100, 0.1% SDS, 0.01 M sodium phosphate pH 7.4, 0.140 M NaCl). We used ^{125}I Con A iodinated to a specific activity of ~1 × 10^9 cpm/mg by the chloramine T procedure (13). (Iodination was done in the presence of 0.2 M α-methyl-mannoside). For labeling experiments, ^{125}I Con A was diluted to 5 × 10^5 cpm/ml in WB plus 1 mg/ml denatured hemoglobin, and incubated with the nitrocellulose paper for 3 h at 4°C on a rotating shaker (We used ~0.2 ml of Con A solution per cm^2 of nitrocellulose paper.) The paper was then given 6 × 5 min washes with WB on a rotating shaker at room temperature, dried on filter paper, and exposed for autoradiography, usually at −80°C with a Dupont Lightning Plus intensifying screen (E.I. dupont De Nemours & Co., Wilmington, DE). Specificity controls involved incubation of nitrocellulose transfers with an ^{125}I Con A solution containing 0.2 M α-methyl-mannoside. When this inhibitor was present, we never observed labeling of any bands on our transfers with ^{125}I Con A.

For labeling of nitrocellulose SDS gel transfers with radioactive antibodies, excess binding sites on the nitrocellulose paper were first saturated by incubation of the transfers for 1 h at 37°C in WB plus 20 mg/ml bovine serum albumin. Subsequently, transfers were incubated for 3 h at 4°C on a rotating shaker with a solution of affinity-purified chicken antibodies diluted to 0.001–0.002 A_{280}/ml in WB plus 20 mg/ml bovine serum albumin. The transfers were then given 6 × 5 min washes with WB as described above, and incubated for 3 h at 4°C with ^{125}I rabbit anti-chicken IgG diluted to 5 × 10^5 cpm/ml in WB plus 20 mg/ml bovine serum albumin. Rabbit anti-chicken IgG was iodinated by the chloramine T procedure (13) to a specific activity of 3 × 10^9 cpm/mg. After the second antibody incubation, transfers were washed as described above, dried on filter paper, and exposed for autoradiography.

Immunofluorescence Microscopy

PtK2 cells (American Type Culture Collection, Rockville, MD) were maintained in monolayer cultures, in Dulbecco's modified Eagle medium (Gibco Laboratories, Grand Island Biological Co., Grand Island, NY) containing 10% fetal calf serum, 100 U/ml penicillin and 100 μg/ml streptomycin. For immunofluorescence staining, cells were plated on #1 coverslips, and following 48–72 h of growth were fixed with 4% paraformaldehyde in 0.01 M sodium phosphate pH 7.4, 0.14 M NaCl, 0.001 M $MgCl_2$ for 3 min at room temperature, followed immediately by 100% methanol for 1 min at −20°C. Coverslips were rinsed several times in PBS, and then in the immunofluorescence solution buffer (IB: 0.01 M sodium phosphate pH 7.4, 0.3 M NaCl). After draining, coverslips were overlaid with various affinity-purified chicken antibodies (diluted to 0.02–0.05 A_{280}/ml and adjusted to the salt composition of IB. Following incubation for 30 min at room temperature in a humid chamber, the coverslips were rinsed four times in IB over a period of 5 min. They were then overlaid with a solution of tetramethylrhodamine isothiocyanate rabbit anti-chicken IgG (Cappel Laboratories Inc., Cochranville, PA) in IB and incubated another 30 min at room temperature. Following rinsing as described above, the coverslips were mounted on slides in a solution containing 25% glycerol, 75% PBS. They were then examined with a Leitz fluorescence microscope equipped with epifluorescence optics. Micrographs were taken with Kodak Tri-X pan film (Eastman Kodak Co., Rochester, NY) developed at an ASA rating of 400.

Immunoferritin Microscopy

We prepared ferritin-conjugated rabbit anti-chicken IgG according to the two-stage glutaraldehyde procedure described by Otto et al. (23), using six times recrystallized ferritin (Miles Laboratories Inc., Elkhart, IN) and DEAE-purified rabbit anti-chicken IgG. After the ferritin-antibody conjugation was accomplished, a "monomeric" fraction containing 1:1 ferritin:antibody conjugates was separated from larger oligomeric species and unconjugated reactants on a Sepharose CL-4B column (Pharmacia Fine Chemicals, Inc.). The "monomeric" peak was stored at 4°C until use. 1 d before ferritin labeling experiments, samples of the "monomeric" ferritin-antibody peak were rechromatographed on a Sepharose CL-4B column, and the fractions eluting again at the "monomeric" position were used for subsequent labeling studies.

Ferritin labeling was performed on Triton-treated rat liver nuclei prepared as described above. All steps before antibody incubations were done at 4°C. Purified nuclei were washed two times by suspension in NB1 (0.25M sucrose, 0.05 M triethanolamine-HCl pH 7.4, 0.025 M KCl, 0.005 M $MgCl_2$, 0.0005 M PMSF, 0.001 M DTT) and centrifugation at 4,000 g_{max} and resuspended a third time in NB1 to a concentration of 50 A_{260}/ml. Aliquots of this suspension (100 μl) were pipetted onto 35-mm Falcon tissue culture petri dishes (Becton, Dickinson & Co., Cockeysville, MD) in 1.5-cm diameter areas circumscribed with a diamond pencil, and allowed to adsorb in the surface of the plastic dish in a humid chamber for 30 min. The dishes were then rinsed in NB2 (8.5% sucrose, 0.01 M triethanolamine-HCl pH 7.4, 0.02 M KCl, 0.005 M $MgCl_2$, 0.0005 M PMSF, 0.001 M DTT) and incubated with 2% Triton X-100 in NB2 for 5 min. Following a subsequent rinse in NB2, dishes were incubated for 15 min in NB2 containing 0.05% glutaraldehyde, and then quenched for 30–60 min in a solution containing 8.5% sucrose, 0.010 M sodium phosphate pH 7.4, 0.1 M NaCl, 0.05 M NH_4Cl, 0.005 M $MgCl_2$. With these conditions, we usually obtained a moderately dense monolayer of nuclei adsorbed to the surfaces of petri dishes, that remained attached to the dishes throughout subsequent manipulations. Before incubation with antibodies, fixed and quenched dishes were rinsed with NB3 (8.5% sucrose, 0.01 M sodium phosphate, pH 7.4, 0.14 M NaCl, 0.002 M $MgCl_2$, 0.0002 M ethylenebis (oxyethylenenitrilo) tetraacetic acid (EGTA), 0.0005 M PMSF). Areas containing the adsorbed nuclei were then overlaid with chicken antibodies diluted to 0.025–0.05 A_{280}/ml (see Figure legends) in a solution adjusted to the composition of NB3. Dishes were incubated for 30 min at room temperature, and subsequently rinsed four times over a period of 5 min with NB3. The dishes were then incubated a second time for 30 min at room temperature with ferritin-conjugated rabbit anti-chicken IgG diluted to 50–100 μg ferritin/ml in NB3. For the ferritin solutions, we assumed that ferritin concentration = OD_{440} × 0.65. Next, dishes were washed four times with NB3, one time with NB1, and fixed for 15 min at room temperature in NB1 containing 2.5% glutaraldehyde. The dishes were then postfixed 60 min at 0°C in a solution containing 0.1 M sodium phosphate pH 7.0, 2% OsO_4. Following a series of graded ethanol dehydration steps, dishes were embedded with Poly Bed 812 (Polysciences, Inc., Warrington, PA). Subsequently, petri dishes were broken from the hardened Poly Bed 812 (containing the nuclei) and 50–60-nm thin sections were cut and mounted on 300-mesh uncoated copper grids. Sections were stained for 15 s in a solution containing equal parts of saturated uranyl acetate, 100% EtOH, and H_2O, followed by 3 min in lead citrate (5). Samples were examined in a Zeiss electron microscope at 80 kV.

