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Colocalization of vertebrate lamin B and lamin B receptor (LBR) in nuclear envelopes and in LBR-induced membrane stacks of the yeast *Saccharomyces cerevisiae*

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ABSTRACT **We have expressed human lamin B and the chicken lamin B receptor (LBR), either separately or together, in yeast and have monitored the subcellular location of the expressed proteins by immunofluorescence microscopy, immunoelectron microscopy, and cell fractionation. At the light microscopic level, the heterologous lamin B localized to the yeast nuclear rim and at electron microscopic resolution was found subjacent to the yeast inner nuclear membrane. These data indicate that vertebrate lamin B was correctly targeted in yeast. Expression of the heterologous LBR, either alone or together with the heterologous lamin B, resulted in the formation of membrane stacks primarily adjacent to the nuclear envelope, but also projecting from the nuclear envelope into the cytoplasm or under the plasma membrane. Double immunoelectron microscopy showed colocalization of the heterologous lamin B and LBR in the yeast nuclear envelope and in the LBR-induced membrane stacks. Cell fractionation showed the presence of the heterologous lamin B and LBR in a subnuclear fraction enriched in nuclear envelopes. The heterologous lamin B was extracted at 8 M urea, but not at 4 M urea, thus behaving as a peripheral membrane protein and indistinguishable from assembled lamins. The heterologous LBR was not extracted by 8 M urea, indicating that it was integrated into the membrane. The observed colocalization and cofractionation are consistent with previously reported *in vitro* binding data and suggest that heterologous lamin B and LBR interact with each other when coexpressed in yeast.**

The nuclear lamina is a peripherally associated superstructure of the inner nuclear membrane. The lamina is composed of the A and B type lamins (1, 2). Primary structure analysis showed that both lamin types belong to the family of intermediate filament proteins (3–5). The lamins are peripheral membrane proteins, but their mode of interaction with the membrane is presently not well understood. Both A and B type lamins are farnesylated at a C-terminal cysteine (6, 7). However, only the lamin B molecules retain this modification during their lifetime, whereas that of lamin A is lost as part of a C-terminal proteolytic cleavage (8). Although the farnesyl moiety of lamin B is likely to help anchor lamin B to the lipid bilayer, stable association of lamin B with the inner nuclear membrane suggested the existence of a specific lamin B receptor (LBR). An integral membrane protein that fulfilled the criteria for a specific high affinity, LBR has indeed been identified in the inner nuclear membrane of avian erythrocytes (9). Thus, in a solution binding assay ^{125}I-labeled lamin B (^{125}I-lamin B) was found to bind in a saturable and specific fashion to nuclear envelopes, with a K_d of 0.2 μM. In ligand blot assays ^{125}I-lamin B was found to bind specifically to an integral membrane protein. This protein, termed the LBR, was subsequently molecularly cloned and sequenced and shown to be a protein of 637 amino acid residues containing two distinct domains: an N-terminal hydrophilic domain of 205 amino acid residues that appears to be exposed on the nucleoplasmic side of the inner nuclear membrane and a C-terminal hydrophobic domain of 432 amino acid residues with eight putative transmembrane segments (10).

An inner nuclear membrane protein with an electrophoretic mobility similar to LBR has been identified with a monoclonal antibody (11). As this protein cross reacts with an LBR-reactive human autoimmune serum (12), it is likely to be identical to LBR. A lamina-associated integral membrane protein that also cosediments with an *in vitro*-assembled lamin B copolymer has been identified and termed LAP 2 (13). This protein has not yet been characterized and may or may not be identical to LBR. Interestingly, two yeast ergosterol biosynthetic pathway enzymes, sterol C-24(28) reductase (Erg4p; encoded by the *ERG4* gene) (14–16) and sterol C-14 reductase (Erg24p; encoded by the *ERG24* gene) (15, 17), appear to be related to LBR. These yeast proteins show similarity across their entire length with the hydrophobic domain of LBR but lack LBR's N-terminal hydrophilic domain. For example, *ERG24*, the gene most closely related to LBR, encodes a protein of 438 amino acid residues that is similar (40% identical) to the 432-amino acid hydrophobic domain of LBR. The cellular location of Erg4p and Erg24p is unknown. The functional implications of the structural relationships between these yeast proteins and the vertebrate LBR remain to be analyzed. For example, does yeast contain an LBR homolog (see below) that also contains LBR's nucleoplasmic domain or does Erg24p also function in lamin B binding? It is not yet known which (or if both) of LBR's two domains functions in lamin B binding.

