Published June 1, 1989

Lamin A, Lamin B, and Lamin B Receptor Analogues in Yeast

Spyros D. Georgatos, Ioanna Maroulakou, and Günter Blobel

Laboratory of Cell Biology, Howard Hughes Medical Institute, The Rockefeller University, New York 10021

Abstract. Previous studies have shown that turkey erythrocyte lamin B is anchored to the nuclear envelope via a 58-kD integral membrane protein termed p58 or lamin B receptor (Worman H. J., J. Yuan, G. Blobel, and S. D. Georgatos. 1988. *Proc. Natl. Acad. Sci. USA.* 85:8531-8534). We now identify a p58 analogue in the yeast *Saccharomyces cerevisiae*. Turkey erythrocyte lamin B binds to yeast urea-extracted nuclear envelopes with high affinity, associating predominantly with a 58-kD polypeptide. This yeast polypeptide is recognized by polyclonal antibodies against turkey p58, partitions entirely with the nuclear fraction, remains membrane bound after urea extraction of the nuclear envelopes, and is structurally similar to turkey p58 by peptide mapping criteria. Using polyclonal antibodies against turkey erythrocyte lamins A and B, we also identify two yeast lamin forms. The yeast lamin B analogue has a molecular mass of 66 kD and is structurally related to erythrocyte lamin B. Moreover, the yeast lamin B analogue partitions exclusively with the nuclear envelope fraction, is quantitatively removed from the envelopes by urea extraction, and binds to turkey lamin A and vimentin. As many higher eukaryotic lamin B forms, the yeast analogue is chemically heterogeneous comprising two serologically related species with different charge characteristics. Antibodies against turkey lamin A detect a 74-kD yeast protein, slightly larger than the turkey lamin A. It is more abundant than the yeast lamin B analogue and partitions between a soluble cytoplasmic fraction and a nuclear envelope fraction. The yeast lamin A analogue can be extracted from the nuclear envelope by urea, shows structural similarity to turkey and rat lamin A, and binds to isolated turkey lamin B. These data indicate that analogues of typical nuclear lamina components (lamins A and B, as well as lamin B receptor) are present in yeast and behave as their vertebrate counterparts.

ALTHOUGH analogues of eukaryotic cytoskeletal elements have been identified in the yeast *Saccharomyces cerevisiae* (1, 5, 15–19, 23, 28, 29), members of either the cytoplasmic or nuclear (lamin) intermediate filament family have yet to be characterized. Unlike higher eukaryotic cells, yeast undergoes closed mitosis; i.e., the nuclear envelope is not disassembled during cell division. Instead, a cytoplasmic bud develops, the nucleus elongates into the bud region and eventually, by a fission/fusion event, a separate nuclear envelope is generated for mother and daughter cell (4, 6, 17, 27). In higher eukaryotic cells, the nuclear lamina is thought to play an important role at interphase by supporting the nuclear membrane, and in mitosis by being disassembled (at prophase) concomitantly with the disassembly of the nuclear envelope (for a review see reference 13). As yeast undergoes closed mitosis, a nuclear lamina may not be required and therefore may not exist. However, if the nuclear lamina functions in organizing interphase chromatin as has been proposed (3, 25), or in anchoring intermediate filaments (8, 10), then these functions should also be indispensable in yeast.

Ioanna Maroulakou's present address is University of Athens, School of Health Sciences, Faculty of Nursing, Department of Biology, Goudi, Athens, Greece.

We report here the presence of analogues of higher eukaryotic lamins A, B, and a recently characterized lamin B receptor (32) in *Saccharomyces cerevisiae*. The occurrence of nuclear lamina building blocks in this organism suggests that the nuclear lamina may be a universal karyoskeletal assembly involved in fundamental functions of all nucleated cells.

Materials and Methods

Cells and Subcellular Fractionation

The protease-deficient haploid *Saccharomyces cerevisiae* strain BJ2168 (a, trp1, leu2, ura3-52, prb1-1122, pep4-3, prc1-407) was obtained from the Yeast Genetic Stock Center, University of California Berkeley (Berkeley, CA). Turkey erythrocytes were obtained from whole turkey blood and fractionated according to Georgatos and Blobel (8). Yeast cells were fractionated by the method of Aris and Blobel (2). After Ficoll gradient centrifugation of a yeast spheroplast lysate, subcellular fractions were collected and processed as follows. (*a*) "Low-density membranes" (essentially plasma membranes that floated to the top of the 20% Ficoll layer) were resuspended in ice-cold buffer A (155 mM NaCl, 15 mM Tris-HCl, 0.3 mM PMSF, pH 7.35), cleared by centrifugation at 2,000 *g* for 2–5 min at 4°C, and then washed once with buffer A. (*b*) The cytoplasmic fraction, contained in the 20% Ficoll layer, was first diluted 1:5 with buffer A, cleared by centrifugation at 8,000 *g* for 10 min and then high-speed centrifuged (365,000 *g* for 45 min at 18°C) to generate a cytosolic supernatant (Sol) and a pellet (P). The supernatant after this centrifugation contained soluble proteins with a

Published June 1, 1989

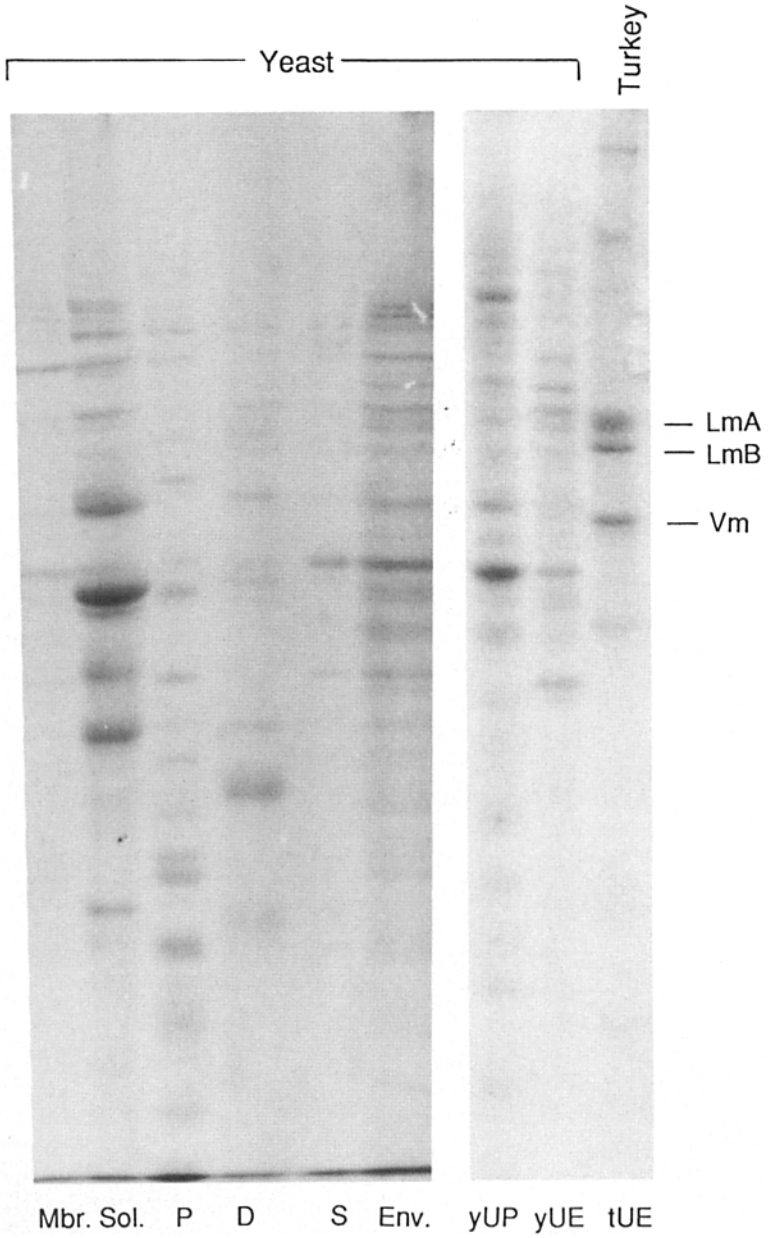


Figure 1. Subcellular fractionation of yeast. Yeast cells were fractionated as specified in Materials and Methods. Equivalent fractions corresponding to 1:500 of one yeast preparation (30–40 g wet weight) were then analyzed by SDS-PAGE and the proteins were visualized by Coomassie blue staining. *Mbr*, light density membranes (plasma membranes); *Sol*, high speed supernatant of cytoplasmic fraction; *P*, pellet after high-speed centrifugation of the cytoplasmic fraction; *D*, DNase I digest of yeast nuclei; *S*, high salt extract of yeast nuclear envelopes; *Env*, salt-extracted yeast nuclear envelopes; *yUP*, urea-extracted yeast envelopes; *yUE*, urea extract of yeast envelopes; *tUE*, urea extract of turkey erythrocyte nuclear envelopes. Bars indicate the positions of turkey lamin A (*LmA*), turkey lamin B (*LmB*), and turkey vimentin (*Vm*).

sedimentation value <16 S. (*c*) Intact nuclei, recovered at the 40% Ficoll layer, were digested with DNase I and salt extracted to prepare nuclear envelopes as previously described (2). The DNase I digest (D) and the salt extract (S) were clarified by high speed centrifugation as above and kept at −70°C. The nuclear envelopes were washed with 30 vol of ice-cold distilled water and then resuspended in 10–20 vol of urea extraction buffer (8 M urea, 10–25 mM Tris-HCl, pH 8.0, 4 mM EDTA, 1 mM DTT, and 0.3–0.6 mM PMSF). After complete resuspension by sonication, the sample was centrifuged at 365,000 *g* for 45 min at 18°C. The supernatant (urea extract) was collected and kept at −70°C. The pellet (yeast nuclear membranes) was gently rinsed with distilled water and kept at −70°C. Electrophoretic profiles of the main fractions are presented in Fig. 1.