RESULTS

A Nuclear Envelope-specific Intrinsic Membrane Glycoprotein

The nuclear envelope is morphologically continuous with the rough and smooth endoplasmic reticulum, and has numerous biochemical similarities to endoplasmic reticulum membranes, including many similar proteins (7, 8). However, the nuclear envelope also contains numerous polypeptides that are not present in membranes of the endoplasmic reticulum (e.g. reference 25). We consider it likely that many of these nuclear envelope-specific proteins occur in either pore complexes or the nuclear lamina, the two prominent structures that are usually unique to the nuclear envelope (7, 10).

To identify potential polypeptide components of the nuclear pore complex, we fractionated nuclear envelopes and endoplasmic reticulum-enriched membranes (microsomal membranes) to obtain peripheral and intrinsic membrane proteins, and examined these fractions by SDS polyacrylamide gel electrophoresis to determine nuclear envelope-specific polypeptides (Fig. 1). In this study, we focused on identifying intrinsic membrane proteins of the pore complex, which can be potentially important for organizing pore complex architecture (4, 18).

We prepared peripheral and intrinsic membrane proteins by conventional procedures (28, 30) involving extraction of membranes with strong protein perturbants (6 M urea and 0.1 N NaOH). According to this operational definition, peripheral proteins are largely or completely solubilized from membranes by these chemical treatments, and appear in a supernatant following centrifugation. Analogously, intrinsic proteins resist

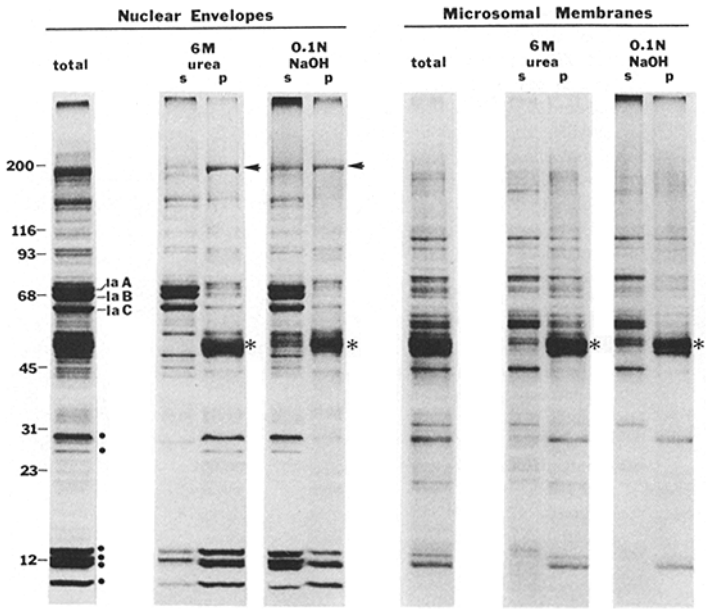

FIGURE 1 Comparison of the peripheral and intrinsic membrane polypeptides of nuclear envelopes and microsomal membranes by SDS gel electrophoresis. Samples of rat liver nuclear envelopes or microsomal membranes were incubated in solutions containing 6 M urea or 0.1 N NaOH as described in Materials and Methods, and centrifuged to yield supernatant (*s*) and pellet (*p*) fractions (representing peripheral and intrinsic membrane proteins, respectively). These fractions, together with samples of unfractionated (total) nuclear envelopes and microsomal membranes, were electrophoresed on a 7.5–15% polyacrylamide gel. The Coomassie Blue-stained gel is shown. Adjacent to the *total Nuclear Envelopes* lane, lamins *A*, *B*, and *C* are labeled, and contaminating histones are indicated by dots. The major 190-kilodalton intrinsic polypeptide specific to nuclear envelopes is designated by arrows. Intrinsic polypeptides common to nuclear envelopes and microsomal membranes that are presumed to be cytochrome P-450 are indicated by asterisks. Numbers in left-hand column are mol wt $\times 10^{-3}$.

extraction from membranes by these chemical perturbants, and appear in the membrane pellet.

Many differences are apparent when the peripheral membrane proteins of nuclear envelopes and microsomal membranes are compared (Fig. 1, *6 M urea and 0.1 N NaOH*: *s* lanes). Notably, the peripheral protein fractions of nuclear envelopes contain the three predominant nuclear envelope bands (lamins A, B, and C). These polypeptides are absent from microsomal membranes, and as shown previously, are the major components of the nuclear lamina (11, 16). The intrinsic protein fractions of nuclear envelopes and microsomal membranes show both similarities and differences (Fig. 1, *6 M urea and 0.1 N NaOH*: *p* lanes). The nuclear envelope intrinsic fractions contain a number of major bands migrating at ~50 kilodaltons that also occur in the corresponding fractions of microsomal membranes (Fig. 1, *6 M urea and 0.1 N NaOH*: *p* lanes, asterisks). From the polypeptide identifications made by other authors in SDS gels of microsomal membranes (15), these bands probably represent multiple forms of cytochrome P-450, a major intrinsic protein of both the endoplasmic reticulum and the nuclear envelope (7, 8).

Aside from these prominent similarities, the nuclear envelope intrinsic fractions contain a major 190-kilodalton species (Fig. 1, *6 M urea and 0.1 N NaOH*: *p* lanes, arrows) that is conspicuously absent from microsomal membranes. Because a major 190-kilodalton band also occurs in the membrane pellets of nuclear envelopes treated with 6 M guanidine-HCl and 4 M $MgCl_2$ (data not shown, see Materials and Methods), this 190-kilodalton component is very likely an intrinsic membrane protein. Here we have focused our attention on this polypeptide, because it appears to be the predominant nuclear envelope-specific intrinsic protein. It should be noted that peripheral membrane polypeptide(s) co-migrate with the 190-kilodalton intrinsic component, because ~50% of the 190-kilodalton band of total nuclear envelope polypeptides is solubilized by the 0.1 N NaOH treatment. (A smaller proportion of this band is extracted by 6 M urea, a condition that results in less complete solubilization of the peripheral proteins). As shown below (Figs. 2 and 4), the 190-kilodalton NaOH-extracted components are clearly distinct from the unextracted band.

The 190-kilodalton intrinsic polypeptide of nuclear envelopes stains heavily when SDS gels are incubated by the periodic acid-Schiff procedure (12), suggesting that it is a glycoprotein (data not shown). We have confirmed this sug-

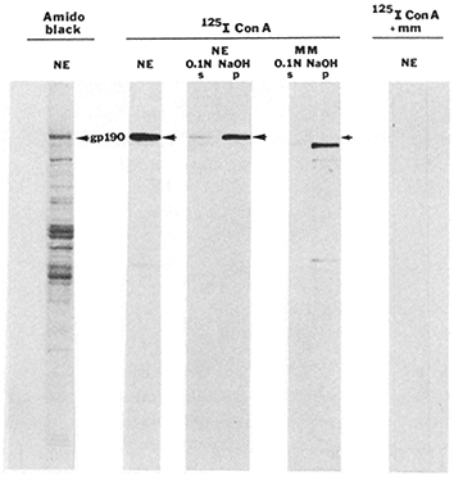

FIGURE 2 Identification of the Con A-binding components of nuclear envelope and microsomal membrane fractions on nitrocellulose gel transfers. Samples of unfractionated nuclear envelopes (*NE*), and the supernatant (*s*) and pellet (*p*) fractions obtained by extraction of nuclear envelopes and microsomal membranes (*MM*) with 0.1 N NaOH (see Fig. 1) were electrophoresed on 7.5–15% SDS gels, and the resolved polypeptides were electrophoretically transferred to nitrocellulose paper (see Materials and Methods). One nuclear envelope lane of this nitrocellulose was stained with Amido black, demonstrating that there is largely faithful transfer of the complete polypeptide profile of nuclear envelopes from SDS gels to nitrocellulose paper with our conditions. Other lanes were incubated with ^{125}I Con A, or ^{125}I Con A plus 0.2 M α-methyl-mannoside (*mm*). Shown are autoradiograms of these lanes. The labeling of bands that we obtained with ^{125}I Con A on these nitrocellulose transfers is carbohydrate specific, since labeling is completely inhibited for nuclear envelope proteins (*^{125}I Con A + mm, NE* lane) and for all other fractions examined (data not shown) by including 0.2 M α-methyl-mannoside in the ^{125}I Con A solution. Large arrows indicate the major Con A-binding, intrinsic membrane polypeptide of nuclear envelopes, gp 190. No Con A-binding component migrates at this molecular weight position in the corresponding gel profile of microsomal membranes (small arrow).