A detailed analysis of the lamin B–LBR interaction would be greatly facilitated if it could be performed in yeast as this cell is readily manipulated by molecular genetic techniques. Putative homologs of both lamins A and B and LBR have been identified in yeast (18). However, these proteins have so far not been further characterized nor have they been localized. Their status as true homologs is at present, therefore, tenuous. Expression of chicken lamin B in the fission yeast *Schizosaccharomyces pombe* has been demonstrated (19); however, the intranuclear location of the expressed lamin B was not established. To both probe for endogenous yeast lamins and yeast LBR and to analyze vertebrate lamin B–LBR interactions we expressed human lamin B and/or the chicken LBR in *Saccharomyces cerevisiae*.

MATERIALS AND METHODS

Construction of Plasmids. Plasmids were constructed using standard cloning procedures (20). To construct pLBR, a 1.9-kb *Bam*HI–*Sac* I fragment encoding the chicken LBR

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Abbreviations: ER, endoplasmic reticulum; HA, hemagglutinin; LBR, lamin B receptor.

cDNA (10) from codon 2 through stop codon 638 was amplified from the plasmid p58FL (21) using the polymerase chain reaction (PCR) with appropriate oligonucleotides and ligated into *Bam*HI–*Sac* I-digested vector, pLGSD5, a 2-μm-based plasmid with a uracil marker (22). The resulting plasmid contained the chicken LBR coding sequence (minus the initiation codon) fused in frame to the second amino acid of the *CYC1* gene under control of the *GAL10* promoter. pLBR also contains a hemagglutinin (HA) epitope (23) inserted in the LBR cDNA after codon 29 (21).

To construct pLmB, a 1.8-kb *Bam*HI fragment encoding the human lamin B cDNA from codon 2 through stop codon 587 was amplified from the plasmid pLAM-2 (24) by PCR with appropriate oligonucleotides and ligated into the *Bam*HI-digested pRS315G vector, which was generated by inserting the 600-bp fragment containing the *GAL10 CYC1* sequences from pLGSD5 (22) into the polylinker of the vector, pRS315, a centomere-based plasmid with a leucine marker (25). The resulting plasmid contained the human lamin B coding sequence (minus the initiation codon) fused in frame with the second amino acid of the *CYC1* gene under the control of the *GAL10* promoter.

Strains and Cell Growth. The yeast strain used was W303 (*Mata/Matα ade2-1/ade2-1 ura3-1/ura3-1 his3-11,15/his3-11,15 trp1-1/trp1-1 leu2-3,112/leu2-3,112 can1-100/can1-100*). Plasmids were transformed into W303 by the lithium acetate method (26) and selected on SM, synthetic minimal medium (27), supplemented with the appropriate amino acids and 2% glucose.

For immunofluorescence, electron microscopy, and cell fractionation of LmB, LBR, and LmB LBR strains, cells were grown to early logarithmic phase in SM supplemented with the appropriate amino acids and 2% glucose. Cells were then pelleted, washed, and resuspended in rich medium (YPM; 1% yeast extract/2% bactopeptone) containing 2% galactose and grown for 5 hr. Cells were harvested and processed as described below.

Immunofluorescence Microscopy. Cells were prepared for immunofluorescence essentially as described (28). Fixation was for 5 min at room temperature in 3.7% formaldehyde (Fluka). Processed spheroplasts were incubated with the following primary antibodies: anti-LBR IgG (anti-p58) (20 μg/ml), an affinity-purified rabbit anti-peptide antibody (21); anti-lamin B antibody (1:200), a rabbit anti-peptide serum (29); undiluted tissue culture supernatant of mouse anti-HA monoclonal antibody (12CA5; Berkeley Antibody, Richmond, CA). For immunofluorescence and immunoelectron microscopy, the anti-LBR and anti-lamin B antibodies were preadsorbed to fixed yeast spheroplasts prepared from wild-type W303 cells as described (30). Cells were incubated with secondary antibodies, mounted, and viewed as described (21).

Electron Microscopy. Yeast cells were fixed in 1% glutaraldehyde in 0.1 M sodium cacodylate (pH 7.4) for 30 min on ice. Spheroplasts were prepared and processed as described (31). Sixty-nanometer-thick sections collected on Formvar carbon-coated copper grids were stained with both uranyl acetate and lead citrate before viewing in the JEOL 100 CX electron microscope (JEOL) operated at 80 kV.

Nuclear envelopes (see below) were diluted to a final concentration of 1 M sucrose with buffer containing 10 mM Bis-Tris (pH 6.5) and 0.1 mM $MgCl_2$ and spun at 57,000 × *g*. The nuclear envelope pellet was fixed in the same buffer containing 2% glutaraldehyde and 1 M sucrose for 1 hr on ice and processed as described above for spheroplasts.