Protein Chemical Procedures

Turkey erythrocyte nuclear lamins A and B, rat liver lamins A/C and B, and erythrocyte vimentin were isolated by ion-exchange chromatography (8, 9). Iodinated derivatives of the various proteins were prepared by reacting appropriate samples with ^{125}I-Bolton-Hunter reagent as specified (9). Affinity matrices were made by coupling isolated turkey lamin B to derivatized agarose (Affigel-15; Bio-Rad Laboratories, Cambridge, MA) as described (8). Limited proteolysis was done by incubating appropriate fractions with *Staphylococcus aureus* V8 protease or a lysine-specific protease (sequencing grade; Boehringer Mannheim Diagnostics, Inc., Houston, TX) for 1 h at room temperature at approximate enzyme to substrate ratios of 1:250–1:500. Limited tryptic digestion was performed using 1:100 (wt/wt) of trypsin (Boehringer Mannheim Diagnostics, Inc.) for 50 min on ice. Chemical cleavages with 2-nitro-5-thiocyanobenzoic acid (NTCB; Sigma Chemical Co., St. Louis, MO) were performed (as in reference 31) with 10 mM NTCB for 18 h at 37°C. Column chromatography of the yeast urea extract was done according to two different methods. Method A: The extract was dialysed against 5 M urea, 10 mM Tris-HCl, 1 mM EDTA, 1 mM DTT, 0.3 mM PMSF, pH 7.35, batch-incubated with DEAE-cellulose (DE-52; Whatman Paper, Maidstone, Kent, England). The slurry was poured into a column and eluted first with a 5–8 M urea gradient (at the same ionic strength and pH as above) and then with a salt gradient of 0–150 mM NaCl in 8 M urea, 10 mM Tris-HCl pH 7.35. For further purification of the yeast lamin A, appropriate fractions from the above column were pooled, dialysed against 8 M urea, 10 mM NaPi, 0.3 mM PMSF, and rechromatographed through a hydroxylapatite column using a 10–100-mM NaPi gradient. Method B:

Published June 1, 1989

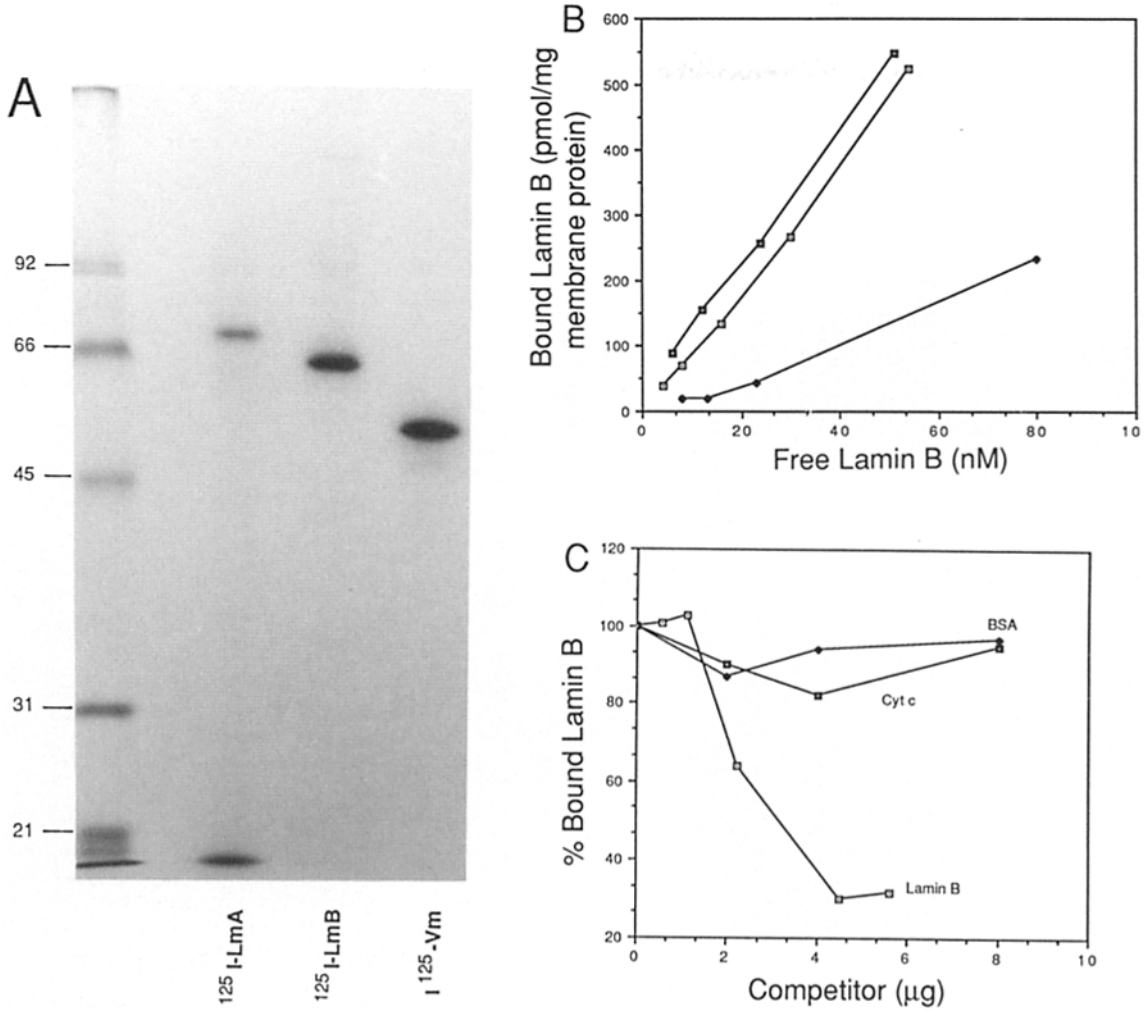


Figure 2. Binding of purified turkey ^{125}I–lamin B to yeast and turkey membrane preparations. (*A*) Radioactive probes used in this study: *^{125}I-LmA*, turkey lamin A probe; *^{125}I-LmB*, turkey lamin B probe; *^{125}I-Vm*, turkey vimentin probe. All three proteins were labeled by the ^{125}I-Bolton-Hunter reagent as described in Materials and Methods, analyzed by SDS-PAGE, and visualized by autoradiography. The first lane on the left of the autoradiogram shows ^{14}C-labeled molecular mass markers with the indicated molecular masses (in kilodaltons). (*B*) Binding isotherms showing the binding of erythrocyte ^{125}I–lamin B to urea-extracted turkey nuclear envelopes (■), urea-extracted yeast nuclear envelopes (⊡) and yeast plasma membranes (■). The membranes were prepared as indicated in Materials and Methods and the assay was done in reference 32. Each point in the curves is an average of at least four independent observations with a variation of <5%. (*C*) Displacement of the bound ^{125}I–lamin B to yeast nuclear membranes by an excess of unlabeled lamin B (*Lamin B*), and lack of displacement by bovine serum albumin (*BSA*), and yeast cytochrome *c* (*Cyt c*). The assay was done as above using constant amounts of yeast nuclear membranes (12 μg), ^{125}I–lamin B (1 μg/ml, 100,000 cpm/μg), and increasing quantities of unlabeled competitor proteins. The nonspecific binding to yeast plasma membranes has been subtracted.

The extract (in 8 M urea extraction buffer pH 8.0, see above) was batch incubated with DEAE-cellulose (DE-53; Whatman Paper) and then eluted in one step with 500 mM NaCl in urea extraction buffer.

Assays

Blot binding assays were performed exactly as described (32), except in the case of the blot shown in Fig. 3 *C*. In this particular experiment, the nitrocellulose sheets were preincubated with 10 μg/ml of purified turkey lamin B in the usual assay buffer (32) for 3 h at room temperature, washed for 2 h with assay buffer, and then probed as described below with specific anti–lamin B antibodies at a dilution of 1:600. Immunoblotting assays were done as specified (8, 32). Solution binding assays were performed as in reference 32. Briefly, purified erythrocyte ^{125}I-lamin B, at specific activities of 22,000–25,000 cpm/μg, in buffer A (see above) was mixed with urea-extracted yeast envelopes, yeast "low density membranes", or urea-extracted turkey erythrocyte envelopes washed and resuspended in buffer A. The assay mixture (100 μl) also contained 200 μg/ml of BSA, pretreated with 1 mM PMSF, and filtered through a 0.2-μm filter. After a 60-min incubation at room temperature, the samples were centrifuged at 16,000 *g* for 30 min at 4°C and processed as previously described (10, 32). Affinity chromatography was done as follows: Fractions of urea extracts of yeast nuclear envelopes enriched in lamin A were radioiodinated with the Bolton-Hunter reagent (see above). Approximately 1 × 10^6 cpm of these preparations were mixed with 20–100 μg of BSA in a volume of 1–2 ml and dialysed against buffer A. The mixture was then combined with 1–2 ml lamin B–agarose (see above) containing ~70 μg of protein per milliliter of beads. The suspension was batch incubated for 90 min at room temperature (end-over-end mixing) and then poured into a column. The flowthrough was collected (nonbound fraction) and the column was washed with 20–25 vol of buffer A until no substantial radioactivity was detected in the wash. Bound material was eluted with 5–10 column volumes of 8 M urea, 20 mM Tris-HCl, 4 mM EDTA, 1 mM DTT, 0.1% SDS, 0.3 mM PMSF, pH 7.6, followed by the same volume of 8 M urea, 1 M NaCl, 0.3 mM PMSF, pH 9.5. Portions of the