gestion by lectin binding analysis (Fig. 2). Incubation of a nitrocellulose transfer of an SDS gel with ^{125}I Con A (a lectin specific for glucosyl and mannosyl residues of glycoproteins [22]) reveals that a 190-kilodalton polypeptide is the predominant Con A-reactive component of total nuclear envelope proteins (Fig. 2, *NE* lane, arrow). Furthermore, because this Con A-binding species occurs almost exclusively in the 0.1 N NaOH pellet of nuclear envelopes (Fig. 2, *NE, 0.1 N NaOH: p* lane, arrow) it corresponds to the 190-kilodalton intrinsic polypeptide. We designate this glycoprotein gp 190. When fractions of microsomal membrane proteins are analyzed by lectin binding, no Con A-reactive polypeptide is detected at 190 kilodaltons (Fig. 2, *MM, 0.1 N NaOH: s and p* lanes). A major Con A-binding component does occur at ~175 kilodaltons in the microsomal intrinsic fraction, but this polypeptide is immunologically distinct from the 190-kilodalton nuclear envelope component, as considered below (Fig. 4).

Gp 190 also binds to *Lens culinaris* agglutinin (Fig. 4), a lectin that (like Con A) is specific for glucosyl and mannosyl residues of glycoproteins (22). However, this glycoprotein apparently does not contain sialyl or terminal N-acetylglucosaminyl residues, since it is not labeled on nitrocellulose gel transfers (data not shown) with ^{125}I wheat germ agglutinin (22). Chromatography of solubilized total membrane proteins on a *Lens culinaris* agglutinin column suggests that ~50% of the 190-kilodalton band seen in electrophoretograms of total nuclear envelope proteins corresponds to gp 190, as considered below (Fig. 4).

Association of gp 190 with the pore complex-lamina fraction

Treatment of salt-washed nuclear envelopes with Triton X-100 at low ionic strength yields a supramolecular protein substructure of the nuclear envelope, comprised of morphologically intact pore complexes attached to the nuclear lamina (the pore complex-lamina fraction; references 2, 5). When nuclear envelopes are incubated with Triton at low ionic strength (0.02 M KCl) to produce the pore complex-lamina fraction, most intrinsic membrane polypeptides of the nuclear envelope are completely solubilized, including the major group of 50-kilodalton bands (Fig. 3 *a*, *NE + 2% Triton: 0.02 M KCl: s and p* lanes, asterisk). In contrast, gp 190 occurs almost quantitatively in the pore complex-lamina pellet under these ionic conditions (Fig. 3 *a* and *b*, *NE + 2% Triton, 0.02 M KCl* lanes, arrowheads). However, gp 190 is not inherently insoluble in Triton X-100, since treatment of nuclear envelopes with Triton at elevated ionic strength (0.15 M KCl) results in the appearance of a major proportion of this protein in the supernatant (Fig. 3 *a* and *b*, *NE + 2% Triton, 0.15 M KCl: s and p* lanes, arrowheads). The observation that gp 190 remains completely associated with the pore complex-lamina material under conditions that solubilize most other major nuclear envelope intrinsic proteins suggests that this polypeptide may be physically associated with either pore complexes or with the lamina.

Production and Characterization of Specific Antibodies

To localize gp 190 by immunocytochemical techniques, we prepared specific antibodies to this polypeptide. Production and purification of these antibodies involved several complementary steps (see Materials and Methods). First, an intrinsic membrane protein fraction of nuclear envelopes (a 0.1 N NaOH pellet) was electrophoresed on preparative SDS gels, and the 190-kilodalton band was eluted and used to immunize chickens. Subsequently, specific anti-gp 190 antibodies were obtained from antisera by affinity chromatography on a column containing lectin-purified nuclear envelope glycoproteins. The gel profile of the glycoprotein fraction used for this affinity column was identical to that shown in Fig. 4 *a* (*Coomassie Blue, lentil lectin: mm* lane).

We characterized our affinity-purified antibody preparation by incubation of nitrocellulose transfers of SDS gels with anti-gp 190, followed by a radioactive second antibody (Fig. 4 *a*). The affinity-purified antibody recognizes a single band at 190 kilodaltons in the gel profile of total nuclear envelope polypeptides (Fig. 4 *a*, ^{125}*I antibody, total* lane). Furthermore, when

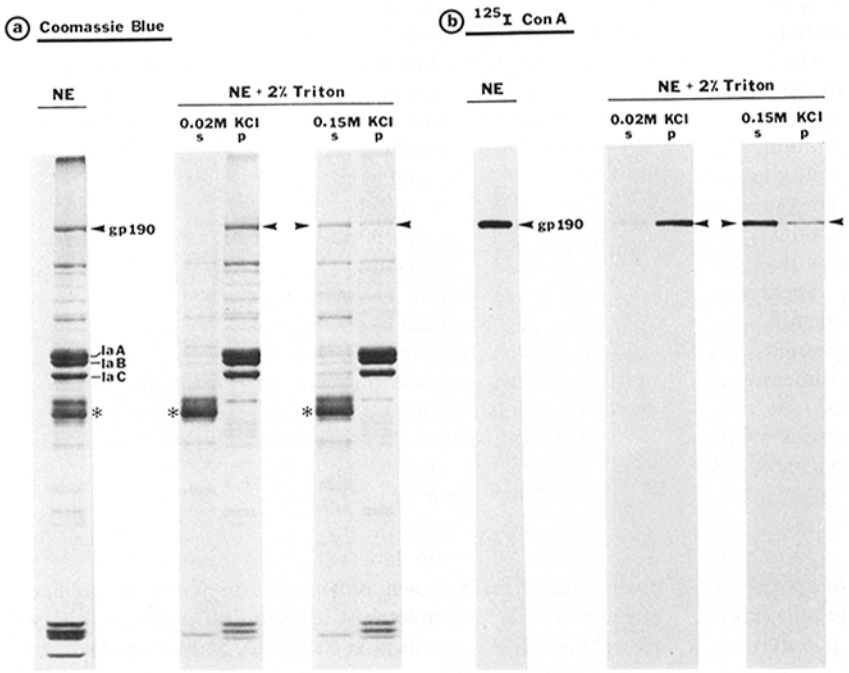

FIGURE 3 Fractionation behavior of gp 190 upon treatment of nuclear envelopes with Triton X-100. Samples of nuclear envelopes were incubated in a buffer containing 2% Triton X-100 and either 0.02 M KCl or 0.15 M KCl, and centrifuged to yield supernatant (*s*) and pellet (*p*) fractions (see Materials and Methods). These fractions, together with a sample of unfractionated nuclear envelopes (*NE*), were electrophoresed on 7.5–15% SDS gels. One set of samples was stained with Coomassie Blue (*a*). A second set was electrophoretically transferred to nitrocellulose paper and incubated with ^{125}I Con A. Shown are autoradiograms of these Con A-incubated lanes (*b*). Gp 190 appears almost entirely in the pellet upon treatment of nuclear envelopes with 0.02 M KCl in Triton, but is largely extracted to the supernatant by Triton plus 0.15 M KCl (arrowheads).

