Targeting of Membranes to Sea Urchin Sperm Chromatin Is Mediated by a Lamin B Receptor-like Integral Membrane Protein

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Abstract. We have identified an integral membrane protein of sea urchin gametes with an apparent molecular mass of 56 kD that cross-reacts with an antibody against the nucleoplasmic NH_2-terminal domain of human lamin B receptor (LBR). In mature sperm, p56 is located at the tip and base of the nucleus from where it is removed by egg cytosol in vitro. In the egg, p56 is present in a subset of cytoplasmic membranes (MV2β) which contributes the bulk of the nuclear envelope during male pronuclear formation. p56-containing vesicles are required for nuclear envelope assembly and have a chromatin-binding capacity that is mediated by p56. Lamin B is not present in these vesicles and is imported into the nucleus from a soluble pool at a later stage of pronuclear formation. Lamin B incorporation and addition of new membranes are necessary for pronuclear swelling and nuclear envelope growth. We suggest that p56 is a sea urchin LBR homologue that targets membranes to chromatin and later anchors the membrane to the lamina.

THE nuclear envelope (NE)[1] consists of a double membrane fenestrated by nuclear pores (Gerace and Foisner, 1994). Nuclear membranes can be divided into three distinct domains, each associated with specific integral and peripheral polypeptides. The outer nuclear membrane is continuous with the ER, studded with ribosomes, and contains ER proteins (Matsuura et al., 1981). The inner nuclear membrane is associated on its nucleoplasmic side with the nuclear lamina, a network of peripheral proteins of the intermediate filament protein family called lamins (Gerace and Blobel, 1980; Aebi et al., 1986; Fisher et al., 1986; McKeon et al., 1986). Since the lamina is discontinuous (Paddy et al., 1990), the inner nuclear membrane may also interact directly with chromatin (Ye and Worman, 1996). The pore membrane domain connects the outer and inner nuclear membranes and is associated with nuclear pore complexes (Hindshaw et al., 1992).

Several integral membrane proteins have been identified that are restricted to the inner nuclear membrane. Lamina-associated polypeptides that bind lamins and chromosomes (Foisner and Gerace, 1993; Gerace and Foisner, 1994) have been cloned (Harris et al., 1994; Furukawa et al., 1995; Martin et al., 1995). The lamin B receptor (LBR) (Worman et al., 1988, 1990; Courvalin et al., 1990; Soullam and Worman, 1993), also named p58 (Simos and Georgatos, 1992) or p54 (Bailer et al., 1991), has been characterized and cloned in chicken and human (Worman et al., 1990; Ye and Worman, 1994; Schuler et al., 1994). LBR has a nucleoplasmic NH_2-terminal domain of ~200 amino acids followed by a hydrophobic region with eight putative transmembrane segments (Worman et al., 1990; Ye and Worman, 1994). In vitro, the NH_2-terminal domain binds lamin B (Worman et al., 1988; Smith and Blobel, 1994; Ye and Worman, 1994), DNA (Ye and Worman, 1994), heterochromatin (Ye and Worman, 1996), and several partially characterized polypeptides (Simos and Georgatos, 1992; Georgatos et al., 1994; Nikolakaki et al., 1996). Binding of LBR NH_2-terminal domain to its ligands may be phosphorylation dependent (Appelbaum et al., 1990; Courvalin et al., 1992; Nikolalaki et al., 1996).

Association of the nuclear membranes with their ligands is dynamic. The membranes vesiculate in prophase of mitosis and reform around daughter nuclei after metaphase. Vesiculation is accompanied by solubilization of lamins A/C into the cytoplasm, whereas lamin B remains associated with vesicles (Gerace and Blobel, 1980). Whether lamin B and LBR colocalize or segregate into distinct vesicles during mitosis is a matter of controversy. After metaphase in HeLa cells, LBR-containing vesicles are targeted to chromatin before lamin B, suggesting that both proteins may

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1. *Abbreviations used in this paper*: DHCC, dihexyloxacarbocyanine iodide; ECL, enhanced chemiluminescence; GST, glutathione-*S*-transferase; LBR, lamin B receptor; LS, lipophilic structure; MV, membrane vesicle; MWB, membrane wash buffer; NE, nuclear envelope; S_{10}, supernatant from a 10,000-*g* spin; S_{150}, supernatant from a 150,000-*g* spin; TN, 10 mM Tris-HCl/150 mM NaCl buffer; TX-100, Triton X-100.

dissociate during mitosis (Chaudhary and Courvalin, 1993). In contrast, in chicken hepatoma cells, LBR and lamin B have been reported to be associated at all stages of the cell cycle (Meier and Georgatos, 1994).

Two models of NE assembly have emerged from in vitro studies. In the first model, illustrated by mammalian cells and *Drosophila* embryos, nuclear reformation requires initial attachment of NE peripheral proteins such as lamins (Burke and Gerace, 1986; Glass and Gerace, 1990; Ulitzur et al., 1992) or pore components (Sheehan et al., 1988) to chromatin for subsequent membrane vesicle (MV) binding. The second model, supported by cell-free experiments performed with *Xenopus* and sea urchin egg extracts, entails initial lamin-independent MV binding to chromatin followed by nuclear pore and lamin assembly (Newport et al., 1990; Meier et al., 1991; Collas et al., 1995). Distinct vesicle populations may cooperate to promote pronuclear formation (Wilson and Newport, 1988; Vigers and Lohka, 1991), but the lack of integral membrane markers has hindered these studies.

In fertilized sea urchin (*Lytechinus pictus*) egg extracts, three lamin epitope-containing peptides have been detected, which include a single B-type lamin with an apparent molecular mass of 65 kD (Collas et al., 1995). In these extracts, sperm chromatin is unpacked in two steps referred to as decondensation and swelling. During the first step that is lamin independent, the chromatin decondenses (Cameron and Poccia, 1994) and binds to vesicles that subsequently fuse to form an NE (Collas and Poccia, 1995). During the second step, the male pronucleus swells, a process requiring fusion of additional MVs and a 65-kD soluble lamin B (Collas et al., 1995). Pronuclear envelope assembly requires contributions from the sperm in the form of apical and basal lipophilic structures (LSs) (Collas and Poccia, 1995, 1996). Three distinct egg MV fractions also participate in NE assembly. The bulk of the NE originates from the most abundant fraction (MV2β), which is enriched in an ER-marker enzyme. Two non-ER minor fractions, MV1 and MV2α, bind specifically to the LS regions and are required for fusion of MV2β vesicles. However, in the sea urchin, as in *Xenopus*, the absence of integral membrane markers has prevented a precise analysis of the chronology of NE assembly.

In the present study, a rabbit polyclonal affinity-purified antibody directed against the NH_2-terminal domain of human LBR was used to identify an integral membrane protein with an apparent molecular mass of 56 kD (p56) in the MV2β egg vesicle fraction, and in the LS regions of sperm nuclei. We show that within the MV2β population, p56 and lamin B are segregated into different vesicle populations that are sequentially targeted to chromatin during NE assembly in vitro. Whereas the early step of membrane binding and fusion around chromatin is p56 dependent and lamin independent, the later step of pronuclear swelling and NE growth requires soluble lamin B.

Materials and Methods

Reagents, Antibodies, and Buffers

The chicken anti–lamin polyclonal antibody (W3-1) was a gift from Dr. Jon Holy (University of Minnesota, Duluth). W3-1 recognizes a single lamin B of 70 kD in *Strongylocentrotus purpuratus* embryos (Holy et al., 1995) and a single lamin B of 65 kD in *L. pictus* egg cytoplasm, sperm, and male pronuclei assembled in vitro (Collas et al., 1995). Rabbit anti–LBR polyclonal antibodies were obtained by immunizing rabbits with a glutathione-*S*-transferase (GST)–LBR fusion protein containing the first 201 amino acids of the NH_2-terminal end of the human LBR (Ye and Worman, 1994). Antibodies were affinity purified using HiTrap NHS-activated columns (Pharmacia, Uppsala, Sweden) containing the immobilized GST–LBR (1–207) fusion protein according to manufacturer's instructions. Egg lysis buffer, membrane wash buffer (MWB), and Tris-HCl/NaCl (TN) buffer were as described (Cameron and Poccia, 1994; Collas and Poccia, 1995, 1996) and contained 1 mM PMSF.