Published June 1, 1989

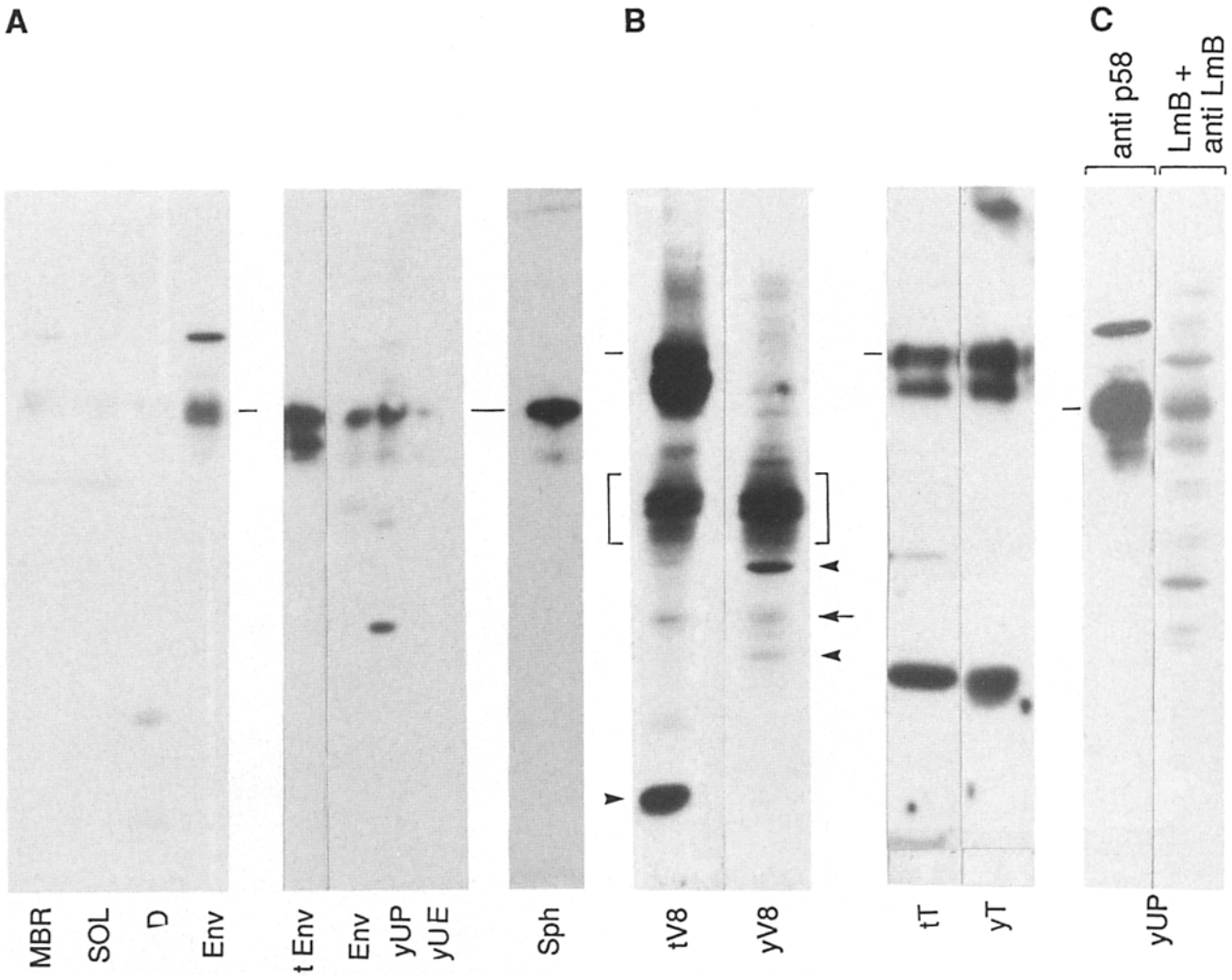


Figure 3. Identification of a lamin B receptor analogue in yeast. (*A*) Immunoblotting of yeast subcellular fractions with anti-p58 antibodies (diluted 1:600). This fraction designation is similar to the one described in Fig. 1. *Sph*, a sample of whole yeast spheroplasts extracted with 8-M urea as specified above for the nuclear envelopes. *tEnv*, a sample of turkey erythrocyte nuclear envelopes. Bars indicate the major 58-kD cross-reactive protein found in the yeast nuclear fractions. (*B*) Comparative peptide mapping of turkey p58 and yeast p58 analogue. Urea-extracted yeast spheroplasts and urea-extracted turkey nuclear envelopes were digested in parallel with V8 protease from *Staphylococcus aureus* or trypsin (see Materials and Methods) and analyzed by SDS-PAGE. The fractionated digests were then transferred to nitrocellulose filters and probed with anti-p58 antibodies (diluted 1:600). *tV8*, turkey membranes digested with V8; *yV8*, yeast membranes digested with V8. *Arrowheads*, the position of unique peptides; *arrows*, the position of minor peptides common in both preparations; and *brackets*, the position of the major common fragments. *tT* and *yT* represent, respectively, samples of turkey and yeast nuclear envelopes digested with trypsin and then processed as above using a 1:500 dilution of the anti-p58 antibodies. (*C*) Binding of turkey erythrocyte lamin B to proteins of yeast urea-extracted envelopes that had been fractionated by SDS-PAGE and transferred to nitrocellulose filters. Note the comigration of a major lamin B binding protein (lane *LmB + anti-LmB*) with the yeast p58 analogue as detected by immunoblotting in a replica gel using anti-p58 antibodies (lane *anti-p58*). For a detailed description of the assay see Materials and Methods.

fractions were diluted 1:1 with distilled water, precipitated with 15% TCA at 0°C, and solubilized in Laemmli sample buffer (21) for electrophoresis and autoradiography.

Other Procedures

Electrophoresis (SDS-PAGE) was according to Laemmli (21), except that in most cases the electrode buffer was twice the ionic strength of the standard buffer to maximize the stacking parameters. All gels shown here are 10% polyacrylamide unless stated otherwise. Two-dimensional gel electrophoresis was performed as described (26). Protein concentrations were estimated using a protein determination kit (Bio-Rad Laboratories).

Results

Identification of a Lamin B Receptor Analogue in Yeast

In previous studies (32), we have established an in vitro binding assay using lamin-depleted erythrocyte nuclear envelopes (i.e., nuclear membranes) and purified ^{125}I-lamin B. Employing this assay, in combination with a blot binding assay (10, 32), we have shown that lamin B associates with the nuclear membrane via a protein receptor (p58). We used a

Published June 1, 1989

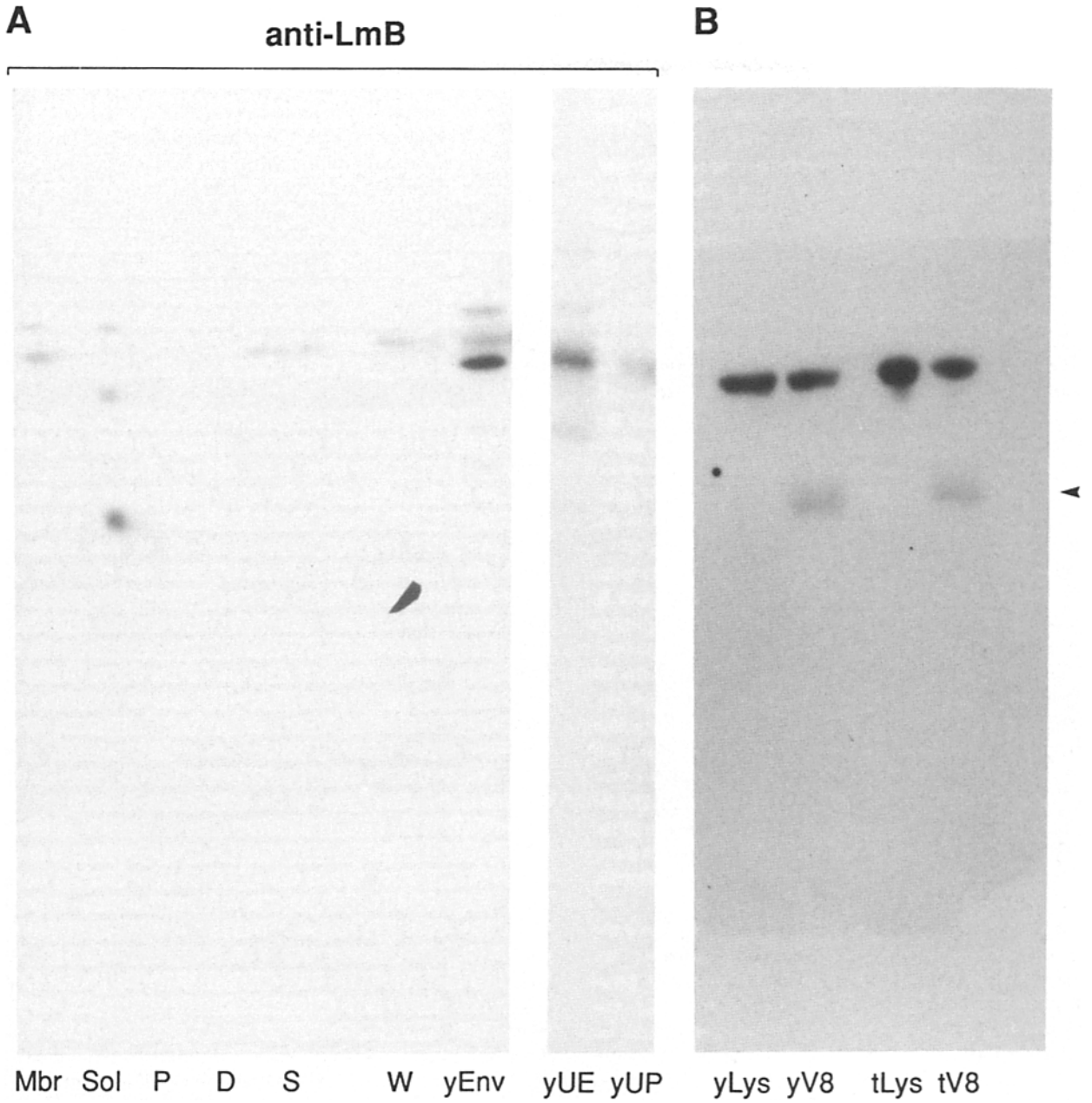


Figure 4. Identification of a yeast lamin B analogue by anti-lamin B antibodies. (*A*) Immunoblotting of yeast subcellular fractions with anti-turkey lamin B antibodies. Fraction processing and designation is as in Fig. 1. *W*, a sample of the water extract of yeast nuclear envelopes. The antibody was diluted 1:600. A bar indicates the position of the yeast 66-kD cross reacting protein. (*B*) Comparative peptide mapping of turkey lamin B and yeast lamin B analogue. Urea extracts of turkey and yeast nuclear envelopes (see Materials and Methods) were diluted with 10 vol of 10 mM Tris-HCl, pH 7.3, and digested with V8 protease from *Staphylococcus aureus* or a lysine-specific protease as described in Materials and Methods. The digests were analyzed by SDS-PAGE, transferred to nitrocellulose filters, and probed with anti-lamin B antibodies (diluted 1:600). *yLys*, lysine protease-treated yeast extract; *yV8*, V8-treated yeast extract; *tLys*, lysine protease-treated turkey extract; *tV8*, V8-treated turkey extract. *Arrowhead*, the position of the major immunoreactive fragments.

similar approach to investigate whether such a receptor for lamin B is also present in yeast nuclear membranes. After cell fractionation (Fig. 1), yeast nuclear envelopes were extracted with 8 M urea (Fig. 1, lane *yUP*) and then tested in parallel with yeast plasma membranes and turkey erythrocyte nuclear membranes for binding of erythrocyte ^{125}I–lamin B (autoradiographic profile of the probe shown in Fig. 2 *A*). Fig. 2 *B* shows that ^{125}I–lamin B bound similarly to both yeast nuclear membranes and turkey nuclear membranes in the linear range of the isotherm (for details see reference 32), whereas much less binding was detected when the plasma membranes from yeast were substituted for the nuclear membranes. Rat liver lamin B exhibited a similar binding (data not shown). Specific binding to the yeast nuclear membranes was concentration dependent and could be effectively competed by an excess of unlabeled ligand but not by unrelated proteins such as BSA or yeast cytochrome *c* (Fig. 2 *C*). The corresponding K_d, estimated from the amount of

Published June 1, 1989

unlabeled lamin B needed to displace 50% of the bound tracer, amounted to 0.22 μM, in agreement with a K_d of 0.20 μM obtained from similar assays done with turkey erythroctye nuclear membranes (32). These data suggested that a specific and high affinity lamin B receptor may be present in yeast urea-extracted nuclear envelopes.