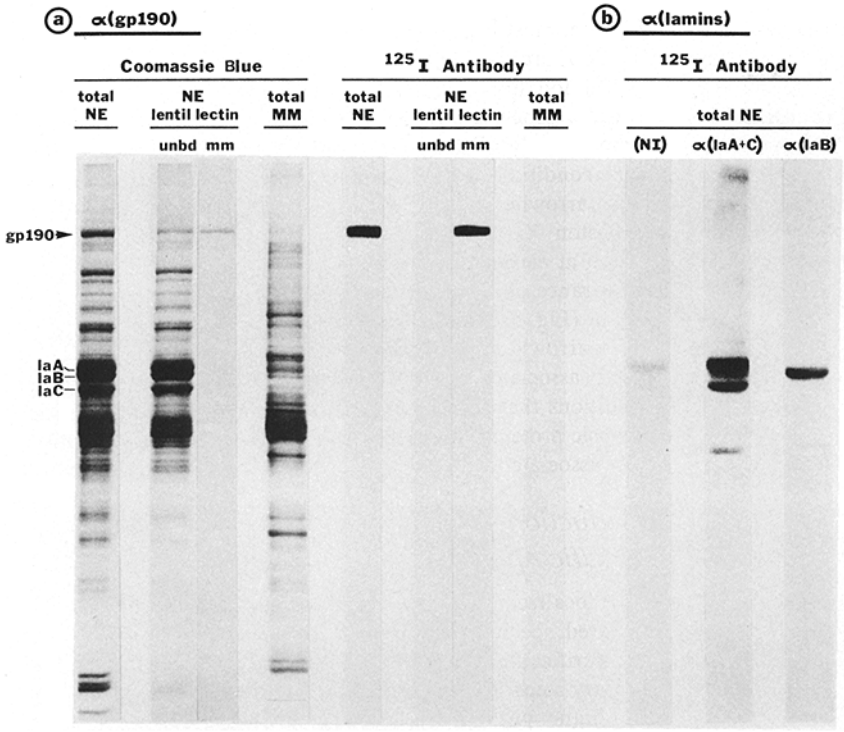

FIGURE 4 Characterization of affinity-purified antibodies to gp 190 and the lamins. A sample of total nuclear envelope polypeptides was solubilized in SDS, and in the presence of excess Triton X-100, was chromatographed on lentil-lectin-Sepharose as described in Materials and Methods. This procedure yielded a nonglycoprotein fraction of unbound (*NE, lentil lectin, unbd*) polypeptides, and a glycoprotein fraction, consisting of bound polypeptides that were eluted from the lectin column with 1 M α-methyl-mannoside (*NE, lentil lectin, mm*). These fractions, together with samples of total nuclear envelope polypeptides (*total NE*) and total microsomal membrane polypeptides (*total MM*) were electrophoresed on 7.5–15% SDS gels. One set of samples was stained with Coomassie Blue (*a*). Another was electrophoretically transferred to nitrocellulose paper. Transfers were then incubated with affinity purified anti-gp 190 (α(gp 190)), antilamins A and C (α(*laA+C*)), antilamin B (α(*laB*)), or nonimmune chicken IgG (*NI*); and subsequently with ^{125}I rabbit anti-chicken IgG. Autoradiograms of the latter samples are shown.

nuclear envelope polypeptides are separated by a *Lens culinaris* agglutinin (lentil lectin) column into an unbound (nonglycoprotein) fraction (Fig. 4*a*, *Coomassie Blue, lentil lectin: unbd* lane) and a bound (glycoprotein) fraction (Fig. 4*a*, *Coomassie Blue, lentil lectin: mm* lane), the anti-gp 190 preparation binds exclusively to the 190-kilodalton polypeptide of the glycoprotein fraction. This antibody does not react with the nonglycoprotein 190-kilodalton nuclear envelope species that co-migrate with gp 190. It also does not react with any polypeptides of microsomal membranes (Fig. 4*a*, *total MM* lane). From these experiments we conclude that this affinity-purified preparation is specific for gp 190.

Because antibodies directed against the lamins are used for controls in our immunocytochemical studies discussed below, we also characterized these preparations by labeling of nitrocellulose gel transfers (Fig. 4*b*). An antibody raised to purified lamin A recognizes both lamins A and C (Fig. 4*b*, α(*laA+C*)), which migrate at 70 and 60 kilodaltons, respectively. As shown previously, lamins A and C are structurally similar (10, 28) and immunologically cross-reacting (11). This preparation also reacts with minor components (at ~68 and 40 kilodaltons) that apparently represent in vivo proteolytic degradation products of lamins A and C (L. Gerace, unpublished observations). The 68-kilodalton species recognized by this preparation does not correspond to lamin B, as shown by antibody labeling of nitrocellulose transfers of two dimensional isoelectric focusing-SDS gels (data not shown). (Lamin B is widely separated from the isoelectrically similar Lamins A and C by two dimensional electrophoresis (9, 28).) An affinity-purified antibody raised to purified lamin B recognizes only lamin B, migrating at 67 kilodaltons, and its apparent proteolytic degradation product, of ~40 kilodaltons (Fig. 4*b*, α(*laB*) lane).

Immunocytochemical Localization

We examined the overall cellular distribution of gp 190 by immunofluorescence staining of PtK2 tissue culture cells (Figs. 5 and 6). In exponentially growing interphase populations, anti-gp 190 (Fig. 5*a* and *b*) gives a prominent nuclear reaction, that is substantially greater than the nonimmune chicken IgG background (Fig. 5*d*). No specific cytoplasmic staining is apparent. In favorable views, the nuclear labeling by anti-gp 190 is characterized by a detectable perinuclear "rim" of enhanced staining intensity (Fig. 6*a*), a pattern suggestive of nuclear envelope antigens (11, 16). However, no identifiable intranuclear structures are labeled. For comparison, cells labeled with antilamins A and C also show prominent nuclear fluorescence, including a strong perinuclear "rim" reaction (Fig. 5*c*). These immunofluorescence results, therefore, suggest that gp 190 is localized in the nuclear envelope of interphase cells, and does not detectably occur in the cytoplasm. Using a cell fractionation approach, we have determined that gp 190 is absent from the nuclear interior. When rat liver nuclei are digested with nucleases to release the internal nuclear contents from nuclear envelopes in a nonsedimenting form (5), negligible quantities of gp 190 appear in the supernatant, as determined by antibody labeling of nitrocellulose SDS gel transfers (data not shown).

During mitosis, the cellular distribution of gp 190 changes dramatically (Fig. 6), analogous to the pattern previously observed for the lamins at cell division (11, 16). Throughout prophase (not shown), gp 190 gradually shifts from its discrete interphase nuclear localization (Figs. 5*a* and 6*a*) to a diffuse cytoplasmic distribution. This protein is completely dispersed throughout the cytoplasm by metaphase (Figs. 6*c* and *d*), with no detectable association with the metaphase chromosomes. Gp 190 remains dispersed throughout the cell until late anaphase and telophase (Figs. 6*e* and *f*), when it gradually returns to a discrete position at the periphery of the condensed telophase chromosome masses. By early G1 (not shown), this polypeptide once again occurs entirely in the nucleus.