Sperm Nuclei, Egg Extracts, and Membrane Vesicles

Sperm nuclei of *L. pictus* were permeabilized as described (Collas and Poccia, 1995). Briefly, sperm heads were extracted for 15 min with 0.1% of Triton X-100 (TX-100) in TN buffer. Demembranated nuclei were pelleted, washed, and resuspended in TN buffer to 10^8 nuclei per ml. Nuclei were diluted 25-fold and added to egg extracts to a final ratio of ~1 sperm nucleus per egg equivalent.

Mature *L. pictus* eggs, arrested in G0 after completion of both meiotic divisions, were fertilized (Poccia and Green, 1986) and G1 cytosolic extracts were prepared essentially as described (Collas and Poccia, 1996). Eggs were homogenized in an equal volume of egg lysis buffer through a 22-gauge needle, the lysate was centrifuged at 10,000 *g*, and the supernatant (S_{10}) centrifuged at 150,000 *g* for 3 h at 4°C (SW28 rotor; Beckman Instruments, Inc., Palo Alto, CA). The clear cytosolic fraction (S_{150}) was frozen in liquid N_2 and stored at −80°C. The 150,000-*g* pellet was washed twice by resuspension in 1 ml of MWB and centrifugation at 150,000 *g* for 30 min. Membranes were fractionated at 150,000 *g* for 24 h in an SW28 rotor, to density equilibrium through a 0.2–2.0 M linear sucrose gradient (Collas and Poccia, 1996). Four fractions were produced (MV1, MV2, MV3, and MV4) and recovered by side puncture. Each fraction was washed in MWB, respun at 150,000 *g*, resuspended in MWB, and frozen at −80°C. The major fraction, MV2, was further fractionated for 4 h in an SW28 rotor through a 0.5–0.8 M sucrose step gradient into a minor light fraction (MV2α) and a major heavy fraction (MV2β; Collas and Poccia, 1996). Each fraction was washed in 1–2 ml of MWB, pelleted at 150,000 *g*, resuspended in a minimal volume of MWB, and frozen.

In Vitro Nuclear Assembly and NE Preparation

These procedures have been described previously (Cameron and Poccia, 1994; Collas and Poccia, 1995, 1996; Collas et al., 1995). Sperm chromatin was decondensed at room temperature in S_{150} containing an ATP-generating system (2 mM ATP, 20 mM creatine phosphate, 50 μg/ml creatine kinase), centrifuged through a 1 M sucrose cushion, and resuspended in TN on ice. MV binding to chromatin and fusion reactions were performed in two ways. (*a*) Decondensed chromatin was incubated in S_{10} extract containing an ATP-generating system to promote MV binding. Chromatin-bound vesicles were referred to as "bound". Fusion of chromatin-bound MVs (referred to as "fused") was induced with 100 μM GTP. The resulting nuclei were small (~4 μm in diam), surrounded by an envelope, but devoid of detectable lamins. Nuclear swelling and lamina assembly were promoted by adding to the assembly reaction 4 mM ATP, 40 mM creatine phosphate, and 100 μg/ml creatine kinase to produce male pronuclei (Collas et al., 1995). Nuclei containing bound or fused membranes and male pronuclei were washed through a 1 M sucrose cushion and resuspended in TN buffer. (*b*) Alternatively, decondensed chromatin was incubated in S_{150} containing MV2β vesicles and an ATP-generating system to promote MV binding. Chromatin with bound vesicles was washed through a 1 M sucrose cushion and resuspended in TN buffer.

NEs were prepared from swollen male pronuclei according to Dwyer and Blobel (1976), except that only one step of DNase/RNase digestion was performed. Nuclease-treated nuclei were sedimented through a 20% sucrose cushion in 20 mM Tris-HCl (pH 7.5) at 10,000 *g* for 10 min at 4°C. The NE pellet was resuspended in TN buffer, respun at 10,000 *g*, and resuspended in TN buffer.

Extraction of Proteins from Vesicles, Nuclei, and NEs

Protein extractions from subcellular fractions by alkali, urea, salt, and nonionic detergent at concentrations indicated in the text were performed in 1 ml of TN for 30 min at 4°C. Extracted vesicles were pelleted at 150,000

g, washed in MWB, and either resuspended in MWB for functional studies or solubilized in SDS sample buffer for biochemical analysis. Since direct solubilization of nuclei in SDS sample buffer resulted in a gel virtually impossible to resolve by SDS-PAGE, nuclei were extracted under conditions indicated in the text, and extracted material was biochemically analyzed. Extracted nuclei were pelleted at 5,000 *g* through a 1 M sucrose cushion made in TN and resuspended in TN. Extracted NE preparations were pelleted at 10,000 *g*, washed in TN, and solubilized in SDS sample buffer. After extraction, solubilized proteins were precipitated with TCA, and the precipitates were washed in acetone and solubilized in SDS sample buffer.

Immunoprecipitation and Membrane Immunodepletion

S_{150} cytosol was immunodepleted from soluble lamin B using the W3-1 antibody as described previously (Collas et al., 1995). Membrane-associated lamin B and p56 were solubilized from MVs or NEs by incubation for 1 h at 4°C in 1 ml of 1% TX-100 and 0.2–0.4 M NaCl as indicated in the text, and then immunoprecipitated by addition of anti-LBR or anti–lamin B antibodies (each at a 1:50 dilution) to the extract for 2 h at room temperature with gentle agitation. Immune complexes were retrieved by binding to protein A–Sepharose (Pharmacia) for 1 h at 4°C and by sedimentation at 4,000 *g* for 10 min. After two washes in TN containing 1% and 0.1% TX-100, immune complexes were solubilized in SDS sample buffer. Immunodepletion of p56- and lamin B–containing vesicles from MV2β was performed starting from 40 μl of concentrated MV2β vesicles diluted to 1 ml in TN. Immunological reactions were performed as above but in the absence of detergent. Immunopelleted vesicles were washed twice in 1 ml of TN and dissolved in SDS sample buffer. The reaction supernatant was spun at 4,000 *g* for 10 min to pellet any residual immune complexes. Vesicles present in the final supernatant were pelleted at 150,000 *g* through 0.5 M sucrose and washed twice in MWB. Immunodepletions were verified by immunoblotting using appropriate antibodies (data not shown).

Immunoblotting, Immunofluorescence, and Fluorescent Membrane Labeling

Proteins were resolved by 10% SDS-PAGE, transferred to a nitrocellulose sheet, and processed for immunoblotting as described (Collas et al., 1995), using anti–lamin B (W3-1) or anti-LBR antibodies at respective dilutions of 1:1,000 and 1:500. In some experiments, as indicated in the figure legends, blotted proteins were also detected by enhanced chemiluminescence (ECL) using the Immun-Lite II Assay kit (Bio Rad Laboratories; Hercules, CA) as described by the manufacturer. Immunofluorescence experiments were performed as described (Collas et al., 1995), except that incubations with first and secondary antibodies were for 30 min. Anti-LBR, anti–lamin B, and secondary antibodies were used at a 1:100 dilution. Nuclei were counterstained with 0.1 μg/ml propidium iodide (Sigma Chemical Co., St. Louis, MO). For fluorescent membrane labeling, 20 μl of concentrated MVs were diluted to 100 μl with MWB containing 10 μg/ml of the lipophilic dye, dihexyloxacarbocyanine iodide (DHCC) (Sigma Chemical Co.). After 30 min, MVs were washed in MWB (Collas and Poccia, 1995). Alternatively, a 5-μl aliquot of nuclear assembly reaction was fixed by mixing with 5 μl of 7% paraformaldehyde containing 20 μg/ml DHCC.