Immunoelectron Microscopy. Spheroplasts (see above) were fixed in 2% paraformaldehyde/0.05% glutaraldehyde in 0.6 M sorbitol/0.1 M phosphate/citrate, pH 7.0, for 1 hr on ice. Pelleted spheroplasts were embedded in 10% gelatin, refixed for 1 hr in the same fixative, infused with 2.3 M sucrose, and frozen in liquid nitrogen (32). Cryosections were collected on Formvar carbon-coated nickel grids and incubated with primary antibodies (see above), followed by goat anti-rabbit or goat anti-mouse IgG conjugated to 10- or 5-nm gold particles (Amersham), respectively. The grids were processed as described (33).

Yeast Cell Fractionation. Ten liters of logarithmic phase LmB LBR cells (≈4 × 10^7 cells per ml) was harvested and incubated in buffer containing 0.1 M Tris (pH 9.4) and 10 mM dithiothreitol for 10 min at 30°C. Spheroplasts, cytosol, and crude and enriched nuclear fractions were prepared as described (34). Nuclear envelopes were prepared by a modification of the procedure described in Kilmartin and Fogg (35) (C. Strambio-de-Castillia, G.B., and M. Rout; unpublished). Nuclear envelopes were extracted with urea as described (9). Protein samples were processed for 10% SDS/PAGE as described (21), transferred electrophoretically to nitrocellulose filters, and incubated with anti-LBR IgG (4 μg/ml) or anti-lamin B serum (1:500), followed by horseradish peroxidase-conjugated goat anti-rabbit IgG (1:1000; Bio-Rad). Detection of immunoreactivity was performed as described in the "ECL" detection system manual (Amersham).

RESULTS

Expression of Human Lamin B and Chicken LBR in Yeast. To express lamin B and LBR in yeast, the cDNAs encoding chicken LBR or human lamin B were placed under the inducible control of the yeast *GAL10* promoter in yeast expression vectors (see *Materials and Methods*). The resulting plasmids, pLBR and pLmB, were used (either alone or together) to transform *Saccharomyces cerevisiae* to yield yeast strains LmB, LBR, and LmB LBR.

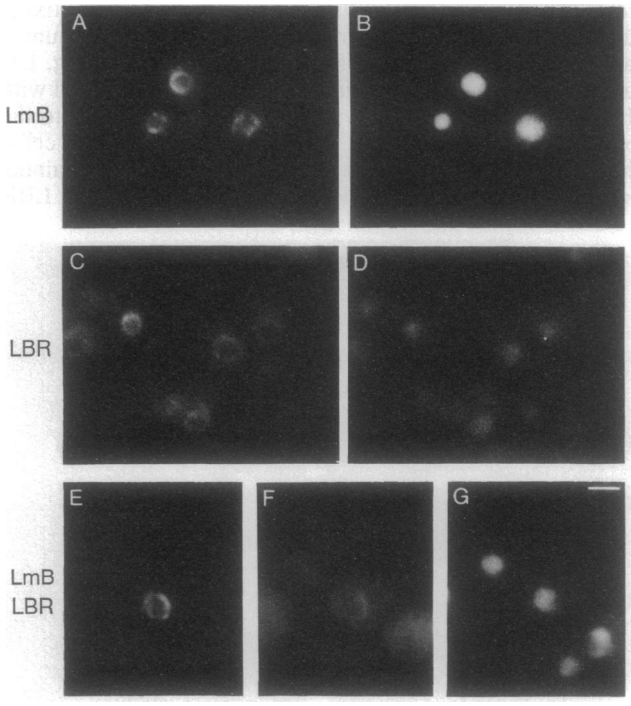

FIG. 1. Indirect immunofluorescence microscopy of human lamin B and chicken LBR expressed in yeast. LmB, LBR, or LmB LBR cells were grown in the presence of galactose and processed for indirect immunofluorescence. LmB cells were stained with rabbit anti-lamin B antibodies (*A*) and 4′,6-diamidino-2-phenylindole (DAPI) (*B*). LBR cells were stained with rabbit anti-LBR antibodies (*C*) and DAPI (*D*). LmB LBR cells were processed for double immunofluorescence by staining with rabbit anti-lamin B antibodies (*E*), mouse anti-HA antibodies (*F*), and DAPI (*G*). (Bar = 2.5 μm.)