We localized gp 190 at the electron microscopic level using indirect immunoferritin labeling of Triton-washed rat liver nuclei (Fig. 7). As shown previously, treatment of isolated nuclei with Triton completely solubilizes the nuclear envelope phospholipids, leaving the pore complexes and lamina exposed

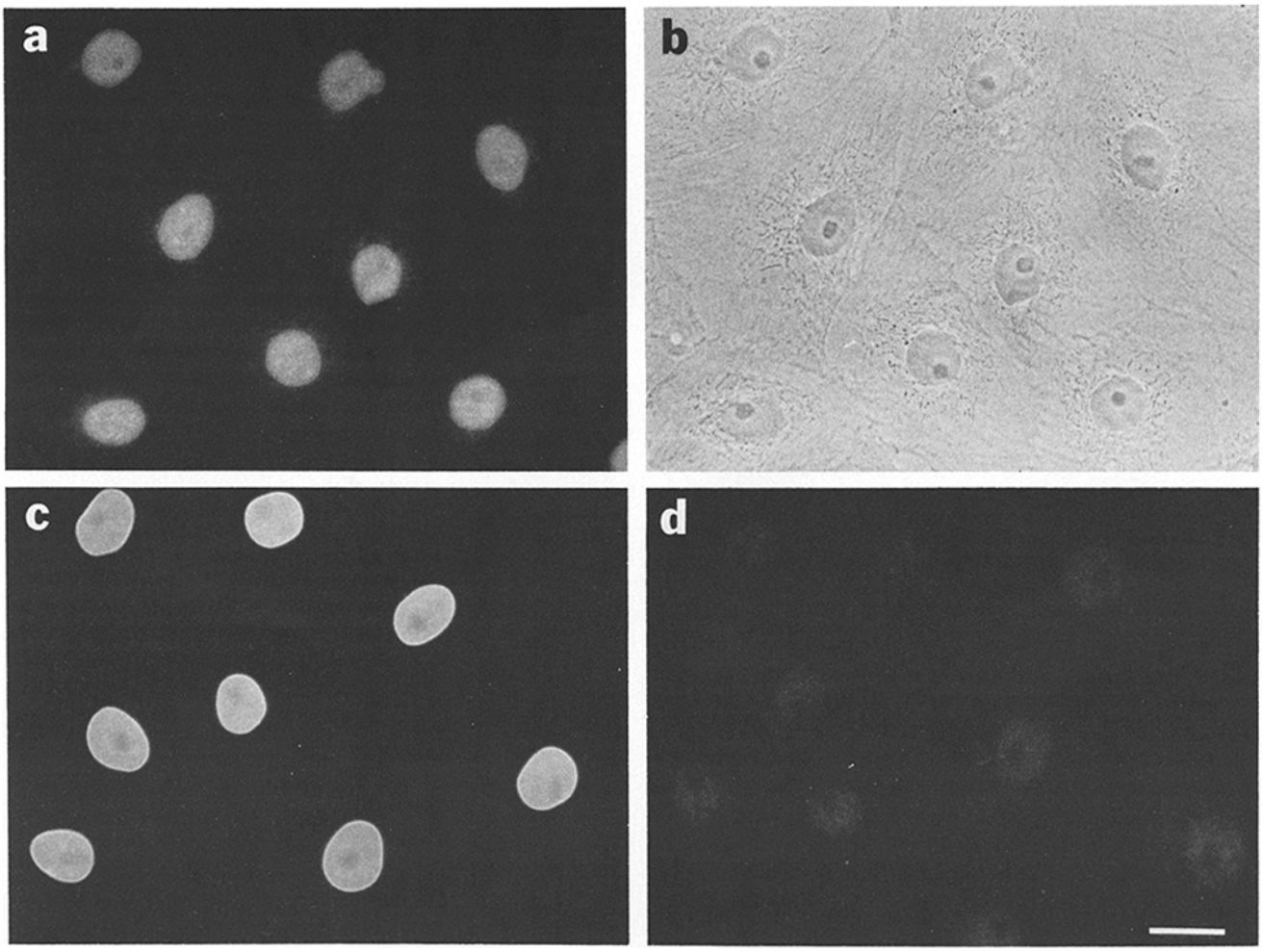

FIGURE 5 Immunofluorescence staining of tissue culture cells with anti-gp 190 and antilamins A and C. Coverslips with exponentially growing PtK2 cells were fixed with 4% formaldehyde followed by 100% methanol, as described in Materials and Methods, and incubated with various chicken IgG samples at a concentration of 0.025 A_{280}/ml, followed by rhodamine-conjugated rabbit anti-chicken IgG. Shown are fluorescence (*a*, *c*, and *d*) and phase-contrast (*b*) micrographs of interphase cells stained with anti-gp 190 (a and *b*), antilamins A and C (c), and nonimmune chicken IgG (*d*). Bar, 20 μm. × 515.

and attached to the Triton-denuded nuclear surface (1). Furthermore, as we demonstrated above (Fig. 3), gp 190 remains quantitatively associated with the pore complex-lamina material under the Triton and salt conditions that we used for our immunoferritin nuclear preparations. Nuclei labeled with anti-gp 190 (Fig. 7 *a–c*) show a highly localized, moderate to heavy deposition of ferritin at the pore complexes (arrowheads in Fig. 7 *a* and *c*), with little labeling of the lamina, which extends continuously over the Triton-washed nuclear surface in the areas between the pore complexes. In contrast, nuclei labeled with antibodies to the lamins (Fig. 8) show a distinct and complementary ferritin distribution to that obtained with anti-gp 190. For both antilamins A and C antibodies (Fig. 8 *a* and *b*) and monospecific antilamin B antibodies (Fig. 8 *c*), labeling occurs approximately uniformly over the nuclear surfaces where the lamina is located, but not at the pore complexes themselves. The ferritin labeling we obtain with nonimmune chicken IgG (Fig. 8 *d*) is negligible. From these results, we conclude that gp 190 is specifically localized in the nuclear pore complex, and is absent from the lamina.

The resolution of our indirect immunoferritin labeling techniques is ~30–40 nm, considering the molecular dimensions of our labeling reagents (16 nm length for IgG, ~10 nm diameter for ferritin). Therefore, although multiple rabbit anti-chicken IgG-ferritin conjugates may often be bound to each chicken IgG molecule in these preparations, the fact that different ferritin particles associated with a single pore complex are separated by as much as 100–120 nm (e.g. Fig. 7 *c*) suggests that multiple copies of gp 190 occur in the pore complex.

High magnification views (Fig. 7 *d–f*) illustrate major features of the ferritin labeling pattern that we obtain with anti-gp 190 in relation to pore complex ultrastructure. (It should be noted that only a fraction of the mass of a pore complex, which has dimensions of ~120 nm × 100 nm (references 7, 20) will appear in one 50-nm diameter thin section.) In most cases, we obtain ferritin labeling only at the cytoplasmic side of the pore complex (Fig. 7 *e*, arrows). Therefore, in a tangential section to the nuclear surface, only pore complexes that have their cytoplasmic regions represented in the section are heavily labeled (Fig. 7 *d*, downward arrows). However, in ~10–15% of morphologically well-preserved pore complexes, there is also a lower level of labeling at the nucleoplasmic pore complex regions (Fig. 7 *f–g*, arrows; also, leftmost upward arrow in Fig. 7 *d*). Despite the diminished level of ferritin labeling that we obtain at the nucleoplasmic side of the pore complex, it is possible that gp 190 occurs to an equivalent extent in both the