Microscopy and Photography

Fluorescence microscope observations were made on a Microphot SA epifluorescence microscope using a PlanApo ×60, 1.4-mm NA oil immersion objective (Nikon, Tokyo, Japan). Images in the green (FITC) and red (rhodamine) channels were captured with a C5810 CCD camera (Hamamatsu Photonics Systems, Bridgewater, NJ) and Adobe Photoshop 3.0 software (Adobe Systems, Inc., Mountain View, CA). Photographs were printed on a dye sublimation printer (Tektronix, Wilsonville, OR).

Results

The Integral Membrane Protein p56 of Egg Cytoplasmic Vesicles Is Immunologically Related to Vertebrate Lamin B Receptor

An antibody raised against the entire NH_2-terminal domain of human LBR was used to probe cytosolic (S_{150}) and MV fractions of sea urchin eggs by immunoblotting. The immune serum reacted with a single protein of 56 kD (apparent molecular mass) in the MV fraction, but not in the cytosol (Fig. 1 *A*). This 56-kD component was also detected in vesicles by antibodies of the same serum that were affinity purified with an immobilized GST–LBR fusion protein (Fig. 1 *B*). The anti–lamin B antibody W3-1 detected on duplicate blots the 65-kD lamin B of the cytosol and a 68-kD lamin component in the MV fraction (Fig. 1 *A*; see Collas et al., 1995).

To determine the mode of association of p56 with the membrane, vesicles were extracted with 1 M NaCl, 0.1 N NaOH, 0.1 M Na_2CO_3 (pH 11.5), or 1% TX-100 and sedimented at 150,000 *g*, and pellets and supernatants were analyzed by immunoblotting using anti-LBR and anti–lamin B antibodies. p56 was recovered in the pellet fractions after salt and alkaline extractions and was released into the supernatant after detergent solubilization of vesicles (Fig. 1 *C*). p56 exhibits extraction properties similar to the LBR, an integral membrane protein (Worman et al., 1988,

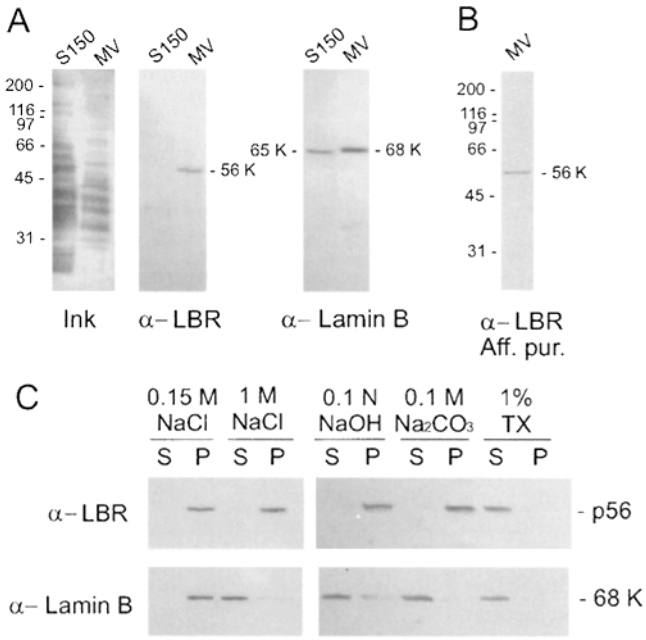

Figure 1. Characterization of an integral membrane protein (p56) in sea urchin eggs that is immunologically related to the vertebrate lamin B receptor. (*A*) Membrane vesicles (*MV*) and S_{150} fraction of egg cytoplasm were resolved by 10% SDS-PAGE, transferred to nitrocellulose, and revealed by ink or immunoblotting with a rabbit immune serum directed against the NH_2-terminal domain of the human lamin B receptor (*α-LBR*), or antibodies directed against sea urchin lamin B (*α-Lamin B*). (*B*) Immunoblotting of MVs with affinity-purified antibodies to LBR. Migration of molecular mass standards is indicated in kD on the left in *A* and *B*. (*C*) MVs were extracted in buffer containing either 0.15 M NaCl, 1 M NaCl, 0.1 N NaOH, 0.1 M Na_2CO_3, pH 11.5, or 1% TX-100, and sedimented at 150,000 *g*. Extracted proteins (*S*) and proteins resistant to extraction (*P*) were analyzed by 10% SDS-PAGE and immunoblotting using anti-LBR (*upper panel*) or anti–lamin B antibodies (*lower panel*). The 56 and 68 kD molecular masses were determined using molecular mass standards. Note that, in contrast to lamin B, p56 displays the characteristics of an integral membrane protein.

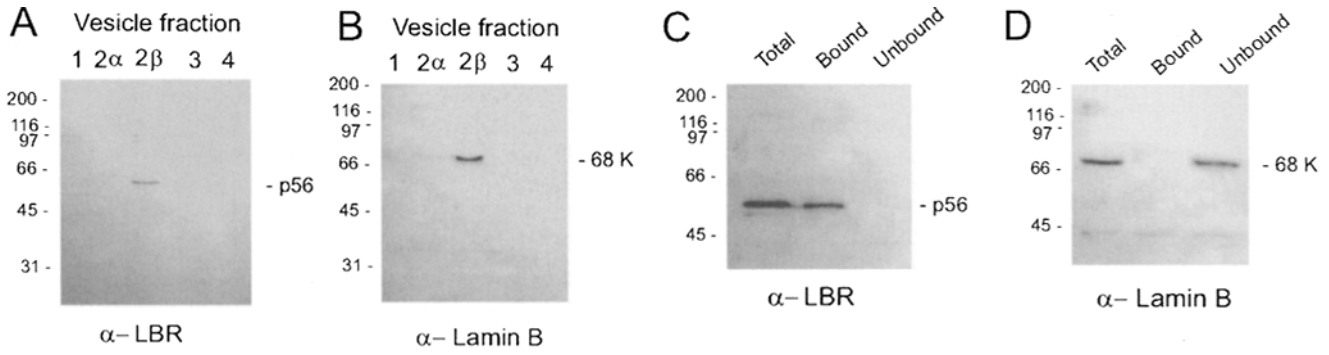

Figure 2. Lamin B and p56 are present in two different fractions of MV2β vesicles. MVs from egg cytoplasm were fractionated by density equilibrium sedimentation on sucrose gradients into five different fractions, MV1, MV2α, MV2β, MV3, and MV4. Proteins in the vesicles were solubilized in 1% TX-100/0.15 M NaCl, and then analyzed by immunoblotting using anti-LBR (*A*) or anti–lamin B antibodies (*B*). Note that p56 and lamin B colocalize in MV2β. Similar results were obtained by ECL detection of proteins on blots (data not shown). (*C*) Demembranated sperm nuclei were incubated in saturating amounts with MV2β vesicle fraction and chromatin-bound MVs recovered by sedimentation through a 1 M sucrose cushion. Membrane proteins in the complex were extracted with 1% TX-100. Proteins from the starting MV2β fraction (*Total*), from the chromatin-bound MV2β fraction (*Bound*), and from unbound MV2β fraction (*Unbound*) were analyzed by immunoblotting using anti-LBR antibodies. (*D*) The same fractions were analyzed using anti–lamin B antibodies. Note that p56 and lamin B were detected in chromatin-bound and unbound MV2β fractions, respectively. Similar data were obtained by ECL detection of proteins on blots (data not shown). Migration of molecular mass standards is indicated in kD at the left of each panel.