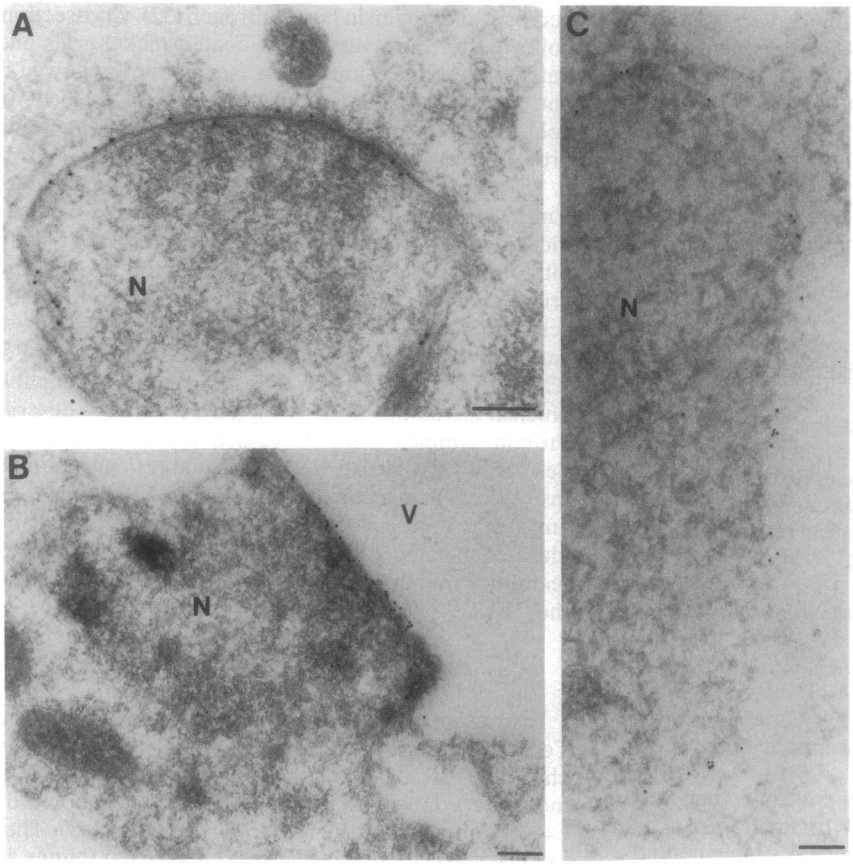

FIG. 2. Localization of human lamin B expressed in yeast by immunoelectron microscopy. LmB cells were grown in the presence of galactose and cryosectioned; the ultrathin sections were incubated with rabbit anti-lamin B antibodies, followed by anti-rabbit IgG conjugated to 10-nm gold particles. (*A*) Lamin B localized to the nuclear membrane. (*B*) In many cells lamin B concentrated in regions where the nuclear envelope membrane was juxtapositioned to the vacuolar membrane. (*C*) Lamin B localized to the nucleoplasmic surface, indicating localization to the inner aspect of the inner nuclear membrane. The nucleus (N) and vacuole (V) are indicated. (Bars = 0.2 μm.)

Indirect immunofluorescence was performed to determine the cellular localization of lamin B and LBR expressed in yeast. The LmB or LBR cells were grown in galactose, fixed, and probed with rabbit anti-peptide antibodies to human lamin B or chicken LBR, respectively. As shown in Fig. 1*A*, lamin B concentrated in a ring-like structure associated with the yeast nucleus. Similarly, LBR localized primarily to the yeast nuclear rim (Fig. 1*C*; note that only a subset of cells, those in the plane of focus, display the nuclear rim staining pattern). To determine the localization of lamin B and LBR coexpressed in the same cells, double immunofluorescence was performed. Here we took advantage of the fact that the

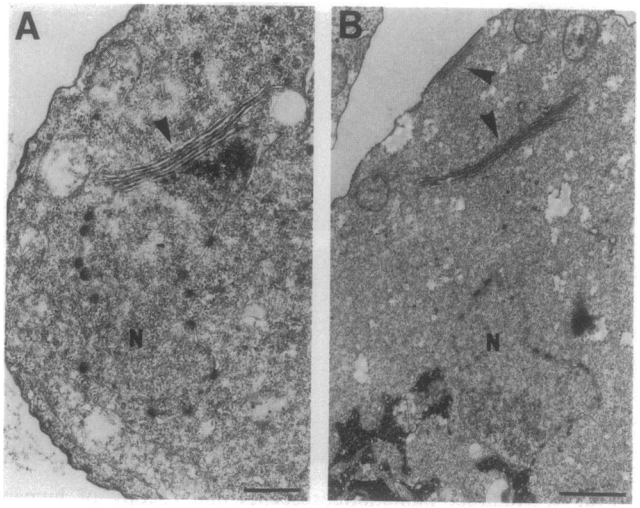

FIG. 3. Thin section morphology of yeast cells containing membrane stacks. LmB LBR cells were grown in the presence of galactose and processed for ultrastructural analysis. Membrane stacks were found closely associated with the nuclear envelope (*A*) or associated with the plasma membrane and in the cytoplasm (*B*). Arrowheads indicate the position of the membrane stacks. The nucleus (N) is indicated. (Bars = 0.5 μm.)