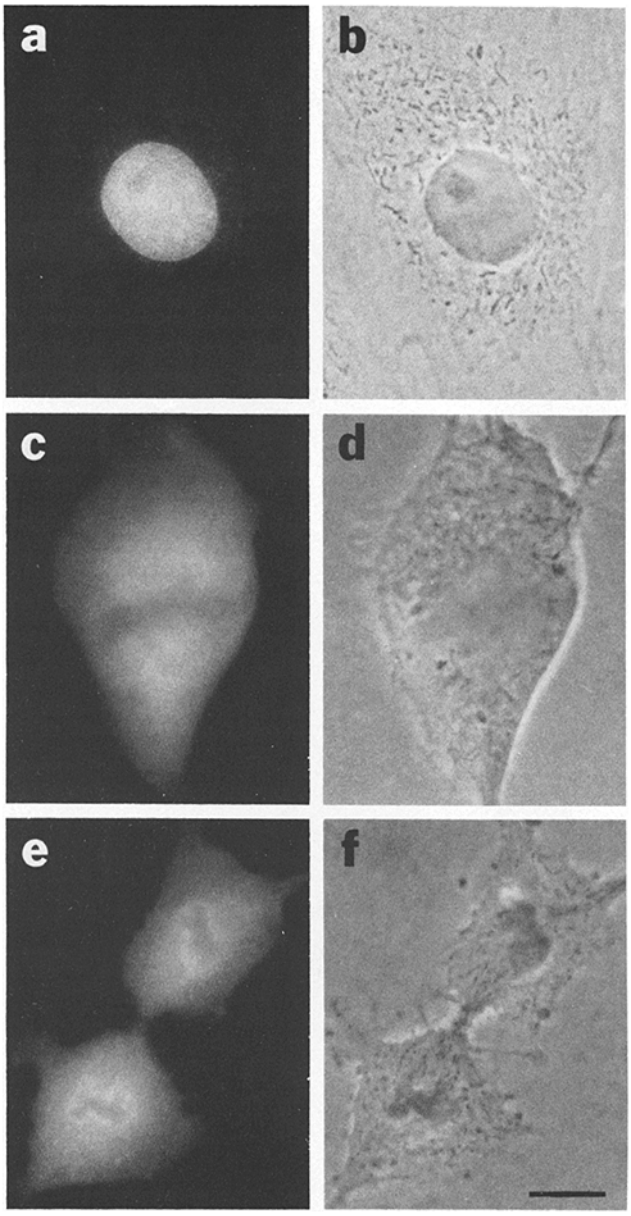

FIGURE 6 Immunofluorescence staining of mitotic cells with anti-gp 190. PtK2 cells growing on coverslips were fixed with formaldehyde/methanol and incubated with anti-gp 190 at a concentration of 0.025 A_{280}/ml, followed by rhodamine-conjugated rabbit anti-chicken IgG. Shown are fluorescence (*a*, *c*, and *e*) and phase-contrast (*b*, *d*, and *f*) micrographs of an interphase cell (*a* and *b*), a metaphase cell (*c* and *d*), and a telophase cell (*e* and *f*). Bar, 10 μm. × 1,010.

cytoplasmic and nucleoplasmic pore complex regions (see Discussion).

We were unable to obtain any significant degree of specific ferritin labeling with anti-gp 190 using nuclear envelopes or nuclei that were not first treated with Triton X-100 (data not shown). We attribute this result to inaccessibility of our labeling reagents to gp 190 in these membrane-containing preparations.

Gp 190 of Other Vertebrates

We examined samples of nuclei from different tissues of several vertebrate species for the presence of gp 190, using radioactive antibody labeling of nitrocellulose SDS gel transfers (Fig. 9). In unfractionated rat liver nuclei (Fig. 9, *RL N* lane), we detect a single antigenic form of gp 190 having an identical molecular weight to the polypeptide of purified rat liver nuclear envelopes (Fig. 9, *RL NE* lane). This indicates that no detectable proteolytic degradation of the protein occurs during the preparation of nuclear envelopes from isolated nuclei. We also detect a ~190-kilodalton polypeptide that cross-reacts with rat liver gp 190 in the nuclei of bovine thymus, canine kidney, and chicken liver (Fig. 9, *BT, CK, and AL* lanes, respectively). Hence, an antigenically similar gp 190 occurs in the nuclei of different tissues and diverse vertebrate species.

DISCUSSION

In this study, we have identified a major nuclear envelope polypeptide (gp 190) that is specifically localized in the nuclear pore complex. Because gp 190 is not extracted from nuclear membranes by a variety of strong protein perturbants, including 0.1 N NaOH and 6 M guanidine-HCl, it is very likely an intrinsic membrane protein (29, 30). Binding studies with Con A and *Lens culinaris* agglutinin indicate that this polypeptide is the major glycoprotein of nuclear envelopes. Gp 190 appears to be an evolutionarily conserved nuclear envelope component, since a cross-reacting polypeptide of the same molecular weight occurs in nuclei of diverse vertebrate species. A high molecular weight Con A-reactive polypeptide was recently detected in a subnuclear fraction of Drosophila cells and in the pore complex-lamina fractions of several vertebrates (6), and probably corresponds to gp 190.

Using specific affinity-purified antibodies, we localized gp 190 by immunofluorescence and immunoferritin microscopy. Immunofluorescence staining of tissue culture cells suggests that gp 190 occurs exclusively in the nuclear envelope during interphase, and is not present in cytoplasmic regions of the cell. In support of these immunofluorescence results, we also cannot detect this protein in an endoplasmic reticulum-enriched membrane fraction by radioactive antibody labeling of nitrocellulose SDS gel transfers. Nevertheless, it is possible that gp 190 is present in trace quantities in the endoplasmic reticulum following biosynthesis, before it is assembled in the pore complex.

Immunofluorescence microscopy shows that gp 190 undergoes changes in its cellular localization during mitosis that resemble the mitotic patterns previously described for other nuclear envelope proteins (the lamins) (11, 16). Specifically, when the nuclear envelope is disassembled during prophase, gp 190 becomes dispersed throughout the cytoplasm. This protein remains diffusely distributed throughout the cell until the nuclear envelope is reconstructed during telophase, when it gradually returns to a localized position at the nuclear periphery. Because gp 190 appears to be an intrinsic membrane protein, we consider it probable that this polypeptide is associated with membrane vesicles derived from the disassembled nuclear envelope when it is dispersed throughout the cell during mitosis.

Our immunoferritin localization results demonstrate that gp 190 occurs exclusively in the pore complex, and is not present in the nuclear lamina. With our indirect immunoferritin procedure, anti-gp 190 labels predominantly the cytoplasmic side of the pore complex, and to a lesser extent, the nucleoplasmic side. Common structural models (7, 21; however, see reference 32), suggest that the pore complex contains two rings or "annuli" (each comprised of eight radially arranged subunits) that lie parallel to the plane of the nuclear envelope, one