Using monospecific polyclonal antibodies against the previously characterized lamin B receptor of turkey erythrocytes (p58, see reference 32), we then looked for a yeast p58 analogue. Subcellular fractionation and immunoblotting revealed a polypeptide with a molecular mass of ~58 kD strongly cross reacting with these antibodies (bars in Fig. 3). A secondary cross-reactivity was also observed at ~80 kD (Fig. 3, lane *Env*), but this signal was not reproducible. Consistent with previous observations concerning the intracellular partitioning of p58, the yeast immunoreactive protein was found in nuclear envelope fractions, in urea-extracted nuclear envelopes, or in urea-extracted spheroplasts, but not in cytosolic fractions, plasma membrane fractions, or urea extracts of nuclear envelopes (Fig. 3 *A*). Thus, the 58-kD polypeptide seemed to represent an integral component of the yeast nuclear membrane, like p58 in turkey erythrocytes.

To confirm the above results, yeast and turkey fractions were digested with proteases and the products analyzed by immunoblotting using anti-p58 antibodies. Tryptic digestion of turkey and yeast fractions yielded almost identical proteolytic patterns (Fig. 3 *B*, lanes *tT* and *yT*). Digestion with V8 protease from *Staphylococcus aureus* yielded two major immunoreactive fragments and several satellite peptides that comigrated in SDS polyacrylamide gels (Fig. 3 *B*, lanes *tV8* and *yV8*, *brackets*). Other proteolytic products also comigrated (Fig. 3 *B*, lanes *tV8* and *yV8*, *arrow*), but a few peptides were either unique or of higher abundance in the turkey preparation (Fig. 3 *B*, lane *tV8*, *arrowhead*). On the basis of these findings it appears that the yeast 58-kD protein is structurally similar to turkey erythrocyte 58.

To examine whether this structural similarity between the two 58-kD proteins has any functional consequences, we probed electrophoretically separated polypeptides of yeast nuclear membranes with ^{125}I-turkey lamin B, using our standard blot-binding assay (32). Under these conditions no substantial binding to the yeast 58-kD component was detected (data not shown). However, because we suspected that the yeast p58 analogue may exhibit a "low-avidity" binding to lamin B, we repeated the assay applying to the yeast substrates unlabeled turkey lamin B, followed by anti-turkey lamin B antibodies and ^{125}I-protein A. Such a "sandwich" method, which would augment low-avidity signals, became an available option because the anti-lamin B antibodies did not react to any significant extent with the components of the yeast urea-extracted envelopes (Fig. 4 *A*, lanes *yUE* and *yUP*). By means of this modified blot assay, the lamin B was found to react predominantly with a 58-kD protein of the yeast nuclear membranes which comigrated with the yeast p58 analogue, as seen after immunoblotting of a replica preparation (Fig. 3 *C*, *LmbB + anti-LmB* and *anti-p58*). Three other polypeptides (of unknown identity) also bound to the avian lamin B to a lesser extent (Fig. 3 *C*). Taken together, these results indicate that the yeast 58-kD protein shares both structural features and functional properties with the turkey erythrocyte p58.

Identification of a Yeast Lamin B Analogue

The identification of a p58 analogue in yeast suggested that a lamin B form may also be present in this organism. To search for lamin B–like proteins, we used a polyclonal antibody directed against turkey erythrocyte lamin B. This antibody reacts strongly with erythrocyte lamin B, weakly with erythrocyte lamin A (see below), and not at all with rat lamins (data not shown). By indirect immunofluorescence, it decorates the turkey erythrocyte nucleus in a rim-like fashion (32). In immunoblotting assays, the antibody against the turkey lamin B detected a yeast polypeptide with a similar molecular mass (66 kD) as turkey erythrocyte lamin B (Fig. 4 *A*, *bars*). Nonimmune sera of guinea pig or rabbit gave no substantial signals when tested on yeast substrates (see below).

The 66-kD cross-reactive protein was detected exclusively in the nuclear envelope fraction, consistent with a nuclear envelope localization (Fig. 4 *A*). After 8 M urea extraction of yeast nuclear envelopes the same polypeptide was found almost quantitatively in the extracted fraction (Fig. 4 *A*, lanes *yUE* and *yUP*), as all authentic lamins (8, 11, 14). To investigate the structural relatedness between the yeast 66-kD band and erythrocyte lamin B, we digested polypeptides of urea extracts from yeast and erythrocyte nuclear envelopes with proteases and examined the cleavage products by immunoblotting. Fig. 4 *B* shows that the turkey lamin B and its yeast analogue yielded one major fragment of a similar size when treated with V8 protease, whereas both molecules were resistant, under these conditions, to a lysine-specific protease. These results strongly suggested that the yeast 66-kD protein and turkey lamin B are structurally related.

To confirm the immunoblotting data by another method, we examined other molecular properties of the putative yeast lamin B analogue. Using the standard blot binding assay (32), we probed the polypeptides of an urea extract of yeast envelopes with erythrocyte ^{125}I-lamin A (autoradiographic profile of probe shown in Fig. 2 *A*). It was found that the 66-kD yeast protein reacts very strongly with this probe (Fig. 5), as one might have expected from the unusually strong heterotypic interactions between rat or *Xenopus laevis* lamins A and B that have been described (9, 20). The specificity and the high affinity of this interaction could be appreciated by considering the small amounts of the yeast protein present in the blot and the compositional complexity of the tested preparation (Fig. 5, *Silver*).

To examine the chromatographic behavior of the yeast lamin B analogue, and to enrich it, the urea extract of yeast nuclear envelopes was fractionated by ion exhange chromatography in the presence of 8 M urea according to the standard method used to purify lamins from vertebrate sources (see Materials and Methods). It was observed that the yeast 66-kD protein eluted in two distinct peaks; an early peak produced by applying a 5–8-M urea gradient (no change in the ionic conditions) and a second peak seen after applying a 0–100-mM NaCl gradient (Fig. 6 *A*, *DE-52-C.B.*). Although the second peak corresponded precisely to the position where a typical (vertebrate) lamin B would be eluted under the same ionic regime (8, 11), the first peak did not correspond to the typical lamin B elution profile. This biphasic profile indicated that the original band was either heteroge-

Published June 1, 1989

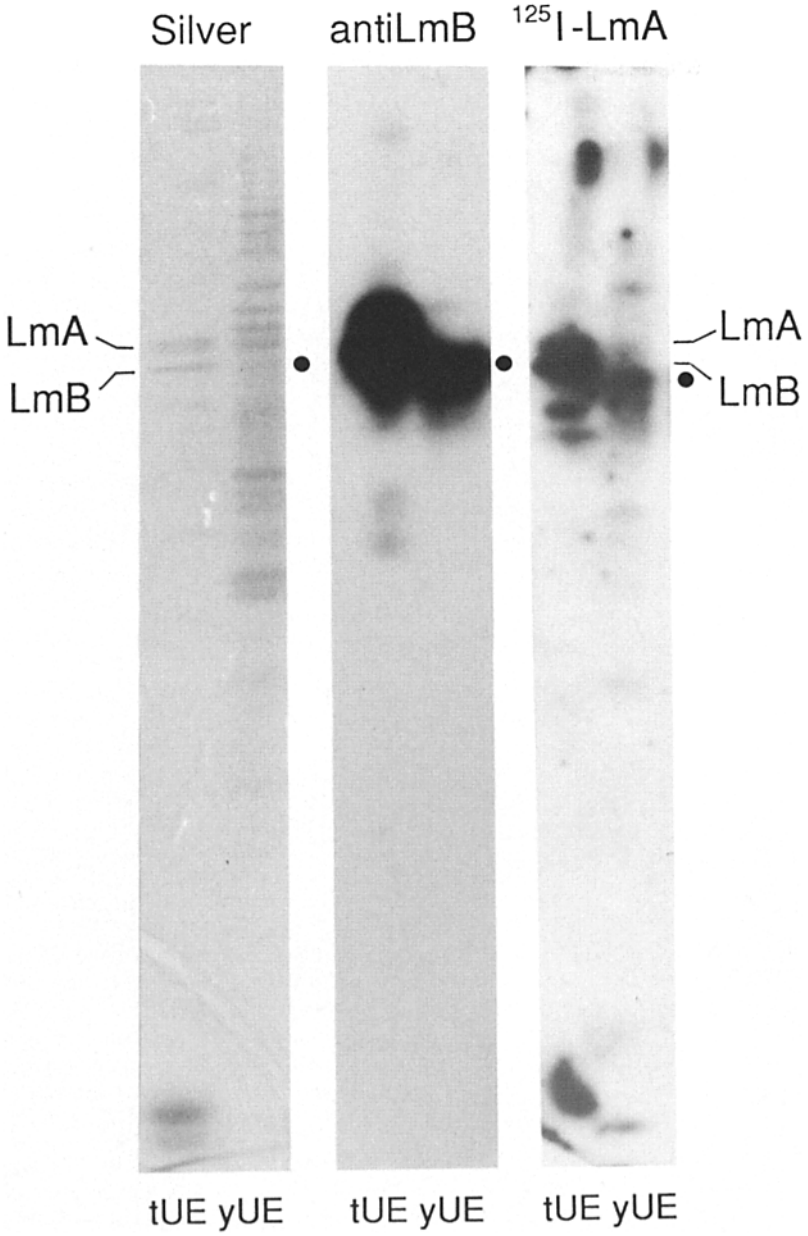


Figure 5. Binding of turkey lamin A to the yeast lamin B analogue. Urea extracts of yeast nuclear envelopes (*yUE*) and turkey urea envelopes (*tUE*) were analyzed by SDS-PAGE (using 1× Laemmli electrode buffer). One of the triplicate gels was stained with silver (*Silver*); the others were transferred to nitrocellulose filters and probed with anti-lamin B antibodies (anti-lamin B) or ^{125}I-lamin A from turkey erythrocytes (^{125}I-lamin A). *LmA*, position of turkey lamin A; *LmB*, position of turkey lamin B. The position of the yeast lamin B analogue is indicated by dots. Note that the yeast lamin B analogue migrates slightly faster than the turkey lamin B when the gels are run with 1× Laemmli electrode buffer.