1990; Courvalin et al., 1990). In contrast, lamin B was solubilized by all these reagents, indicating that it is a peripheral protein. The presence of lamin B in the high speed supernatant of TX-100 extract shows that it was present in vesicles in unpolymerized form.

p56 Is Located in a Subset of Vesicles That Binds to Chromatin In Vitro and Does Not Contain Lamin B

To determine if p56 and lamin B were present in all egg cytoplasmic MV fractions separated by density equilibrium sedimentation (Collas and Poccia, 1996), equivalent amounts (~30 μg protein) of each vesicle fraction were analyzed by immunoblotting using anti-LBR and anti–lamin B antibodies. Both p56 and lamin B were detected exclusively in the MV2β fraction (Fig. 2 *A* and *B*).

To ascertain if LBR was found in the subfraction of MV2β vesicles that was able to bind to decondensed chromatin in vitro (Collas and Poccia, 1996), the MV2β fraction was incubated for 1 h with an excess of chromatin (20 demembranated decondensed sperm nuclei per egg equivalent, corresponding to 4,000 nuclei per μl S_{150}; Collas and Poccia, 1996), and chromatin-bound and unbound vesicles were sorted by sedimentation. Immunoblotting of both vesicle fractions showed that p56 was exclusively found in the chromatin-bound fraction of MV2β (Fig. 2 *C*), whereas lamin B was detected only in the unbound fraction (Fig. 2 *D*). Under these experimental conditions, nuclei were decondensed but not swollen.

Identification and Localization of p56 in Sperm Nuclei

The presence of p56 in sperm nuclei was assessed by immunofluorescence, in parallel with that of lamin B. In intact sperm heads, anti-LBR antibodies decorated only both nuclear poles, whereas lamin B labeling was observed over the entire nuclear surface (Fig. 3 *A*). Extraction of nuclei with 0.1% TX-100/0.15 M NaCl before fixation did not alter LBR and lamin B labeling (Fig. 3 *A*). When extraction was performed with 1% TX-100/0.15 M NaCl, bipolar labeling with both anti-LBR and anti–lamin B antibodies persisted, whereas lamin B was completely extracted from the lateral aspects of the chromatin (Fig. 3 *A*; see Collas et al., 1995). Extraction with 1% TX-100/0.4 M NaCl eliminated all lamin and LBR labeling (Fig. 3 *A*).

The differential extraction of lamin B and p56 from sperm nuclei was further documented by biochemical analysis. Sperm heads (10^5 per sample) were extracted for 1 h in TN containing 0, 0.1, or 1% TX-100, each with 0.2, 0.3, or 0.4 M NaCl, and extracted proteins were analyzed by immunoblotting using anti-LBR and anti–lamin B antibodies. Whereas lamin B was largely extracted in 1% TX-100/0.2 M NaCl, a p56 component reacting with anti-LBR antibodies was extracted only with 1% TX-100/0.4 M NaCl (Fig. 3 *B*). That the 56-kD sperm component and p56 of egg cytosol were the same polypeptide was verified by their exact comigration in 10% SDS-PAGE, as judged by immunoblotting using anti-LBR antibodies (data not shown). Since no other protein of the sperm envelope was revealed on blots by the anti-LBR antibodies, we conclude that the immunoreactive component observed by immunofluorescence in sperm heads with this antibody was p56. Colocalization of p56 and lamin B at both poles of sperm nuclei after extraction in 1% TX-100/0.15 M NaCl was also observed by double immunofluorescence (Fig. 3 *C*). Immunoblotting and immunofluorescence experiments have shown that all sperm lamins (Collas et al., 1995) and p56 (data not shown) are removed from sperm nuclei within 15 min of incubation in egg extract. This removal precedes the formation of the new male pronuclear envelope (data not shown).

p56 and Lamin B Are Sequentially Targeted to Chromatin In Vitro

To document the targeting to chromatin of p56 and lamin B during pronuclear formation in vitro, sperm chromatin

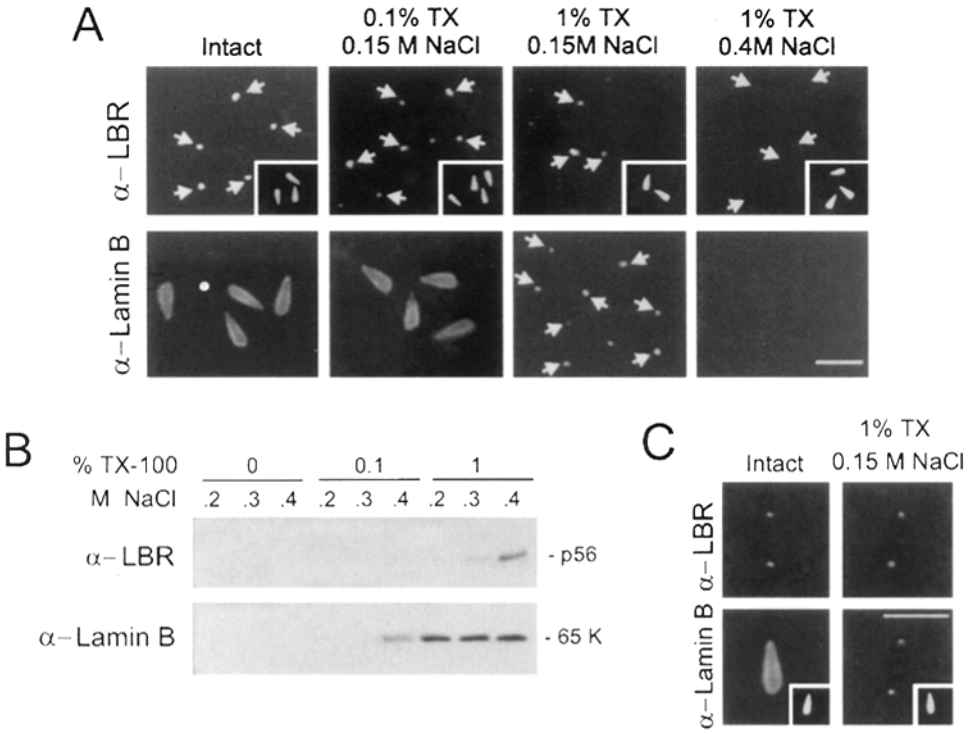

Figure 3. Partial colocalization and differential extraction of p56 and lamin B in sea urchin sperm nuclei. (*A*) Intact sperm heads, or sperm heads extracted with 0.1% TX-100/0.15 M NaCl, 1% TX-100/0.15 M NaCl, or 1% TX-100/0.4 M NaCl, were fixed in 3% paraformaldehyde and processed for indirect immunofluorescence using anti-LBR (*upper panel*) or anti–lamin B antibodies (*lower panel*). Nuclear DNA was counterstained with 0.1 μg/ml propidium iodide (*insets*, *upper panel*). Arrows point to nuclear poles. (*B*) Sperm heads were extracted with TX-100 as indicated, and extracted proteins were resolved by 10% SDS-PAGE and immunoblotting using anti-LBR (*upper panel*) or anti-lamin B antibodies (*lower panel*). (*C*) Double immunofluorescence detection of p56 (*α-LBR*) and lamin B in intact and 1% TX-100/0.15 M NaCl extracted sperm nuclei. Lamin B and p56 were detected in the same nuclei using secondary antibodies conjugated to TRITC for anti-LBR and to FITC for anti-lamin antibodies. DNA was visualized with 0.1 μg/ml Hoechst 33342 (*insets*). Note that p56 and lamin B only colocalize in the regions of the lipophilic acrosomal and basal structures of the sperm nucleus. Note also the differential extraction of lamin B according to its polar or lateral location. Bars, 5 μm.

was incubated in S_{10} under conditions promoting either vesicle binding to chromatin, binding and membrane fusion, or binding and fusion followed by pronuclear swelling with formation of a complete NE (see Materials and Methods). Duplicate groups of nuclei were then settled on coverslips and analyzed by immunofluorescence using anti-LBR and anti-lamin antibodies. Fig. 4 *A* shows that both proteins were targeted to chromatin, creating a perinuclear labeling specific for proteins of the NE. However, conditions promoting their respective targeting to chromatin were strikingly different. Whereas p56 was targeted to chromatin during the early steps of NE formation (Fig. 4 *A*; *Bound* and *Fused*), lamin B was only targeted at the later stage of pronuclear swelling (Fig. 4 *A*; *NE*). This sequential targeting to chromatin of p56 and lamin B was confirmed by double immunofluorescence (data not shown) and by immunoblotting analysis of proteins extracted from nuclei at different stages of reconstitution (Fig. 4 *B*). Demembranated sperm nuclei decondensed for 1 h in S_{150}, i.e., in the absence of MVs, did not contain any detectable p56 or lamin B (Fig. 4).