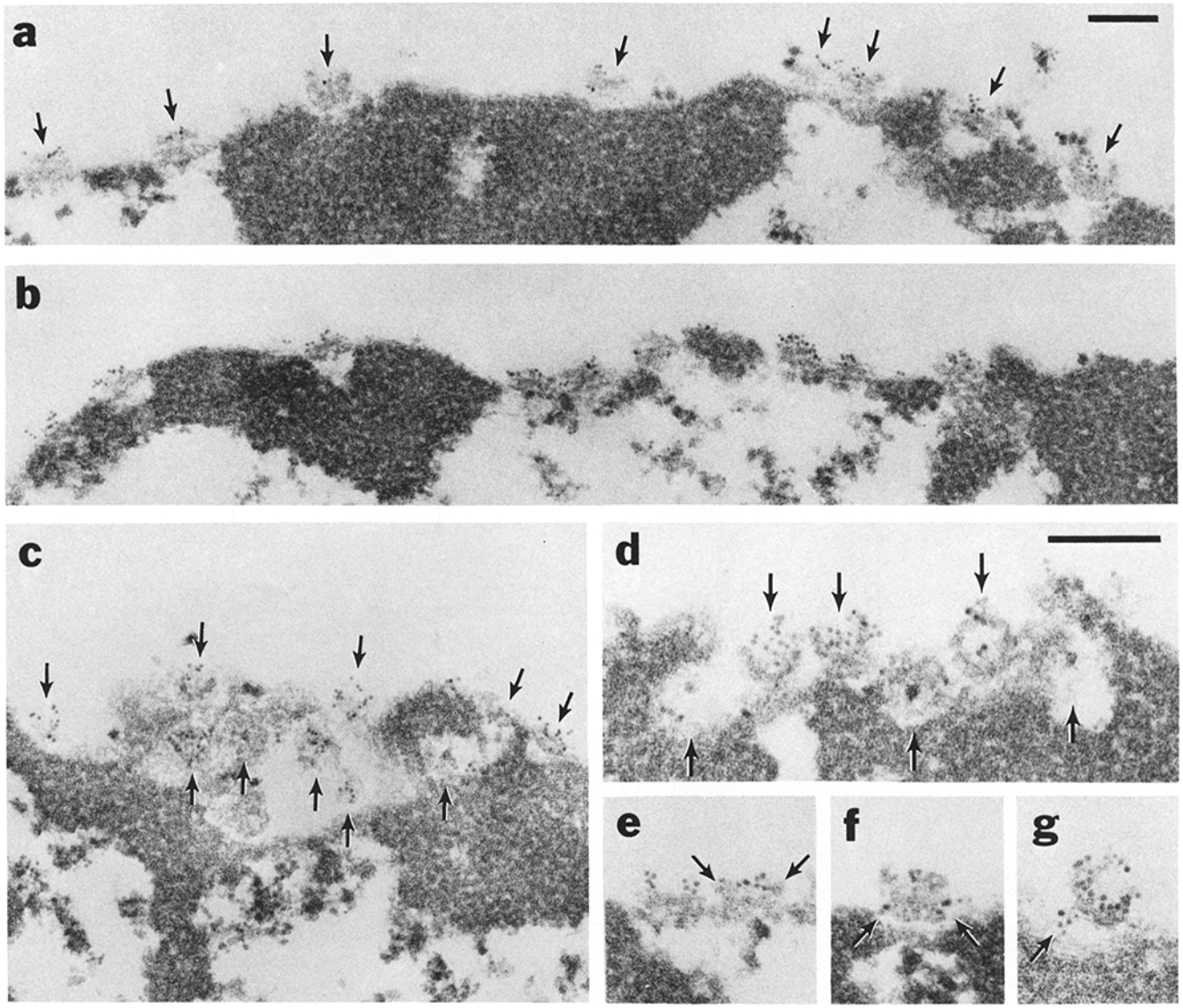

FIGURE 7 Immunoferritin labeling of Triton-treated nuclei with anti-gp 190. Rat liver nuclei were briefly treated with Triton X-100 and fixed with 0.05% glutaraldehyde as described in Materials and Methods. Samples were then incubated with anti-gp 190 at a concentration of 0.025 A_{280}/ml, followed by ferritin-conjugated rabbit anti-chicken IgG. Electron micrographs of the surfaces of Triton-treated nuclei are shown in thin sections perpendicular (*a* and *b*) and tangential (*c*) to the nuclear surface. Pore complexes remain attached to the surfaces of nuclei during Triton treatment, and in some fields (*a* and *c*) are identified by arrows. Higher magnification micrographs of selected pore complexes illustrate the major features of pore complex labeling that we obtained (*d–g*). A thin section tangential to the nuclear surface (*d*) shows both cytoplasmic regions of pore complexes, which are heavily labeled with ferritin (*d*, downward-pointing arrows), as well as nucleoplasmic areas of pore complexes, which show little or no ferritin labeling (*d*, upward-pointing arrows). Transverse sections to the nuclear surface (*e–g*) demonstrate the same features. In ~85–90% of morphologically well-preserved pore complexes viewed in transverse section, labeling occurs only at the cytoplasmic side of the pore complex (arrows in *c*). However, in ~10–15% of morphologically well-preserved pore complexes, labeling also occurs at the nucleoplasmic side (arrows in *f* and *g*). Bars, 150 nm. *a–c* × 70,000; *e–g* × 115,000.

annulus on the nucleoplasmic side of the pore, and another on the cytoplasmic side. Additional structural components are believed to interconnect these two annuli and form a diaphragm in the central pore region. Considering these structural models, our labeling data are consistent with the possibility that gp 190 is a component of the cytoplasmic pore complex annulus. Gp 190 may also occur to an equivalent extent in the nucleoplasmic annulus, since the cytoplasmic and nucleoplasmic annuli may be structurally similar (7, 21, 32). The lower degree of nucleoplasmic labeling that we obtain with anti-gp 190 could be a result of diminished steric accessibility of our labeling reagents to nucleoplasmic pore complex regions, compared to the cytoplasmic areas. Alternately, it is possible that gp 190 is exclusively confined to the cytoplasmic annulus, and that a minor degree of antigen relocalization is responsible for the nucleoplasmic labeling that we observe.

We were unable to obtain a significant level of specific ferritin labeling with anti-gp 190 in intact (non-Triton-treated) nuclear envelopes or nuclei, presumably due to inaccessibility of our labeling reagents to gp 190 in these membrane-containing preparations. However, we consider it extremely unlikely that any major degree of long-distance antigen redistribution occurs during Triton treatment of nuclei, in view of the precise specificity of our ferritin labeling, and the negligible solubili-