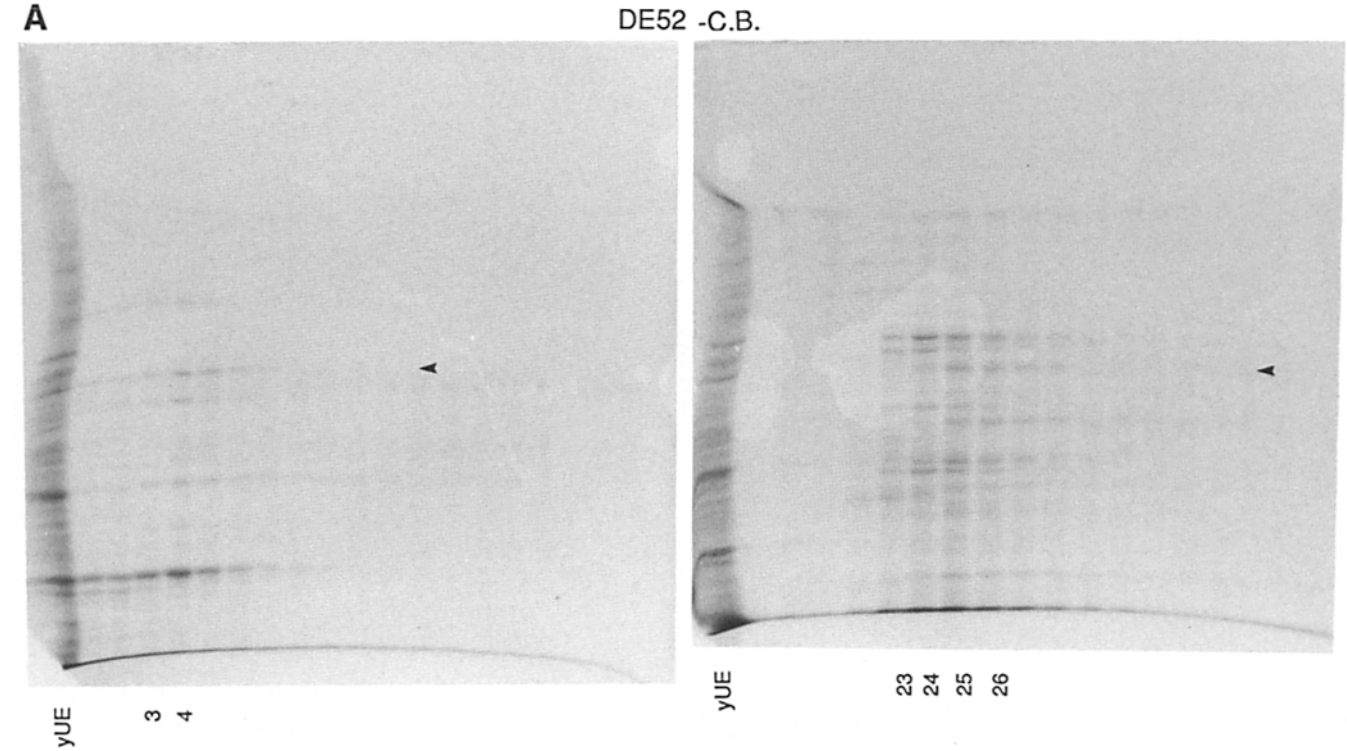
neous (comprising two different proteins), or that it contained two distinct forms of the yeast lamin B analogue. To differentiate between these two possibilities, samples from the two peaks were probed with anti-lamin B antibodies. Fig. 6 *B* shows that the 66-kD bands in both peaks were immunoreactive, suggesting that in both cases the eluted material was serologically related to lamin B. Two-dimensional gel electrophoresis and immunoblotting of a urea extract of yeast nuclear envelopes confirmed this conclusion (data not shown). Posttranslational modifications, principally phosphorylation, may account for the charge heterogeneity of the two yeast lamin B forms. Such heterogeneities have been described previously for lamin B forms occurring in several rat tissues (31).

Having obtained these lamin B–enriched preparations, we then tested the lamin B analogue for binding to erythrocyte vimentin, a property exhibited by both mammalian and avian lamin B (8, 10). When the yeast fractions (in parallel with a sample of urea extracts of yeast or turkey nuclear envelopes) were probed with erythrocyte ^{125}I-vimentin (autoradiographic profile of the probe in Fig. 2 *A*), we observed a strong binding to the corresponding 66-kD bands (Fig. 6 *B*). As expected, the ^{125}I-vimentin also reacted with erythrocyte vimentin and erythrocyte lamin B. We therefore inferred that the yeast lamin B analogue behaves functionally as a lamin B isotype.

Identification of a Yeast Lamin A Analogue

To identify lamin A–like proteins in yeast we used a poly-

Published June 1, 1989



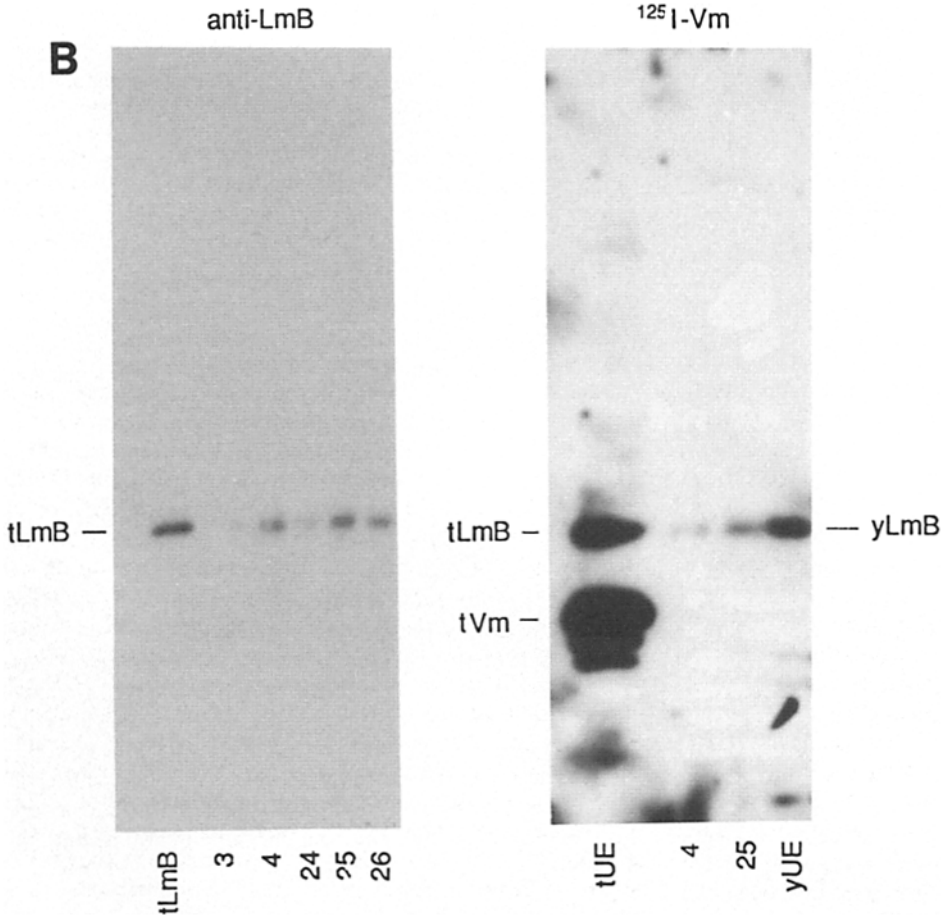


Figure 6. (*A*) Fractionation of urea extracts of yeast nuclear envelopes by ion-exchange chromatography. A urea extract of yeast nuclear envelopes was chromatographed on DEAE-cellulose (DE-52) as specified in method A in Materials and Methods. Samples of successive fractions (only a few of which are indicated here by numbers) were analyzed on 7.5% acrylamide gels and stained with Coomassie blue. Note the biphasic elution of the yeast lamin B analogue (*arrowhead*). (*B*) Probing of the peak column fractions shown in *A* by anti-lamin B antibodies and turkey vimentin. The polypeptides of the indicated fractions from the DE-52 column were analyzed by SDS-PAGE, in parallel with isolated turkey lamin B (*tLmB*), polypeptides of a urea extract of turkey nuclear envelopes (*tUE*), and of a urea extract of yeast nuclear envelopes (*yUE*). The proteins were then transferred to nitrocellulose filters and probed with anti-lamin B antibodies (1:600; *anti-LmB*) or purified turkey erythrocyte ^{125}I-vimentin (*^{125}I-Vm*). Bars indicate the positions of turkey lamin B (*tLmB*), yeast lamin B analogue (*yLmB*), and turkey erythrocyte vimentin (*tVm*).