Since p56 may interact with DNA, chromatin, and lamin B, we examined the strength of these interactions at the different stages of pronuclear formation by extracting membranes under conditions of increasing stringency. Free MV2β vesicles, chromatin-bound MVs, and NEs prepared from swollen pronuclei were extracted with 1% TX-100 and increasing NaCl concentrations, and extracts were analyzed for the presence of p56 by immunoblotting. To verify the completion of extractions, extracted MV2β vesicles and NEs were pelleted, washed, dissolved in SDS sample buffer, and analyzed by immunoblotting using anti-LBR antibodies. p56 was completely extracted from free MV2β vesicles with 1% TX-100/0.2 M NaCl, whereas its extraction from pronuclear membranes required 0.4–0.5 M NaCl (data not shown). The strength of interaction of lamin B with free MV2β vesicles and with NEs of pronuclei was also evaluated by extraction with increasing urea concentrations. Lamin B was extracted from MV2β with 2 M urea, whereas 8 M urea was required for extraction from NEs (data not shown). These results indicate that, at an early stage of NE assembly, p56 strongly interacts with a chromatin component and may contribute to stabilizing membrane–chromatin interactions. At a later stage, both p56 and lamin B are difficult to extract, suggesting lamin B may interact with p56, and perhaps other lamin-binding proteins.

p56 and Lamin B Are Coimmunoprecipitated from NEs But Not from Free Vesicles

To confirm the interaction of p56 and lamin B in pronuclei, immunoprecipitation experiments of both proteins were performed. MV2β was chosen as a control since both proteins were previously shown to be segregated into different subsets of this MV fraction (see Fig. 2, *C* and *D*). MV2β vesicles were solubilized in 1% TX-100/0.2 M NaCl and immunoprecipitated from the extract with anti-LBR or anti-lamin B antibodies, and immunoprecipitates were immunoblotted with each antibody to reveal each protein. Blots of Fig. 5 *A* show that anti-LBR antibodies precipitated p56 but not lamin B, and, conversely, that anti-lamin

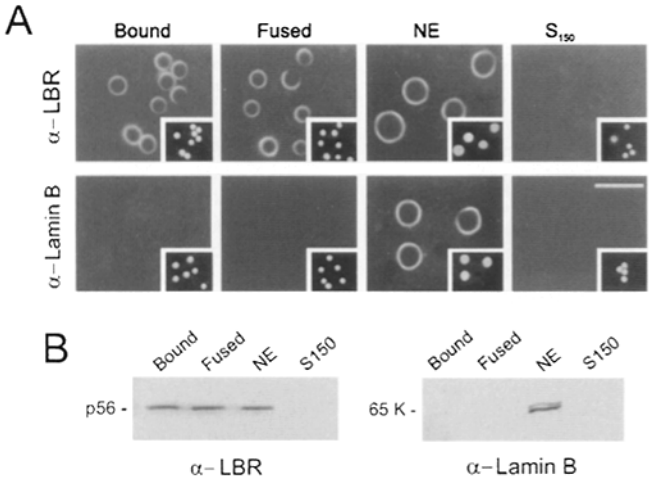

Figure 4. Sequential targeting to chromatin of p56 and lamin B during in vitro assembly of sea urchin male pronuclei. (*A*) Demembranated and decondensed sperm nuclei were incubated in S_{10} cytoplasmic extract under conditions where MVs were either bound to chromatin but not fused (*Bound*), bound to chromatin and fused (*Fused*), or fused in nuclear envelopes surrounding swollen nuclei (*NE*). A control experiment (*S150*) was performed in the absence of MVs. Chromatin was sedimented on coverslips and processed for immunofluorescence using anti-LBR (*upper panel*) or anti–lamin B antibodies (*lower panel*) as in Fig. 3. DNA was labeled with propidium iodide (*insets*). (*B*) The same nuclear preparations were extracted with 1% TX-100/0.4 M NaCl, and extracted proteins were resolved by 10% SDS-PAGE and immunoblotted using anti-LBR (*left*) or anti–lamin B (*right*) antibodies. Similar results were obtained by ECL detection of proteins on blots (data not shown). Note the lamin B–independent targeting of p56 containing MVs during the early stages of NE formation. Bar, 10 μm.

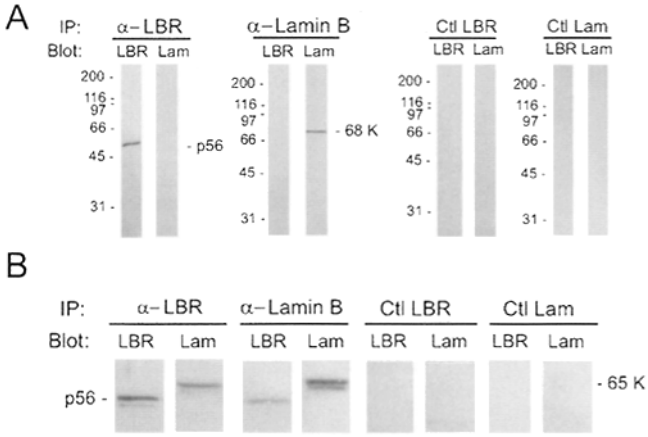

Figure 5. Lamin B and p56 coprecipitate when extracted from NEs but not when extracted from cytoplasmic MVs. MV2β membrane fraction was solubilized in 1% TX-100/0.2 M NaCl (*A*), and NEs isolated from reconstituted nuclei were extracted with 1% TX-100/0.4 M NaCl (*B*). Solubilized extracts were immunoprecipitated (*IP*) with either anti-LBR or anti–lamin B antibodies as indicated. The precipitated material obtained with each antibody was further analyzed by immunoblotting (*Blot*) with both antibodies as indicated (*A* and *B*, *four left lanes*). Control blots (*Ctl*) were performed with the respective preimmune sera as indicated (*A* and *B*, *four right lanes*). Migration of molecular mass standards is indicated in kD on the left in *A*.

antibodies precipitated lamin B but not p56. Neither p56 nor lamin B was detected in material precipitated with preimmune sera. These results confirm that p56 and lamin B are not physically associated in free MV2β vesicles.