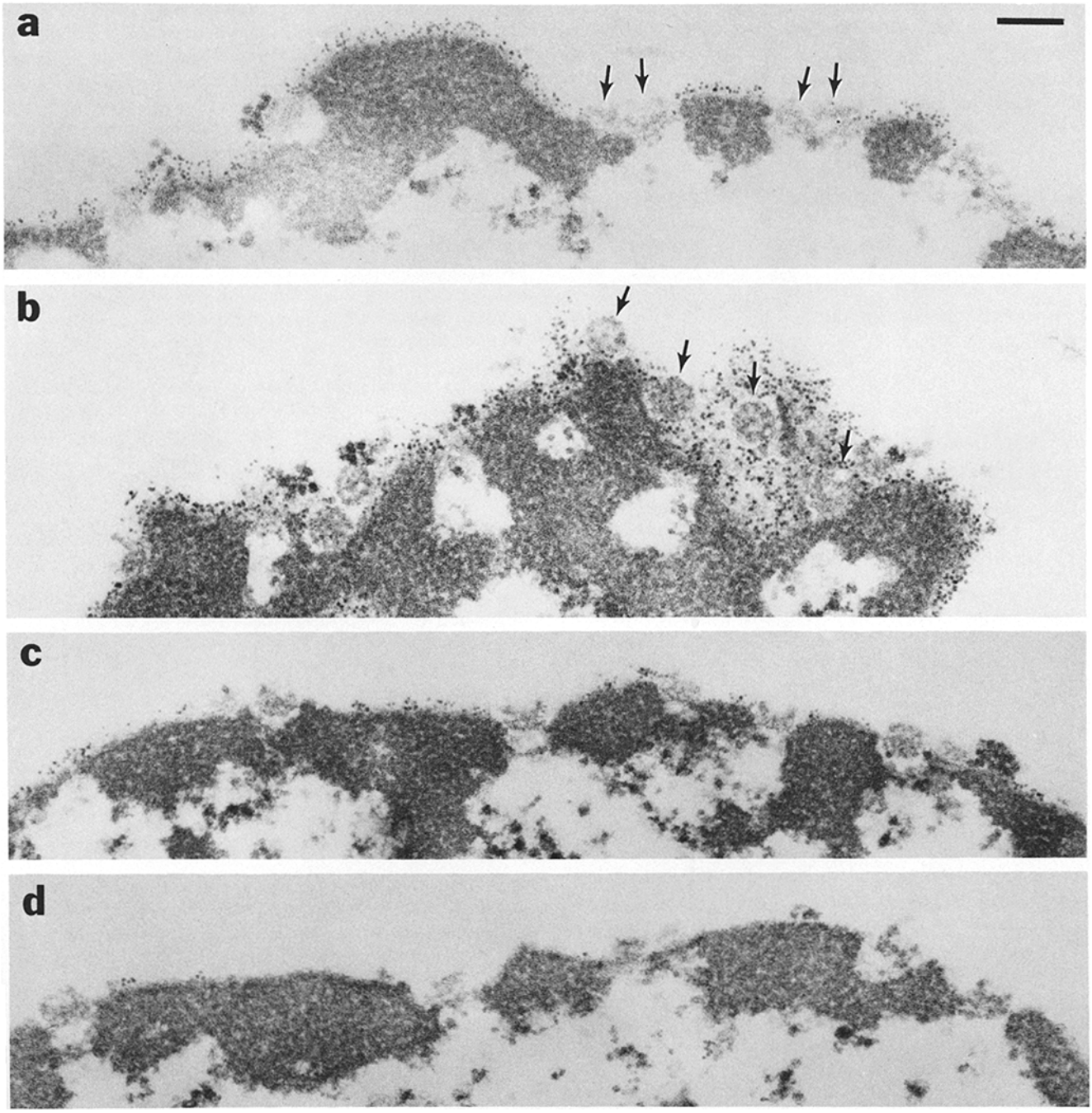

FIGURE 8 Immunoferritin labeling of Triton-treated nuclei with antibodies to the lamins. Rat liver nuclei were treated briefly with Triton X-100 and fixed with 0.05% glutaraldehyde, as described in Materials and Methods. Samples were then incubated with antilamins A and C at a concentration of 0.025 A_{280}/ml (*a* and *b*), antilamin B at a concentration of 0.025 A_{280}/ml (*c*), and nonimmune chicken IgG at a concentration of 0.05 A_{280}/ml (*d*). Subsequently, these samples were incubated with ferritin-conjugated rabbit anti-chicken IgG. Shown are thin section electron micrographs of fields perpendicular (*a*, *c* and *d*) and partially tangential (*b*) to the nuclear surfaces. Examples of nuclear pore complexes are indicated by arrows in *a* and *b*. Bar, 150 nm. × 70,000.

zation of gp 190 with our conditions of Triton extraction.

Considering the resolution of our indirect immunoferritin technique (see Results), the ferritin labeling patterns that we obtain suggest that multiple copies of gp 190 occur in the pore complex. We favor the idea that the number of molecules of this polypeptide per pore complex is related to the pore complex eightfold symmetry (7, 21, 32). Hence, a pore complex may contain 8, 16, or some further eightfold multiple copies of this protein. A calculation of the number of molecules of gp 190 per pore complex can be made from the quantity of this polypeptide isolated from nuclear envelopes by lentil lectin chromatography. Based on Coomassie Blue staining of an SDS gel of a lectin-purified fraction (Fig. 4), we estimate that ~1 μg of gp 190 is derived from 10 A_{260} units of nuclei. Furthermore, assuming that 1 A_{260} unit of nuclei contains 3×10^6 nuclei (1), and that an average rat liver nucleus contains 4×10^3 pore complexes (21), we calculate that there are ~25 molecules of gp 190 per pore complex. Considering the number

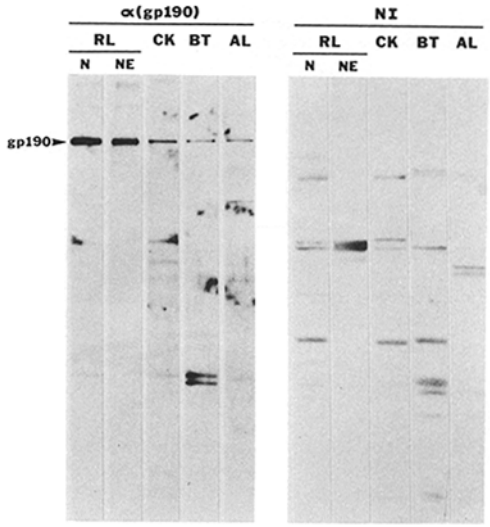

FIGURE 9 Presence of gp 190 in diverse vertebrate species. Samples of rat liver (*RL*) nuclei (*N*) and nuclear envelopes (*NE*), and nuclei from canine kidney (*CK*), bovine thymus (*BT*), and chicken liver (*AL*) were electrophoresed on a 7.5–15% SDS gel, and electrophoretically transferred to nitrocellulose paper. The transfers were then incubated with affinity-purified anti-gp 190 (*α(gp 190)*) at a concentration of 0.0015 A_{280}/ml, or with nonimmune chicken IgG (*NI*), 0.01 A_{280}/ml, followed by ^{125}I rabbit anti-chicken IgG. Shown are autoradiograms of these samples. The nonimmune lanes were incubated with a higher concentration of chicken IgG than were the immune lanes, to more strongly reveal nonspecific background bands. Immune lanes show a specific labeled band at 190 kilodaltons (arrow).

of estimations that are involved in determining this figure, this calculation must be considered to be only approximate.

In previous studies (11), we localized the nuclear lamins by electron microscopic immunoperoxidase cytochemistry using several antibody preparations that react with all three lamins. These earlier studies are extended by the immunoferritin localization of these polypeptides described in this paper, where we used an antibody that is specific for lamins A and C (which are structurally similar [10, 28] and strongly cross-reacting [11]), and a second antibody that is monospecific for lamin B. With both antilamin preparations, we observed uniform labeling of the Triton-treated nuclear surfaces in the areas between the pore complexes where the lamina occurs, but no labeling of the pore complexes themselves. From these data, it can be concluded that lamin B is distributed approximately uniformly throughout the lamina, and is not segregated in any restricted lateral domains (e.g. adjacent to pore complexes). Furthermore, lamins A and C considered together also appear to have a uniform lamina distribution.

The biochemical data described in this paper provide insight on the membrane topology of gp 190. This polypeptide resists extraction from nuclear membranes by a number of strong protein perturbants, and therefore presumably has a lipid-integrated domain (29). Furthermore, it remains associated with the pore complex-lamina fraction when nuclear envelopes are treated with Triton X-100 at low ionic strength, in contrast to most other nuclear envelope intrinsic proteins. This indicates that a portion of gp 190 is physically associated with the pore complex, and that a second domain of this polypeptide may be on the pore side of nuclear membranes. Finally, gp 190 has a carbohydrate-bearing region. By analogy to endoplasmic reticulum glycoproteins, for which the carbohydrate-containing domains appear to be exclusively confined to the cisternal side of the endoplasmic reticulum (26), the carbohydrate-bearing portion of gp 190 most likely occurs in the perinuclear cisternal space (which is equivalent to, and continuous with the endoplasmic reticulum cisternae). Therefore, gp 190 may be a transmembrane glycoprotein.

Because gp 190 appears to be a predominant nuclear envelope-specific intrinsic polypeptide, it may have a primarily structural role in the pore complex. In analogy to the organization of several model membranes (4, 18), gp 190 may be involved in "anchoring" the pore complex (via the cytoplasmic and possibly nucleoplasmic annuli) to nuclear membranes in the region of the pore. The characteristics of gp 190 described in the present paper suggest that this polypeptide will provide a useful molecular handle in further investigations of pore complex structure.

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Note Added in Proof: It has recently been reported (Stick, R. and G. Krohne, 1982. *Exp. Cell Res.* 138:319–330) that a major polypeptide of amphibian oocyte nuclear envelopes is localized in both the nuclear lamina and in pore complexes.

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