Published June 1, 1989

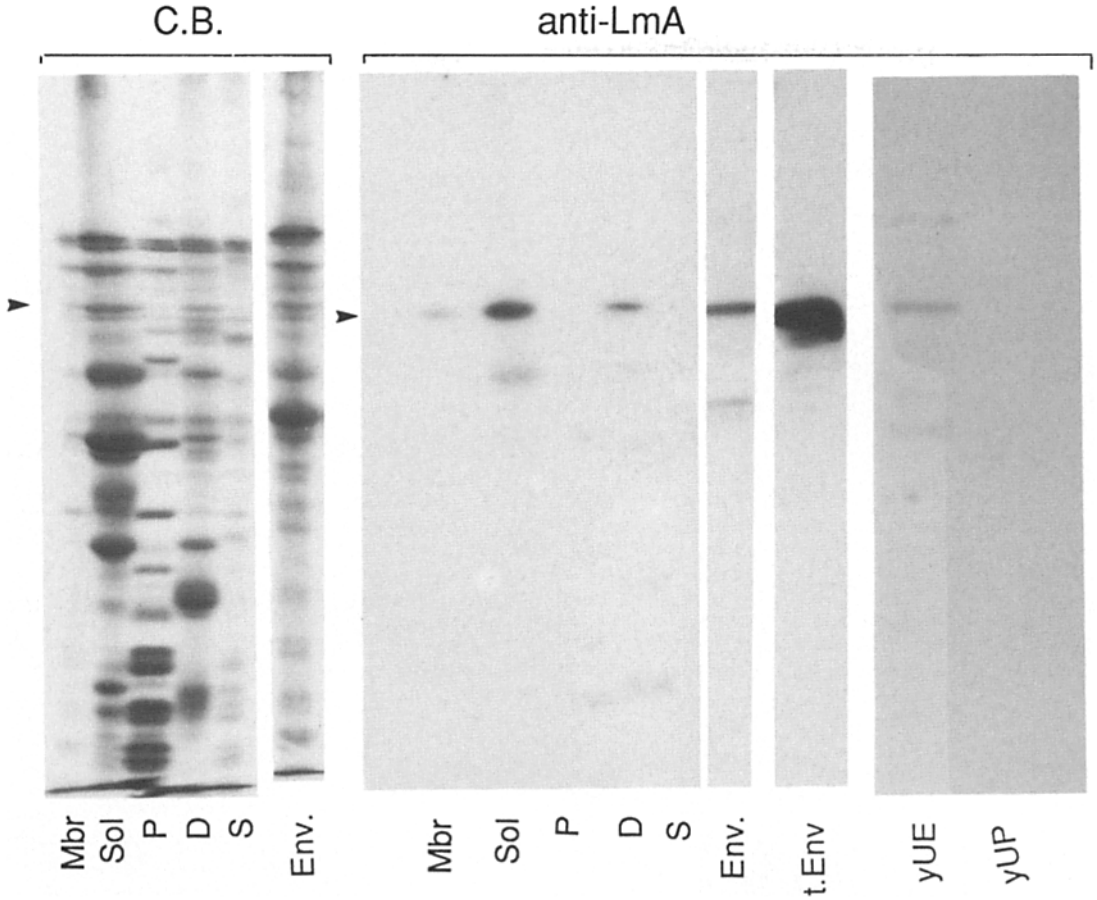


Figure 7. (*A*) Identification of a yeast lamin A analogue by anti-turkey lamin A antibodies. Polypeptides of subcellular fractions from yeast (designations as in Fig. 1) were analyzed by SDS-PAGE and either stained by Coomassie blue (*CB*) or transferred to nitrocellulose filters and probed with anti-lamin A antibodies (*anti-LmA*) (diluted 1:300). *Arrowhead*, the position of the yeast lamin A analogue. Note the partitioning of the cross-reactive 74-kD band with the soluble cytosol, the DNase I digest, the nuclear envelope fraction and the urea extract of nuclear envelopes.

clonal antibody against turkey erythrocyte lamin A. This reagent reacts on blots exclusively with turkey lamin A (see below). Probing subcellular fractions from yeast revealed a 74-kD band in both the nuclear envelope fraction and a soluble cytoplasmic fraction containing elements with a sedimentation coefficient (S value) of <16 S (Fig. 7). Small amounts of this protein were released from the yeast nuclei during digestion with DNase I, but no further removal was seen after high salt washing or water washing. Compared to the previously described lamin B analogue, the 74-kD protein occurred in higher amounts. Upon extraction of the nuclear envelopes with 8-M urea, all the envelope-associated 74-kD protein was solubilized (Fig. 7, lanes *yUE* and *yUP*), consistent with the behavior of a peripheral nuclear envelope protein. Fractionation of the urea extracts by DEAE-cellulose chromatography revealed that the yeast lamin A is an acidic protein (it binds to the anion exchanger DE-53) that is eluted in a single peak by 0.5 M NaCl. The 74-kD protein in the column fractions could be specifically decorated by anti-lamin A antibodies but not by nonimmune sera (Fig. 8, *A* and *B*).

To confirm the identity of the putative yeast lamin A analogue we incubated urea extracts of rat, turkey, and yeast envelopes with NTCB, a reagent that cleaves proteins specifically at Cys residues (9). The cleavage products were then analyzed by SDS-PAGE and probed with specific antibodies. To probe the rat material we used an anti-lamin A/C polyclonal antibody that has been previously characterized (3, 14). Because this antibody does not recognize the turkey lamins (data not shown), we employed the anti-turkey lamin A antibody to test the two nonmammalian samples. Comparative immunoblotting revealed in all three preparations a single 60-kD immunoreactive fragment (Fig. 9, *F60* and *F'60*), indicating a substantial similarity between the various lamin A forms. Inspecting the reported sequence of mammalian lamins A and C (7, 24), we noticed that the first cysteine residue in these two molecules is found at position 522. In lamin A, there are four more Cys residues at its carboxy-terminal tail which are closely spaced. Therefore, the 60-kD fragment of rat lamins A and C could only correspond to a peptide extending from their common amino terminus to residue 522 (see schematic representation in Fig. 9). That a single 60-kD peptide was also detected in both the turkey and the yeast digests suggests that the spacing of the cysteine

Published June 1, 1989

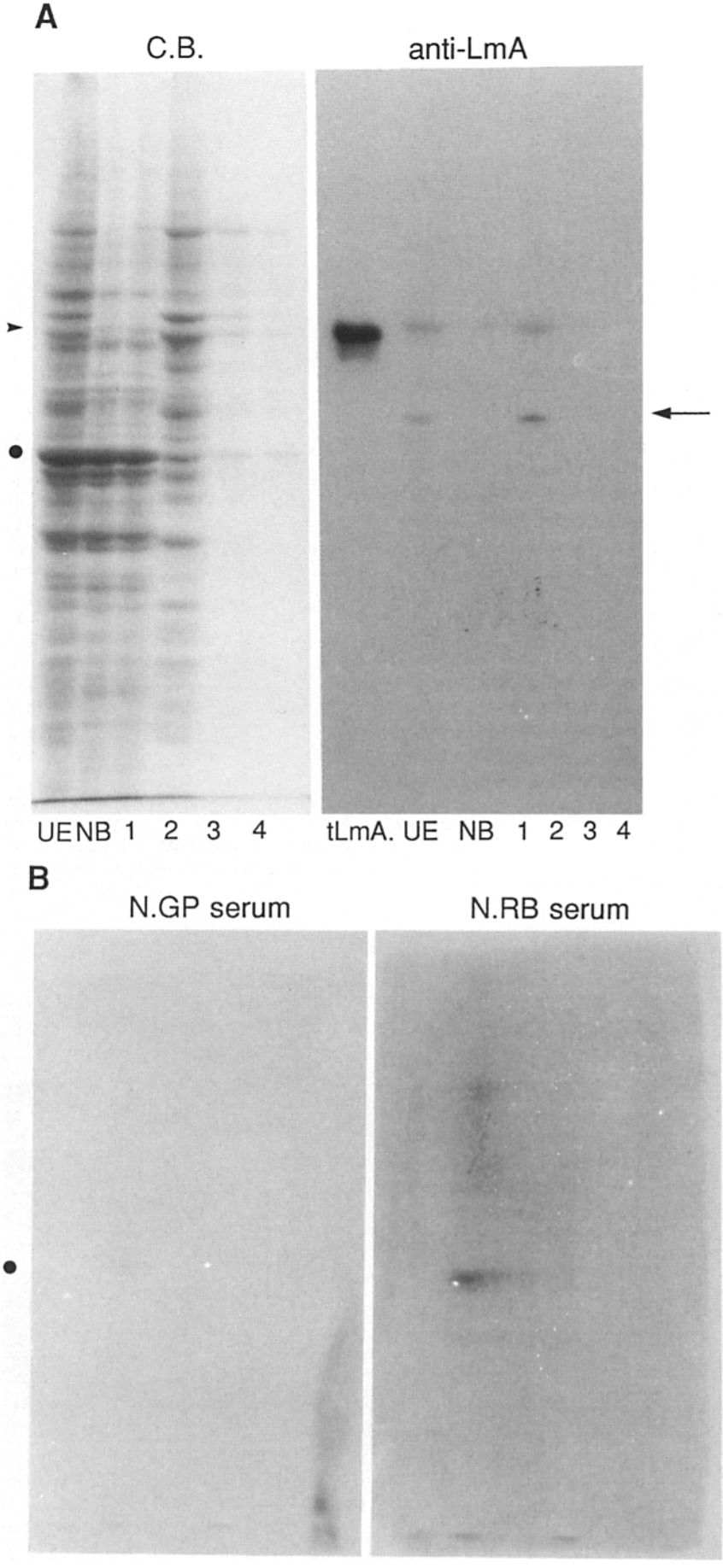


Figure 8. (*A*) Chromatographic fractionation of urea extracts of yeast nuclear envelopes and elution of the yeast lamin A analogue. Urea extracts of yeast nuclear envelopes were chromatographed according to method B as described in Materials and Methods. Polypeptides were analyzed by SDS-PAGE and either stained with Coomassie blue (*C.B.*) or transferred to nitrocellulose and probed with anti-lamin A at a dilution of 1:600 (*anti-LmA*). *UE*, Urea extract of yeast nuclear envelopes; *NB*, material nonbound to the DE-53 column; *1*, *2*, *3*, and *4*, successive column fractions; *tLmA*, purified turkey lamin A. The position of the yeast lamin A analogue is shown by an arrowhead. *Arrow*, a proteolytic product of the yeast lamin A at ~45 kD. (*B*) Immunoblots of the fractions shown in *A* by a nonimmune guinea pig serum (*N.GP serum*, dilution 1:200) and a nonimmune rabbit serum (*N.RB serum*, dilution 1:100). Note the lack of cross-reaction. Dots indicate a prominent protein of the urea extract of yeast nuclear envelopes (also shown in *A*) that exhibits background reactivity.