NEs were then prepared from swollen pronuclei and solubilized in 1% TX-100/0.4 M NaCl, and the extracted material was analyzed as described above. p56 and lamin B were coprecipitated regardless of the antibody used for immunoprecipitation, whereas no precipitation was observed with preimmune sera (Fig. 5 *B*). p56 was not detected in material that was not immunoprecipitated, suggesting that all p56 was coprecipitated with lamin B. These experiments indicate that p56 and lamin B, which are not associated in free MV2β vesicles, are physically associated in male pronuclear envelopes.

p56-Containing MV2β Vesicles Are Necessary for Nuclear Envelope Assembly

Although p56-containing MVs are targeted to chromatin during the first step of in vitro NE assembly (Fig. 4), they may be dispensable at this stage, as other vesicles with similar functions may also bind. To investigate this question, attempts were made to assemble pronuclei using MV2β membranes previously immunodepleted from p56-containing MVs. Immunodepletion was complete as judged by immunoblotting of protein A–bound and unbound material with anti-LBR antibodies (Fig. 6 *A*, *top*), and selective since duplicate blots with anti–lamin B antibodies showed that lamin B–containing MVs were not coprecipitated (Fig. 6 *A*, *bottom*). Neither p56- nor lamin B–containing vesicles were precipitated by a preimmune rabbit serum (Fig. 6 *A*; *Ctl*). Chromatin was incubated in S_{150} for 1 h with MV2β fraction immunodepleted of p56-containing vesicles, or mock depleted, and processed for immunofluorescence and immunoblotting using anti-LBR antibodies. A similar chromatin-binding assay was performed in parallel with immuno- or mock-depleted MVs prelabeled with the lipophilic dye DHCC. Fig. 6 *B* shows that, after depletion of p56-containing MVs, no MV2β vesicles were bound to chromatin as judged by DHCC fluorescence. As expected, no p56 was detected on nuclei by either immunofluorescence (Fig. 6 *B*) or immunoblotting (Fig. 6 *C*). In the control experiment performed with mock-depleted MV2β, the chromatin was surrounded by p56-containing membranes (Fig. 6, *B* and *C*). These data show that p56-containing vesicles are necessary for pronuclear assembly in vitro.

The above results do not imply that all egg cytoplasmic MVs must contain p56 to bind to chromatin. MV1 and MV2α membrane fractions (which do not contain p56) mixed with p56-depleted MV2β vesicles were able to bind to chromatin. However, binding was restricted to the LS regions, as detected by DHCC staining (data not shown; see Collas and Poccia, 1996). Once MV2β vesicles containing p56 bind to chromatin, MV2β vesicles lacking p56 can incorporate into the nuclear membrane. When an unlabeled, untreated, MV2β fraction mixed with a p56-immunodepleted MV2β fraction labeled with DHCC was used in the chromatin-binding assay, a fluorescent perinuclear labeling was observed (data not shown). Therefore, vesicles

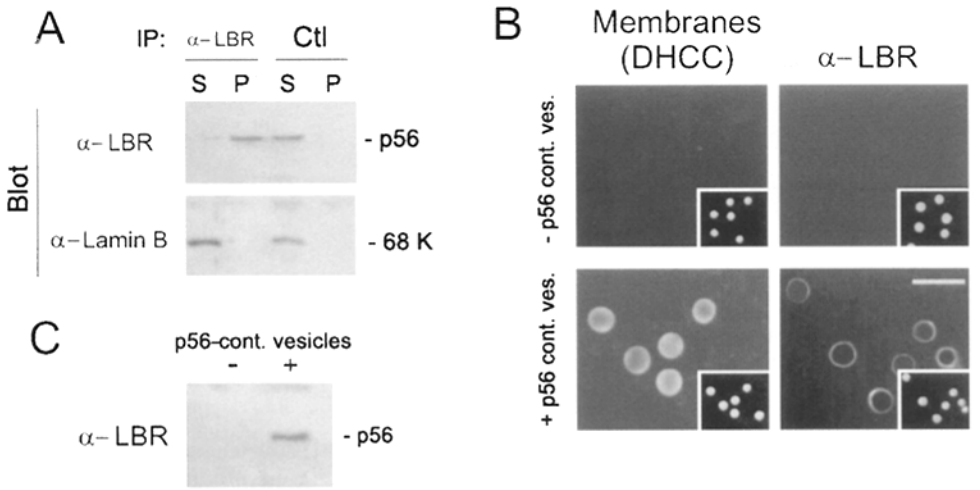

Figure 6. Immunodepletion of p56-containing vesicles from MV2β membrane fraction prevents MV2β binding to chromatin. (*A*) MV2β was immunoprecipitated (*IP*) with protein A–Sepharose beads coupled to anti-LBR antibodies or preimmune rabbit serum (*Ctl*). Beads were sedimented and extracted with SDS sample buffer, and the extract (*P*) was analyzed by immunoblotting using anti-LBR (*upper panel*) or anti–lamin B (*lower panel*) antibodies. Vesicles present in the supernatants (*S*) were solubilized in 1% TX-100, and solubilized proteins were analyzed by immunoblotting as above. (*B*) MV2β depleted of p56-containing MVs (*−p56-cont. ves.*) or mock depleted (*+p56-cont. ves.*) were either labeled with the lipophilic dye DHCC or unlabeled, and then incubated with chromatin in S_{150} for 1 h. After washing, unlabeled nuclei were processed for immunofluorescence using anti-LBR antibodies (*right*), and DHCC-labeled nuclei were viewed to assess membrane binding (*left*). DNA was stained with propidium iodide (*insets*). (*C*) Aliquots of nuclei assembled as in *B* were sedimented and extracted with 1% TX-100/0.4 M NaCl, and the extract was analyzed by immunoblotting using anti-LBR antibodies (10^5 nuclei per lane). Immunoblotting results were confirmed by ECL (data not shown). Bar, 10 μm.

in the MV2β fraction devoid of p56, although unable to bind to chromatin, can contribute to the NE, likely by fusion with chromatin-bound vesicles.

p56 Is Required for Targeting MV2β Vesicles to Chromatin

Although p56 is present in a subset of MV2β vesicles necessary for NE formation, its role in targeting these vesicles to chromatin is uncertain. This potential role of p56 was investigated by incubating MV2β vesicles in S_{150} containing either anti-LBR antibodies, anti–lamin B antibodies, or preimmune rabbit serum, for 20 min before and during the chromatin-binding reaction. After a 1-h incubation, aliquots were taken to visualize MVs bound to chromatin with DHCC (data not shown), or assess the presence of p56 by immunofluorescence and immunoblotting (Fig. 7). The data indicate that MV2β binding to chromatin was completely inhibited by incubation with anti-LBR antibodies, whereas anti-lamin antibodies and preimmune serum did not inhibit binding. Inhibition of MV2β binding to chromatin with anti-LBR antibodies was not caused by a nonspecific effect of the antibodies because MV1 and MV2α membrane fractions, which do not contain p56, were able to bind to the LS regions of sperm chromatin when incubated in the presence of anti-LBR antibodies (data not shown). These results suggest that LBR is responsible for the targeting of MV2β vesicles to chromatin.

Vesicle-associated and Soluble Lamin B Are Not Required for Targeting of p56-Containing Vesicles to Chromatin

Since lamins are absent from pronuclei during the early step of pronuclear formation in vitro (see Fig. 4), their involvement in targeting MVs to chromatin is unlikely. This hypothesis was tested by incubating chromatin with an MV2β fraction that was immunodepleted or mock depleted of lamin B–containing MVs using anti–lamin B antibodies or a preimmune serum, respectively. Immunodepleted and control MVs were incubated for 1 h with chromatin in S_{150} or S_{150} previously immunodepleted of soluble lamin B, each containing an ATP-generating system. p56 in chromatin-bound MVs was examined by immunoblotting using anti-LBR antibodies. p56 was detected in chromatin-bound MVs under each experimental condition (data not shown), indicating that neither vesicle-associated nor soluble lamin B was required for targeting p56-containing vesicles to chromatin.