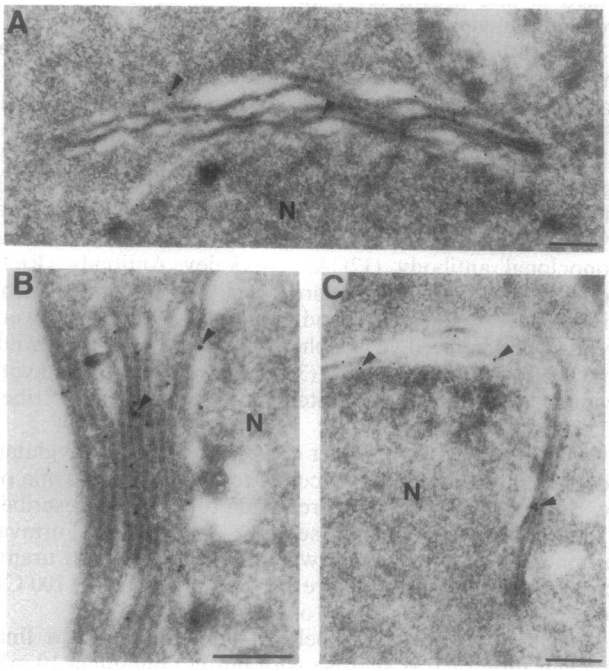

FIG. 4. Localization of human lamin B and chicken LBR coexpressed in yeast by immunoelectron microscopy. LmB LBR cells were grown in the presence of galactose, cryosectioned, and probed with rabbit anti-lamin B antibodies and mouse anti-HA monoclonal antibody; this was followed by incubation with anti-rabbit IgG conjugated to 10-nm gold particles and anti-mouse IgG conjugated to 5-nm gold particles. *A–C* show colocalization of lamin B (10-nm gold, arrowheads) and LBR (5-nm gold) to the inner and outer nuclear membranes and the membrane stacks. The nucleus (N) is indicated. (Bars = 0.2 μm.)