Published June 1, 1989

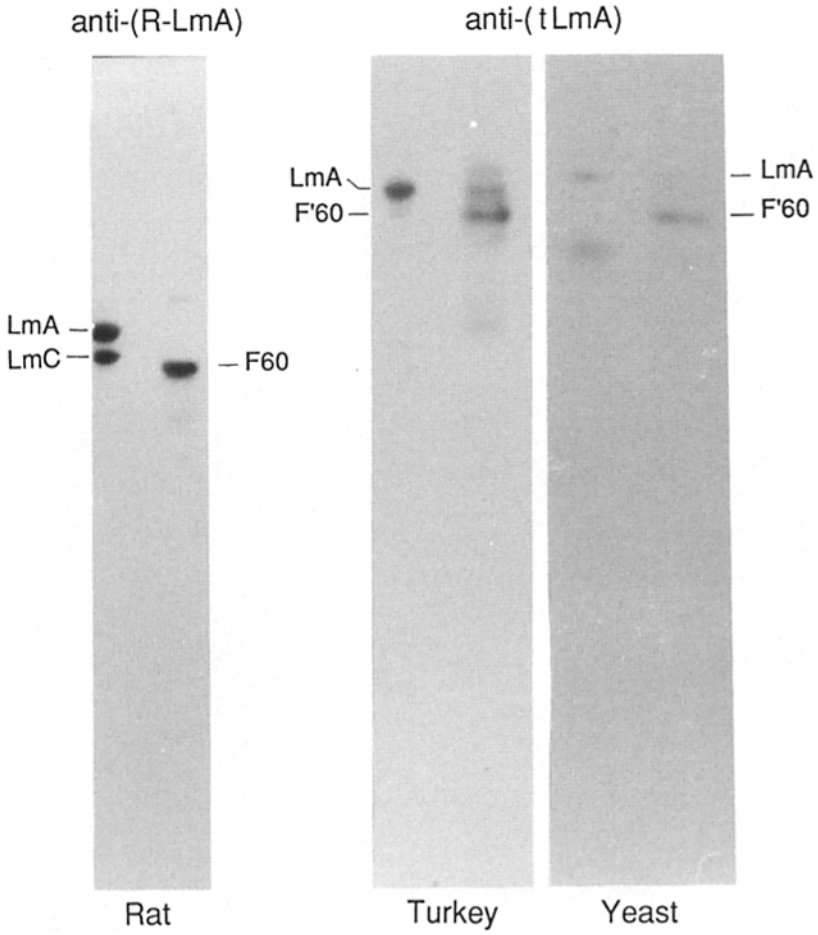


Figure 9. Comparative peptide mapping of rat lamins A and C, turkey lamin A, and yeast lamin A analogue. Urea extracts of isolated nuclear envelopes from rat liver, turkey erythrocytes, and yeast cells were treated with NTCB as specified in Materials and Methods and in reference 31. Polypeptides were then analyzed by SDS-PAGE, transferred to nitrocellulose filters, and probed with the corresponding antibodies. *Anti-(R-LmA)*, rat urea extract (*left lane*) and digest (*right lane*) probed with anti-rat lamin A and C antibodies. *Anti-(tLmA)*, turkey and yeast urea extracts (*left lanes*) and digests (*right lanes*) probed with anti-turkey lamin A antibodies. The positions of rat lamins A and C, turkey lamin A, and yeast lamin A are indicated. *F60* and *F'60* denote the major 60-kD cleavage products in rat or turkey, and yeast, respectively. The turkey and yeast samples were run on replica gels while the rat sample was analyzed on a separate gel. The schematic diagram on top shows the distribution of cysteine residues in the human lamin A and C molecules (see references 7 and 24).

residues along the molecules is conserved, in spite of the apparent immunochemical (i.e., primary structure) differences in the same stretch of the polypeptide chain.

To further characterize the putative yeast lamin A, we examined its ability to associate with erythrocyte lamin B. When we probed urea extracts of yeast nuclear envelopes with ^{125}I-turkey lamin B, the decoration of the 74-kD component was weaker than the corresponding reaction between ^{125}I-turkey lamin A and yeast lamin B (data not shown). Therefore, we approached the same question by affinity chromatography. For these purposes, we first prepared a yeast lamin A–enriched fraction by ion exchange chromatography (profile shown in Fig. 10 *A*, *DE53/HAP-C.B.*). The entire fraction was radiolabeled with the ^{125}I-Bolton-Hunter reagent and applied together with an excess of unlabeled BSA to a turkey lamin B affinity column. As seen in Fig. 10 *C*, >70% (see figure legend) of the labeled 74-kD protein was retained specifically by the lamin B affinity column, whereas the albumin control protein was quantitatively recovered in the flowthrough (Fig. 10 *B*, *C.B.*). A major labeled species of low molecular weight was excluded from the column as were several other labeled proteins (Fig. 10 *C*, *dots*). However, more than one band was seen in the bound fraction. The nature of the additional polypeptides is not known, but it is conceivable that some may represent degradation products of the yeast lamin A analogue.

Discussion

Occurrence of Lamins and a Lamin B Receptor in Yeast

Using several criteria, we have characterized lamin A and B as well as lamin B receptor analogues in *Saccharomyces cerevisiae*. These findings open the door to study the function of the nuclear lamins by the powerful genetic methods that are available in yeast.

Evidence for a yeast lamin B receptor analogue is mani-

Published June 1, 1989

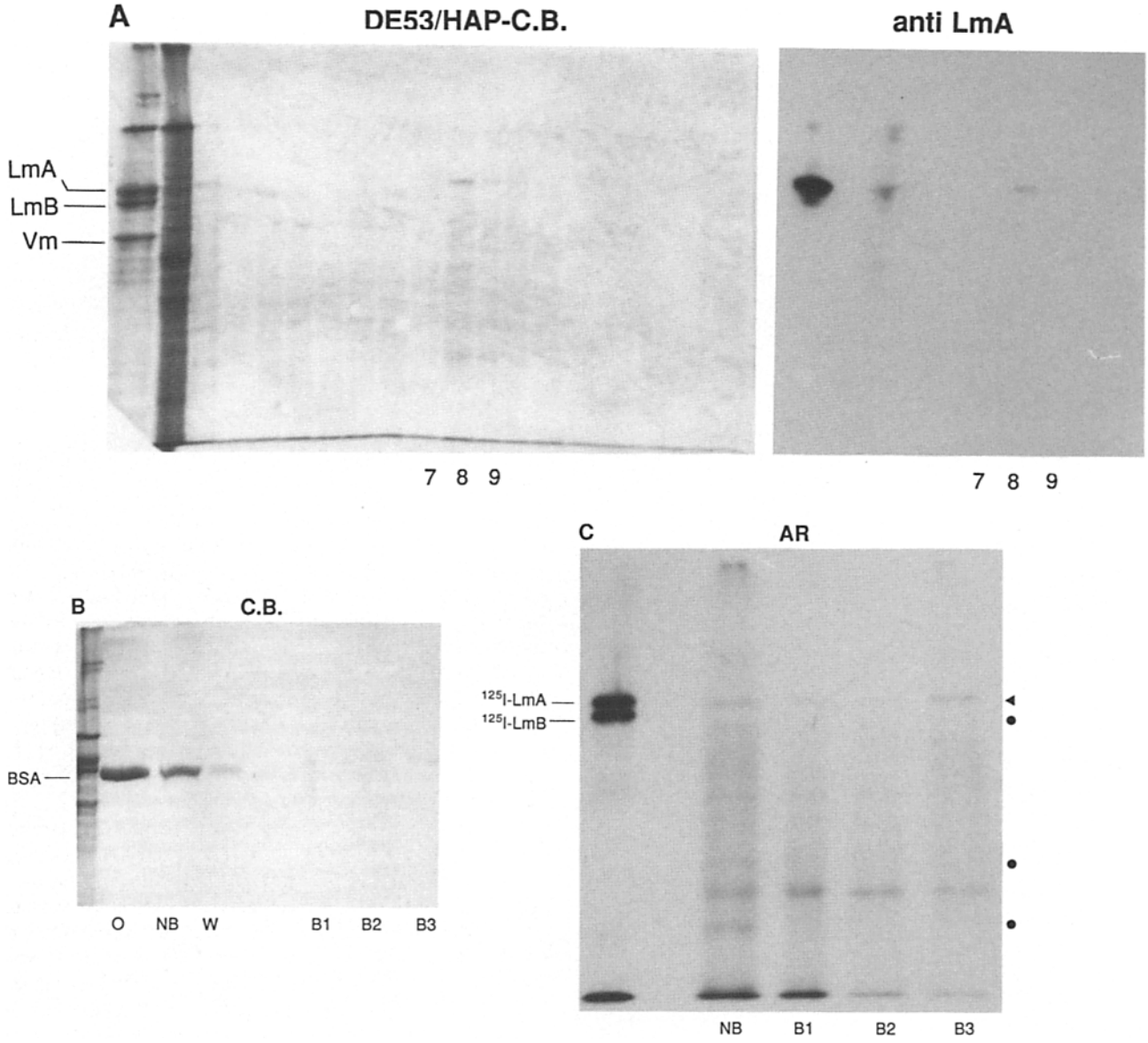


Figure 10. (*A*) Partial purification of yeast lamin A analogue. An urea extract of yeast nuclear envelopes was chromatographically fractionated as described in Materials and Methods using method A (DEAE-cellulose and hydroxyl-apatite chromatography). Successive fractions from the second column were analyzed by SDS-PAGE and either stained with Coomassie blue (*DE-53/HAP-C.B.*) or probed with anti-lamin A antibodies (*anti LmA*). The numbers (*7*, *8*, and *9*) denote the column fractions where the yeast lamin A analogue is eluted. The first two left lanes shown on the Coomassie blue-stained gel show profiles of urea extracts of turkey nuclear envelopes and of yeast nuclear envelopes, respectively. The positions of the turkey lamins and turkey vimentin are indicated. (*B* and *C*) Detection of yeast lamin A analogue-turkey lamin B complexes by affinity chromatography. The partially purified yeast lamin A was radiolabeled by the ^{125}I-Bolton-Hunter reagent and then passed through a lamin B affinity column after mixing with an excess of unlabeled BSA (for details see Materials and Methods). Equal volumes of the original sample (*O*), the column flowthrough (*NB*), the wash (*W*), and successive peak fractions of the eluted material (*B1*, *B2*, and *B3*) were analyzed by SDS-PAGE and either stained with Coomassie blue (*C.B.*) or analyzed by autoradiography (*AR*). The position of BSA eluting in the NB fraction is indicated by a bar. The first lane in the Coomassie blue-stained gel shows a profile of a urea extract of turkey nuclear envelopes. The sample analyzed in the *NB* fraction corresponds to 50% of the total radioactivity present in the nonbound fraction, while the total amount analyzed in *B1*, *B2*, and *B3* samples corresponds to ~25% of the total radioactivity in the bound fraction. Note, the binding of the yeast lamin A analogue to the column (*arrowhead*) and the exclusion of several other labeled species (*dots*).

fold: The candidate protein partitions with the nuclear envelope, behaves as an integral nuclear membrane protein, reacts with specific antibodies against a lamin B receptor (p58) of turkey nuclear envelopes, produces similar cleavage products as p58 upon limited proteolysis, and binds to lamin B. Evidence for lamin A and B analogues in yeast has been obtained by studying the partitioning and solubility properties of the candidate proteins, their cross-reactivity with specific antibodies, their structural similarity with authentic lamins, and their interactions with diagnostic reagents such

Published June 1, 1989

as heterologous lamins and vimentin (an intermediate filament protein). By all of these criteria (except for the soluble lamin A, see below), the yeast lamin analogues behave as typical peripheral proteins of the nuclear envelope exhibiting many of the characteristic properties of lamin isoforms. So far, lamin B appears to be a ubiquitous element of the nuclear lamina (22, 31). In addition, recent studies demonstrate that this component is anchored to the nuclear membrane via a receptor protein (reference 32, see also supporting evidence in reference 14) and not through a direct association with the lipid bilayer. Therefore, it makes sense that lamin B and its receptor are coexpressed in yeast.