Nuclear Swelling Requires Soluble Lamin B and Vesicles Not Necessarily Containing Lamin B

Lamin B is stored in egg cytoplasm in soluble and membrane-associated pools (see Figs. 1 *A* and 2 *B*) and required for sea urchin male pronuclear swelling in vitro (Collas et al., 1995). The relative contributions of each pool to the nuclear lamina were examined. Nuclei surrounded by a membrane but devoid of lamins (Collas et al., 1995) were produced in an S_{10} extract, and then resuspended in either intact S_{150} (containing soluble lamins) or S_{150} immunodepleted of soluble lamin B, each containing MV2β either mock depleted or immunodepleted of lamin B–containing vesicles. MV fusion with nuclear membranes and nuclear swelling were promoted with an ATP-generating system and GTP. Nuclear swelling was assessed by measuring nuclear diameters, and incorporation of lamin B into nuclei was examined by immunofluorescence using anti–lamin B antibodies. Membrane fusion was monitored in parallel experiments by the uniform spreading over the nuclear surface of DHCC fluorescence from prelabeled immuno- and mock-depleted MV2β vesicles. The results are shown in Fig. 8 *A*. In intact S_{150}, mock- and lamin-depleted MV2β vesicles fused with nuclear membranes, nuclear lamin B was detected, and nuclear swelling occurred. As expected, none of these events occurred when MVs were omitted from the assay, or in the absence of

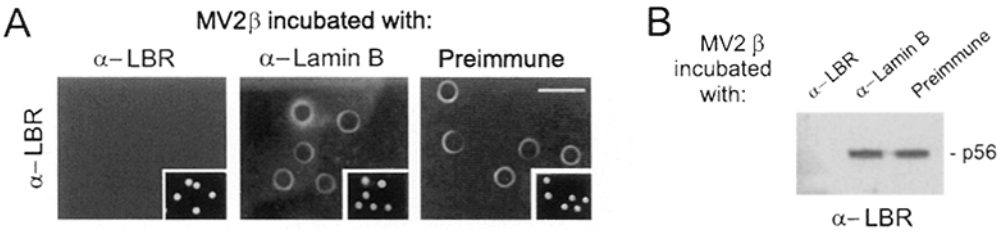

Figure 7. Antibodies to LBR prevent binding of p56-containing vesicles to chromatin. MV2β membrane fraction was incubated in S_{150} with 1:24 dilutions of either anti-LBR antibodies, anti–lamin B antibodies, or preimmune rabbit serum for 20 min before, and during, the chromatin-binding reaction. (*A*) Nuclei were settled on coverslips and analyzed by immunofluorescence using anti-LBR antibodies. DNA was labeled with propidium iodide (*insets*). (*B*) Aliquots of nuclei assembled as in *A* were sedimented and extracted with 1% TX-100/0.4 M NaCl, and the extract was analyzed by immunoblotting using anti-LBR antibodies (10^5 nuclei per lane). Immunoblotting results were confirmed by ECL (data not shown). Bar, 10 μm.

GTP (data not shown). In lamin B–depleted S_{150}, mock-depleted MV2β vesicles fused with nuclear membranes, a weak nuclear lamin B signal was detected, but nuclei did not swell. Lamin-depleted MV2β vesicles also fused with nuclear membranes, but neither nuclear lamin labeling nor nuclear swelling was detected. In each experiment, immunofluorescence data were confirmed by immunoblotting analysis of extracted nuclear membranes using anti-lamin antibodies (Fig. 8 *B*). Furthermore, nuclear lamin B incorporation and nuclear swelling, both abolished in lamin B–depleted S_{150}, were restored to a full extent by adding fresh, untreated S_{150} (data not shown; see Collas et al., 1995). These results indicate that nuclear lamin B incorporation as well as nuclear swelling require soluble lamin B, and additional membranes not necessarily containing lamin B.

A likely mode of incorporation of cytosolic lamins into the nucleus during pronuclear formation is import through nuclear pores, a process reversibly inhibited by WGA (Finlay et al., 1987; Newport et al., 1990). To investigate this putative mechanism, nuclei produced in an S_{10} extract were incubated in intact S_{150} containing 0,5 mg/ml WGA and MV2β fraction either mock depleted or immunodepleted of lamin B–containing vesicles. Membrane fusion, nuclear lamin B incorporation, and nuclear swelling were induced and monitored as in the previous experiment. Data in Table I show that, with WGA, nuclear incorporation of lamin B and nuclear swelling were prevented, although vesicle fusion with nuclear membranes occurred. Nuclear lamin incorporation and nuclear swelling were restored in control S_{150} containing WGA and 1 mM of the WGA ligand *N,N′,N″*-triacetylchitotriose (Dabauvalle et al., 1988). Lima bean agglutinin substituted for WGA in the assay had no inhibitory effect. These results indicate that nuclear lamina assembly and nuclear swelling depend on the import of soluble lamin B via nuclear pore complexes, and fusion of vesicles that do not necessarily contain lamin B.

Discussion

We have characterized an integral membrane protein of sea urchin gametes with an apparent molecular mass of 56 kD that cross-reacts with affinity-purified antibodies directed against vertebrate LBR. In the sperm nucleus, p56 is localized in the basal and apical LS regions of the NE. In egg cytoplasm, p56 is contained in a subset of cytoplasmic vesicles required for pronuclear envelope formation. p56 may play a role in targeting these vesicles to chromatin, stabi-

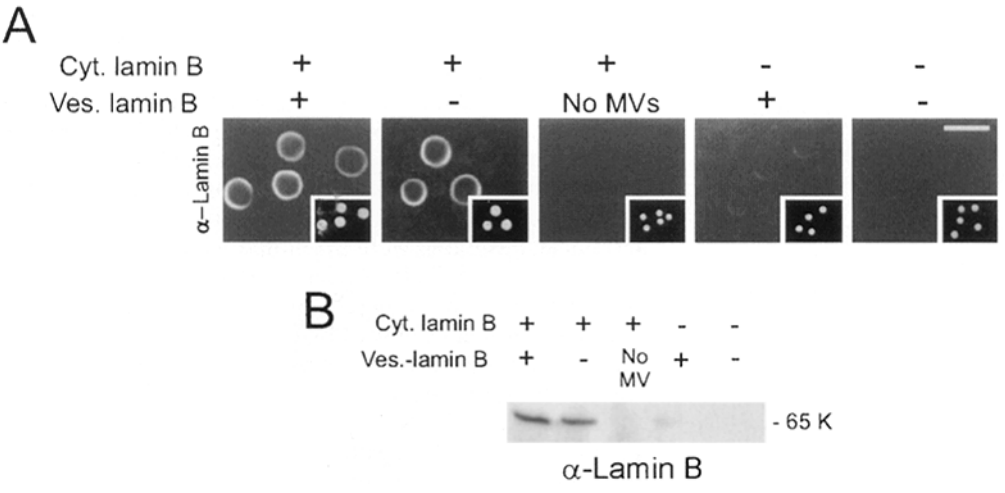

Figure 8. Respective contributions of cytosolic and vesicle-associated lamin B to the formation of the nuclear lamina. Assembled nuclei containing fused membranes but no lamina were incubated in S_{150} and MV fractions in different combinations. S_{150} was either immunodepleted of lamin B (*Cyt. lamin B −*) or mock depleted (*Cyt. lamin B +*). MV2β membrane fraction was either immunodepleted of lamin B–containing MVs (*Ves. lamin B −*) or mock depleted (*Ves. lamin B +*). Membrane fusion and pronuclear swelling were promoted by the addition of an ATP-generating system and GTP (see Material and Methods). After sedimentation through a 1 M sucrose cushion, nuclei were analyzed for the presence of lamin B by immunofluorescence (*A*) and immunoblotting of the material extracted with 1% TX-100 (*B*). Immunoblotting data were confirmed by ECL (data not shown). In *A*, DNA was labeled with propidium iodide (*insets*). Bar, 10 μm.