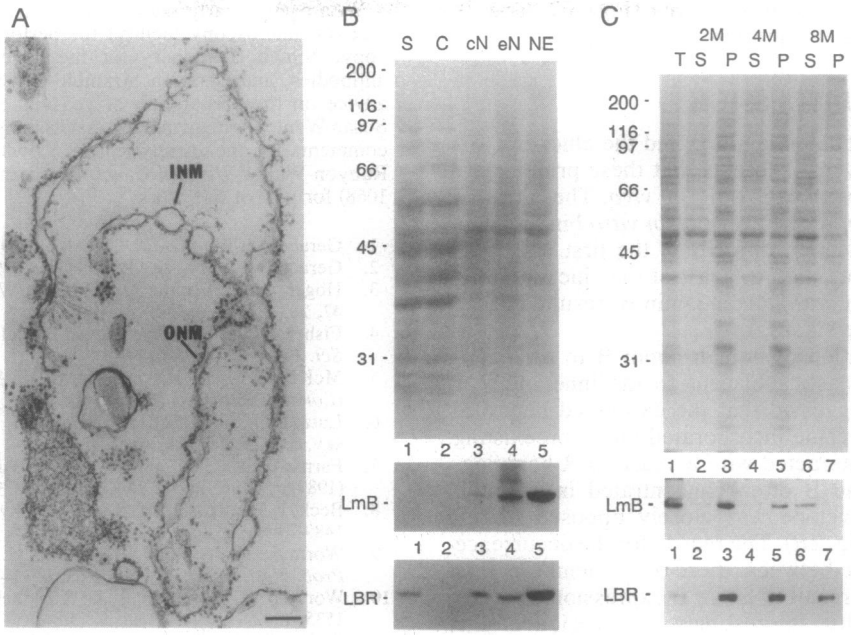

FIG. 5. Analysis of yeast nuclear envelopes purified from LmB LBR cells. LmB LBR cells were grown in the presence of galactose and subjected to subcellular fractionation. (*A*) Electron micrograph of nuclear envelopes purified from LmB LBR cells. The inner nuclear membrane (INM) can be clearly distinguished from the outer nuclear membrane (ONM), which is studded with ribosomes. (Bar = 0.2 μm.) (*B*) Immunoblot analysis of fractions across the nuclear envelope purification. Twenty-five micrograms of protein from each step of the purification was separated by SDS/PAGE, transferred to nitrocellulose, and stained with amido black (*Top*) and processed for immunodetection with rabbit anti-lamin B antibodies (*Middle*) or rabbit anti-LBR antibodies (*Bottom*). Lanes: S, spheroplasts; C, cytosol; cN, crude nuclei; eN, enriched nuclei; NE, nuclear envelopes. Molecular mass markers are indicated in kDa. (*C*) Urea extraction of purified nuclear envelopes. Nuclear envelopes (T, total) were extracted with 2 M, 4 M, or 8 M urea and separated by centrifugation into supernatant (S) and pellet (P) fractions. Proteins were separated by SDS/PAGE and stained with Coomassie brilliant blue (*Top*) or transferred to nitrocellulose and processed for immunodetection with rabbit anti-lamin B antibodies (*Middle*) or rabbit anti-LBR antibodies (*Bottom*). Molecular mass markers are indicated in kDa.

chicken LBR protein contained an HA epitope inserted into its N terminus. The LmB LBR cells were grown in galactose, fixed, and probed with rabbit anti-peptide antibodies to human lamin B and a mouse monoclonal antibody to the HA tag. As shown in Fig. 1, lamin B (Fig. 1*E*) and LBR (Fig. 1*F*) colocalized to the yeast nuclear rim.

Lamin B Localizes to the Inner Aspect of the Nuclear Envelope. For immunoelectron microscopy, LmB cells were grown in galactose, fixed, cryosectioned, and probed with rabbit anti-peptide antibodies to human lamin B. Immunogold labeling of ultrathin sections showed that lamin B localized primarily to the inner nuclear membrane (Fig. 2 *A* and *C*). In many cells (≈40–50% of the lamin B-expressing cells) lamin B accumulated in an unusual subcompartment of the nuclear envelope, the region where the vacuolar membrane was juxtapositioned to the nuclear envelope (Fig. 2*B*).

Expression of LBR Induces the Formation of Membrane Stacks. When LBR or LmB LBR cells were grown in galactose, fixed, and thin sectioned and the thin sections were analyzed by electron microscopy, striking membrane stacks were observed. As shown in Fig. 3 (for LmB LBR cells) the membrane stacks were most often observed to be closely associated with the nuclear envelope (Fig. 3*A*) but were occasionally found in the cytoplasm and associated with the plasma membrane (Fig. 3*B*). Similar membrane stacks were found in LBR cells but were never observed in LmB or wild-type yeast cells. Immunogold labeling (see below) indicated that these stacks were enriched in LBR.

Lamin B and LBR Colocalize to the Nuclear Envelope and Membrane Stacks. We used double immunogold labeling to determine whether lamin B and LBR colocalized when coexpressed in yeast. LmB LBR cells were grown in galactose, fixed, cryosectioned, and probed with rabbit anti-peptide antibodies to lamin B and the monoclonal antibody to the HA-tagged LBR. The bound primary antibodies were then decorated with specific secondary antibodies conjugated to 5- or 10-nm gold particles, yielding labeling of LBR with 5-nm gold and lamin B with 10-nm gold. Shown in Fig. 4 are examples of nuclear envelopes and perinuclear membrane stacks labeled with both 5- and 10-nm gold, indicating colocalization of lamin B and LBR.

Localization of lamin B in yeast actually appeared to be influenced by the coexpression of LBR; the accumulation of lamin B in the vacuolar-associated region of the nuclear envelope, which was frequently observed in LmB cells (see Fig. 2*B*), was never observed in LmB LBR cells.

Lamin B and LBR Coenrich with Yeast Nuclear Envelopes. The LmB LBR cells were grown in the presence of galactose and subjected to cell fractionation. A nuclear subfraction was obtained that by electron microscopy was enriched in nuclear envelopes (Fig. 5*A*). Proteins in the various cellular fractions were separated on SDS/PAGE and analyzed by immunoblotting (Fig. 5*B*). Lamin B and LBR were found to coenrich to the yeast nuclear envelope fraction.