Table I. Inhibition of Nuclear Lamin B Uptake and Nuclear Swelling by WGA

Exp. Conditions		Observations		
WGA	Lamin B-cont. vesicles*	MV fusion‡	Nuclear lamin staining§	Nuclear swelling‖
+	+	+	±	−
+	−	+	−	−
+	no MVs	−	−	−
+, TCT	−	+	+	+
−, LBA	−	+	+	+

*MV2β vesicle fraction was either mock depleted (+) or immunodepleted (−) of lamin B–containing vesicles.
‡Determined by spreading of DHCC fluorescence from prelabeled vesicles over the nuclear surface.
§Assessed by immunofluorescence using anti–lamin B antibodies; (±) refers to weak labeling above background.
‖Nuclear diameters ranged from 6.2–7.4 μm (+), and from 3.7–4.2 μm (−) ($P < 0.01$, t test; $n = 40$ per sample).
WGA, 0.5 mg/ml WGA included in the assay; *TCT*, 1 mM *N,N',N''*-triacetylchitotriose; *LBA*, 0.5 mg/ml lima bean agglutinin.

lizing membrane–chromatin interaction, and, at a later stage of pronuclear development, anchoring lamin B to the inner nuclear membrane. Soluble lamin B is dispensable at the membrane binding and fusion steps of NE assembly, but it is required for subsequent growth of the NE.

p56 Is a Putative Sea Urchin Lamin B Receptor

In vertebrate somatic cells, interactions between inner nuclear membrane, lamina, and chromatin are mediated by integral membrane proteins including LBR (Worman et al., 1988; Ye and Worman, 1994, 1996). Although the structural relationship between sea urchin p56 and LBR has not been substantiated by sequence data, several lines of evidence suggest that p56 may be a sea urchin analogue of LBR: (*a*) p56 is recognized by an affinity-purified antibody directed against human LBR; (*b*) p56 is an integral membrane protein that localizes at the periphery of pronuclei as judged by immunofluorescence; (*c*) extraction of p56 from chromatin-bound vesicles by nonionic detergent requires high salt as does vertebrate LBR; and (*d*) p56 in in vitro–assembled male pronuclei is physically associated with lamin B since both proteins can be coimmunoprecipitated.

In sperm, p56 and lamin B also colocalize in the apical and basal specialized regions of the NE, where both proteins and membrane lipids (the LSs) require high concentrations of nonionic detergent and salt for extraction (Fig. 3; Collas et al., 1995). A strongly anchored lamin B–LBR complex at the sperm nuclear poles may account for the persistence of sperm NE in the polar regions in vivo after fertilization (Longo and Anderson, 1968). It may also account for the ability of isolated LSs to specifically bind back to these sites of sperm chromatin stripped of LSs in vitro (Collas and Poccia, 1995).

p56 and Lamin B Are Located in Different Egg Cytoplasmic Vesicles

In fertilized sea urchin egg lysates, p56 and lamin B are present in different MV populations, since (*a*) they do not coimmunoprecipitate using either anti-LBR or anti–lamin B antibodies, and (*b*) they can be sorted by a chromatin-binding assay that selectively retrieves p56-containing MVs. However, it cannot be excluded that a minor fraction of lamin B, not detectable by immunoblotting or immunofluorescence, may also exist in chromatin-bound p56-containing MVs. Previous in vitro and in vivo studies suggest that colocalization of LBR and lamin B in MVs may depend on the system examined. In *Xenopus* meiotic egg extracts, vesicles containing a 58-kD integral membrane protein (p58) recognized by mAbs against LBR (Bailer et al., 1991; see Soullam and Worman, 1995) and vesicles containing lamin XB_3 do not cofractionate, whereas p58- and lamin XB_2–containing vesicles do (Lourim and Krohne, 1994). In vivo, LBR and lamin B apparently colocalize at all stages of the cell cycle in chicken hepatoma cells (Meier and Georgatos, 1994), whereas in HeLa cells they almost completely segregate in anaphase and early telophase (Chaudhary and Courvalin, 1993).

Binding of p56-Containing Vesicles to Chromatin and Vesicle Fusion Precede Lamin Incorporation into the Nucleus

During the stepwise assembly of the sea urchin male pronuclear envelope in vitro in the presence of all NE precursors, p56-containing MVs are targeted to chromatin at the early stage of vesicle binding, whereas lamin B is targeted at a later stage characterized by pronuclear swelling and NE growth. Lamin-independent targeting of p56-containing vesicles to chromatin is further demonstrated by its occurrence after immunodepletion of lamin B–containing vesicles or of soluble lamin B, or in the presence of anti-lamin antibodies in the binding reaction. The data support a model of lamin-independent NE assembly mediated by direct binding of integral membrane proteins to chromatin (Newport et al., 1990; Meier et al., 1991; Chaudhary and Courvalin, 1993; Collas et al., 1995), rather than a lamin-dependent process (Burke and Gerace, 1986; Ulitzur et al., 1992; Lourim and Krohne, 1993; Meier and Georgatos, 1994). Here we show that the assembly of the male pronuclear envelope in a sea urchin egg extract is a sequential process where both envelope proteins are independently targeted to the chromatin. The contribution of distinct vesicle populations to the pronuclear envelope in vitro has been shown previously (Wilson and Newport, 1988; Vigers and Lohka, 1991; Collas and Poccia, 1996); however, to our knowledge, this is the first report of sequential NE assembly and lamina formation in vitro demonstrated using a marker of the inner nuclear membrane.

Two nonmutually exclusive pathways can be proposed for the incorporation of lamins into the nucleus after NE assembly. Lamins may be incorporated by fusion of lamin-containing vesicles with the NE, or by nuclear import of soluble lamins. In vitro, lamins contributing to the lamina originate primarily from a soluble pool in the sea urchin (Collas et al., 1995; this study). Import of soluble lamins through nuclear pores has been shown by the reversible inhibition of lamina assembly with the lectin WGA (Newport et al., 1990; this study). Although the contribution of vesicle-associated lamins to the lamina cannot be excluded (Fig. 8), our data indicate that MVs harboring lamin B are not required for lamina formation. Nuclear uptake of lamins requires additional vesicles whose composition and

role have not been elucidated. These vesicles may provide membranes necessary for NE growth or for nuclear pore assembly (Chaudhary and Courvalin, 1993).

A Role for p56 in Vesicle Targeting to Chromatin?

The ability of LBR to bind to DNA in vitro, and chromatin in vitro and in vivo (Ye and Worman, 1994, 1996), suggests a possible targeting function for this integral membrane protein. Immunodepletion of p56-containing MVs from the assay and antibodies to LBR in the binding reaction both prevent MV2β vesicle binding to chromatin and NE formation. These results suggest that binding to chromatin of vesicles harboring p56 is a prerequisite for subsequent binding of additional MV2β vesicles. However, no binding of intact MV2β vesicles occurs if LSs are absent from the sperm nuclear poles (Collas and Poccia, 1996), suggesting that binding of p56-containing MVs to chromatin may depend on prior MV binding to the poles. After binding of p56-containing vesicles to chromatin, other MVs that do not contain p56 are able to incorporate into nuclear membranes. Whether these vesicles directly bind to chromatin has not been elucidated.

Based on our current and previous results, the formation of the sea urchin male pronuclear envelope in vitro appears as a multistep process requiring several membrane populations. Upon sperm chromatin decondensation, two minor membrane fractions, MV1 and MV2α, bind to the polar regions of nuclei containing LSs (Collas and Poccia, 1996), in a lamin- and p56-independent manner. Simultaneously or subsequently, p56-containing vesicles of the MV2β fraction, constituting the bulk of the NE, are targeted to chromatin. GTP triggers fusion of MV1, MV2α, and MV2β vesicles to form a sealed nuclear membrane (Collas and Poccia, 1996). Further ATP hydrolysis promotes incorporation of soluble lamins that are required for pronuclear swelling and NE growth.

This work was supported by grant DCB 9004170 (to D.L. Poccia) and by the F.E.G.E.F.L.U.C. and Ligue Contre le Cancer (to J.-C. Courvalin).

Received for publication 25 June 1996 and in revised form 28 October 1996.

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