The proteins of the nuclear envelope fraction were further analyzed as to their extractability by urea, a strong chaotrope that solubilizes peripheral, but not integral, membrane proteins. The nuclear envelope fraction from LmB LBR cells was extracted with increasing concentrations of urea. The total (T) as well as the extracted (S) or unextracted (P) proteins were separated by SDS/PAGE and analyzed by immunoblotting with anti-lamin B or anti-LBR antibodies (Fig. 5*C*). With 2 M or 4 M urea (conditions which extract many peripheral membrane proteins, including a number of nucleoporins) (36), lamin B remained unextracted (Fig. 5*C*, lanes 3 and 5). This may reflect assembly of lamin B into a lamina-like structure and/or its membrane association. Solubilization of lamin B from the nuclear envelope required extraction with 8 M urea (Fig. 5*C*, lane 6). As expected, LBR

remained unextracted even at 8 M urea (Fig. 5C, lane 7), indicating its membrane integration.

DISCUSSION

The colocalization of human lamin B and the chicken LBR when coexpressed in yeast suggests that these proteins are capable of interacting with each other *in vivo*. These *in vivo* results are consistent with the previous *in vitro* binding data that led to the identification of LBR in the first place (9). Unexpectedly, the expression in yeast of the chicken LBR, either alone or together with human lamin B, resulted in the induction of membrane stacks.

The correct localization of human lamin B to a lamina-equivalent locale in yeast, subjacent to the inner nuclear membrane (Fig. 2), suggested that the expressed heterologous lamin B either became incorporated into a preexisting yeast lamina and/or interacted with a yeast LBR homolog. The heterologous lamin B often concentrated in a region where the nuclear envelope was closely opposed to the vacuolar membrane (Fig. 2B). The reason for the occurrence of a close association between these two membranes is unknown. It is not the result of lamin B expression as it can occur in wild-type cells. Interestingly, this region of the nuclear envelope has been shown by serial sectioning to be devoid of nuclear pore complexes (37). Thus, this region of the nuclear envelope may provide a site for the accumulation of excess heterologous lamin B that results from lamin B overexpression. Consistent with this interpretation is the finding that heterologous lamin B accumulation subjacent to the vacuole-associated nuclear envelope region was not observed when human lamin B was coexpressed with chicken LBR. Presumably the concomitantly expressed LBR would provide additional sites for the expressed lamin B, thereby preventing accumulation in the vacuole-associated nuclear envelope region.

A surprising result of the expression in yeast of chicken LBR was the induction of membrane stacks, located near the nuclear envelope, in the cytoplasm and subjacent to the plasma membrane. The locations are typical of yeast endoplasmic reticulum (ER). The simplest scenario suggests that the overexpression of chicken LBR amplifies preexisting ER: the chicken LBR would be integrated into the ER and then probably sorted to the inner nuclear membrane via its first transmembrane sequence (21). Once the inner membrane sites are occupied, additional LBR will remain in the ER, resulting in ER amplification and formation of membrane stacks to accommodate the overexpressed LBR. It should be noted that some ER proteins have previously been reported to form membrane stacks after overexpression in yeast (for review see ref. 38).

An important question to be addressed in future experiments is whether the chicken LBR cDNA can complement an *ERG24* gene disruption in yeast. If so, this would suggest that LBR may also function in sterol biosynthesis in chicken. Alternatively, the *ERG24* gene and the LBR gene may have diverged from a common ancestor. Both may have retained a binding pocket (perhaps formed by some of its eight transmembrane segments) for the farnesyl moiety of lamin B, in the case of LBR, or for a sterol biosynthetic intermediate, in the case of Erg24p.

Note Added in Proof. We found that the chicken LBR cDNA was unable to complement an *ERG24* gene disruption in *Saccharomyces cerevisiae* (S.S., G.B., M. Lai, and D. Kirsch; unpublished).

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1. Gerace, L., Blum, A. & Blobel, G. (1978) *J. Cell Biol.* **79**, 546–566.
2. Gerace, L. & Blobel, G. (1980) *Cell* **19**, 277–287.
3. Hoger, T. H., Krohne, G. & Franke, W. W. (1988) *Eur. J. Biochem.* **47**, 283–290.
4. Fisher, D. Z., Chaudhary, N. & Blobel, G. (1986) *Proc. Natl. Acad. Sci. USA* **83**, 6450–6454.
5. McKeon, F. D., Kirschner, M. W. & Caput, D. (1986) *Nature (London)* **319**, 463–468.
6. Lutz, R. J., Trujillo, M. A., Denham, K. S., Wenger, L. & Sinensky, M. (1992) *Proc. Natl. Acad. Sci. USA* **89**, 3000–3004.
7. Farnsworth, C. C., Wolda, S. L., Gelb, M. H. & Glomset, J. A. (1989) *J. Biol. Chem.* **264**, 20422–20429.
8. Beck, L. A., Hosick, T. J. & Sinensky, M. (1990) *J. Cell Biol.* **110**, 1489–1499.
9. Worman, H. J., Yuan, J., Blobel, G. & Georgatos, S. D. (1988) *Proc. Natl. Acad. Sci. USA* **85**, 8531–8534.
10. Worman, H. J., Evans, C. D. & Blobel, G. (1990) *J. Cell Biol.* **111**, 1535–1542.
11. Bailer, S. M., Eppenberger, H. M., Griffiths, G. & Nigg, E. A. (1991) *J. Cell Biol.* **114**, 389–400.
12. Courvalin, J. C., Lassoued, K., Worman, H. J. & Blobel, G. (1990) *J. Exp. Med.* **172**, 961–967.
13. Foisner, R. & Gerace, L. (1993) *Cell* **73**, 1267–1279.
14. Chen, W., Capieaux, E., Balzi, E. & Goffeau, A. (1991) *Yeast* **7**, 305–308.
15. Lai, M. H., Bard, M., Pierson, C. A., Alexander, J. F., Goebl, M., Carter, G. T. & Kirsch, D. R. (1994) *Gene* **140**, 41–49.
16. Shimanuki, M., Goebl, M., Yanagida, M. & Toda, T. (1992) *Mol. Biol. Cell* **3**, 263–273.
17. Lorenz, R. T. & Parks, L. W. (1992) *DNA Cell Biol.* **11**, 685–692.
18. Georgatos, S. D., Maroulakou, I. & Blobel, G. (1989) *J. Cell Biol.* **108**, 2069–2082.
19. Enoch, T., Peter, M., Nurse, P. & Nigg, E. A. (1991) *J. Cell Biol.* **112**, 797–807.
20. Sambrook, J., Fritsch, E. F. & Maniatis, T. (1989) *Molecular Cloning: A Laboratory Manual* (Cold Spring Harbor Lab. Press, Plainview, NY), 2nd Ed.
21. Smith, S. & Blobel, G. (1993) *J. Cell Biol.* **120**, 631–637.
22. Guarente, L., Yocum, R. R. & Gifford, P. (1982) *Proc. Natl. Acad. Sci. USA* **79**, 7410–7414.
23. Wilson, I. A., Niman, H. L., Houghten, R. A., Cherenson, A. R., Connolly, M. L. & Lerner, R. A. (1984) *Cell* **37**, 767–778.
24. Pollard, K. M., Chan, E. K. L., Grant, B. J., Sullivan, K. F., Tan, E. M. & Glass, C. A. (1990) *Mol. Cell. Biol.* **10**, 2164–2175.
25. Sikorski, R. S. & Hieter, P. (1989) *Genetics* **122**, 19–27.
26. Ito, H., Fukuda, Y., Murata, K. & Kimura, A. (1983) *J. Bacteriol.* **153**, 163–168.
27. Sherman, F., Fink, G. R. & Hicks, J. B. (1986) *Methods in Yeast Genetics* (Cold Spring Harbor Lab. Press, Plainview, NY).
28. Pringle, J. R., Alison, E. M., Adams, D. G., Drubin, D. G. & Haarer, B. K. (1991) *Methods Enzymol.* **194**, 565–602.
29. Chaudhary, N. & Courvalin, J.-C. (1993) *J. Cell Biol.* **122**, 295–306.
30. Roberts, C. J., Raymond, C. K., Yamashiro, C. T. & Stevens, T. H. (1991) *Methods Enzymol.* **194**, 644–661.
31. Small, G. M., Imanaka, T., Shio, H. & Lazarow, P. B. (1987) *Mol. Cell. Biol.* **7**, 1848–1855.
32. Tokuyasu, K. T. (1973) *J. Cell Biol.* **57**, 551–565.
33. Griffiths, G., Simons, K., Warren, G. & Tokuyasu, K. T. (1983) *Methods Enzymol.* **96**, 466–485.
34. Rout, M. P. & Kilmartin, J. V. (1990) *J. Cell Biol.* **111**, 1913–1927.
35. Kilmartin, J. V. & Fogg, J. (1982) in *Microtubules in Microorganisms*, eds. Cappuccinelli, P. & Morris, N. R. (Decker, New York), pp. 157–169.
36. Radu, A., Blobel, G. & Wozniak, R. W. (1993) *J. Cell Biol.* **121**, 1–9.
37. Severs, N. J., Jordan, E. G. & Williamson, D. H. (1976) *J. Ultrastruct. Res.* **54**, 374–387.
38. Wright, R. (1993) *Curr. Biol.* **3**, 870–873.