Despite repeated efforts to immunolocalize these components, we have been unable to specifically decorate any particular structure in yeast cells using the antibodies against the turkey antigens. A detailed immunolocalization will have to await a morphological survey using antibodies directed against yeast antigens.

Conservation and Diversity in the Features of Yeast Lamins

Although the anti-turkey lamin antibodies readily detected the yeast lamin analogues, the same reagents were unreactive when tested with rat liver substrates or with material derived from mammalian tissue culture cells. Correspondingly, all of the antibodies against rat liver lamins that we have tested were unreactive with turkey or yeast substrates (Georgatos, S. D., unpublished results). Thus, there seems to be a schism in the lamin immunogenicity among mammals and nonmammals. The basis of this immunological divergence, in the context of a well-documented primary structure homology between mammalian and nonmammalian lamins (see references 20 and 30), is not clear. However, a possible explanation may be that only a limited number of sites in the various lamin molecules are sufficiently immunogenic, and that these potential epitopes are distinctly modified in different cellular environments.

In contrast to this immunological difference, lamin–lamin associations, lamin B–lamin B receptor binding, and lamin B–intermediate filament interactions seem to be conserved. The fact that the same type of molecular associations between intermediate filament proteins and lamin B are detectable in organisms as diverse as yeast and rat implies their involvement in fundamental cellular functions. Another important analogy between yeast and higher eukaryotes concerns the composition and stoichiometry of the yeast lamins. As in all nonmammalian cells (8, 22), no lamin C was detected in yeast. In addition, as in most cases with mammalian and nonmammalian cells (with the exception of rat liver; see reference 31), the type "A" and type "B" lamins occurred in nonstoichiometric ratios, with lamin B being chemically heterogeneous and limiting in relation to lamin A.

A number of observations indicate a conservation of structural and functional principles between the yeast and the avian p58 analogues. At the same time, also apparent is a substantial divergence in the corresponding primary structures. Upon limited (tryptic) digestion both proteins produce almost identical proteolytic patterns. However, upon more extensive degradation (with V8 protease), one notices a few differences in the proteolytic patterns. Furthermore, by means of the blot binding assay, the yeast protein seems to bind avian lamin B with low avidity, whereas the avian form does not show such a behavior. Genuine differences in the valency of the two receptors, or an incomplete renaturation of the yeast p58 after exposure to SDS, explain equally well this finding. In both cases the underlying differences could be due to variations in the primary structure.

Cytoplasmic Pool of Yeast Lamin A

An unexpected finding in this study concerns the pool of cytoplasmic lamin A that we have detected after subcellular fractionation and immunoblotting. It appears from the relative quantities of the yeast lamin A analogue in the various fractions that a substantial amount of this protein occurs in a soluble state (aggregate size <16 S), and does not seem to be in a polymerized or organelle-associated form. This observation is puzzling. It suggests that, either a sizable amount of assembly-incompetent lamin A is stored in the cytoplasm to fuel a rapid remodelling of the lamina, or that the cytosolic lamin A is the product of a dissociation reaction that relates to the mitotic state of a fraction of the yeast cells (3, 12, 14).

Yeast undergoes closed mitosis, and the nuclear envelope, instead of disassembling, maintains its continuity and elongates during cell division. It is therefore possible that during this elongation process the nuclear surface area is greatly increased whereas the number of lamin A binding sites at the nuclear envelope (i.e., lamin B–lamin B receptor) remains the same. If the lamin A–lamin B interaction is a cooperative one, as we suspect, a redistribution of lamin B along a greater surface area may reduce its local concentration, result in a weakening of lamin A–lamin B interactions (or possibly lamin A–lamin A interactions, see reference 11) and cause the dissociation of lamin A from the nuclear lamina. Since such a dissociation could be achieved without invoking superphosphorylation of lamin A, which does occur in higher eukaryotes (12, 14), the same protein could reassemble or disassemble during mitosis when and where the surface density of lamin A binding sites along the lamina favors this process (i.e., in a localized fashion). From a structural point of view, the above interpretation ("stoichiometric" lamin A assembly/disassembly) makes good sense because, via this mechanism, the nuclear lamina could be locally remodelled during the process of nuclear elongation. Another possibility could be that the soluble lamin A form exists in a typically mitotic, systemically superphosphorylated state and is unable to assemble. Preliminary data do not support the second alternative.

We would like to thank C. Klein for skillful technical help; J. P. Aris for advice and assistance; H. J. Worman and J. Yuan for their assistance; Parkash Cashmore for her expert secretarial skills; and T. Melese (Columbia University, New York) for providing yeast material at the initial stages of this project and for helpful discussions. Data shown in Fig. 9 were generated in part using an anti-rat liver lamin A/C antibody kindly provided by L. Gerace (Research Institute of Scripps Clinic, La Jolla, CA). We appreciate the critical comments of one of the reviewers of this manuscript.

The paper is dedicated to Elias Brountzos.

Received for publication 20 December 1988 and in revised form 20 February 1989.

References

1. Adams, A. E. M., and J. R. Pringle. 1984. Relationship of actin and tubulin distribution to bud growth in wild-type and morphogenetic-mutant *Sac-*

charomyces cerevisiae. J. Cell Biol. 98:934–945.
2. Aris, J. P., and G. Blobel. 1988. Identification and characterization of a yeast nucleolar protein that is similar to a rat liver nucleolar protein. *J. Cell Biol.* 107:17–31.
3. Burke, B., and L. Gerace. 1986. A cell free system to study the reassembly of the nuclear envelope at the end of mitosis. *Cell.* 44:639–652.
4. Byers, B., and L. Goetsch. 1974. Duplication of spindle plaques and integration of the yeast cell cycle. *Cold Spring Harbor Symp. Quant. Biol.* 38:123–131.
5. Byers, B., and L. Goetsch. 1976. A highly ordered ring of membrane-associated filaments in budding yeast. *J. Cell Biol.* 69:717–721.
6. Carter, B. L. A. 1978. The yeast nucleus. *Adv. Microb. Physiol.* 17: 243–302.
7. Fisher, D. Z., N. Chaudhary, and G. Blobel. 1986. cDNA sequencing of nuclear lamins A and C reveals primary and secondary structural homology to intermediate filament proteins. *Proc. Natl. Acad. Sci. USA.* 83:6450–6454.
8. Georgatos, S. D., and G. Blobel. 1987. Lamin B constitutes an intermediate filament attachment site at the nuclear envelope. *J. Cell Biol.* 105:117–125.
9. Georgatos, S. D., D. C. Weaver, and V. T. Marchesi. 1985. Site-specificity in vimentin-membrane interactions: intermediate filament subunits associate with the plasma membrane via their head domains. *J. Cell Biol.* 100:1962–1967.
10. Georgatos, S. D., K. Weber, N. Geisler, and G. Blobel. 1987. Binding of two desmin derivatives to the plasma membrane and the nuclear envelope of avian erythrocytes: evidence for a conserved site-specificity in intermediate filament-membrane interactions. *Proc. Natl. Acad. Sci. USA.* 84:6780–6784.
11. Georgatos, S. D., C. Stournaras, and G. Blobel. 1988. Heterotypic and homotypic associations between the nuclear lamins: site specificity and control by phosphorylation. *Proc. Natl. Acad. Sci. USA.* 85:4325–4329.
12. Gerace, L., and G. Blobel. 1980. The nuclear lamina is reversibly depolymerized during mitosis. *Cell.* 19:277–287.
13. Gerace, L., and B. Burke. 1988. Functional organization of the nuclear envelope. *Annu. Rev. Cell Biol.* 4:335–374.
14. Gerace, L., C. Comeau, and M. Benson. 1984. Organization and modulation of nuclear lamina structure. *J. Cell Sci.* 1(suppl.):137–160.
15. Greer, G., and R. Schekman. 1982. Actin from *Saccharomyces cerevisiae. Mol. Cell Biol.* 2:1270–1278.
16. Jacobs, C. W., A. E. M. Adams, P. J. Szaniszlo, and J. R. Pringle. 1988. Functions of microtubules in the *Saccharomyces cerevisiae* cell cycle. *J. Cell Biol.* 107:1409–1426.
17. Kilmartin, J. V., and A. E. M. Adams. 1984. Structural rearrangements of tubulin and actin during the cell cycle of the yeast *Saccharomyces. J. Cell Biol.* 98:922–933.
18. Kilmartin, J. V. 1981. Purification of yeast tubulin by self-assembly in vitro. *Biochemistry.* 20:3629–3633.
19. Kotelianski, V. E., M. A. Glukhova, M. V. Bejanian, A. P. Surguchov, and V. N. Smirnow. 1979. Isolation and characterization of actin-like protein from yeast *Saccharomyces cerevisiae. FEBS (Fed. Eur. Biochem. Soc.) Lett.* 102:55–58.
20. Krohne, G., S. L. Wolin, F. D. McKeon, W. W. Franke, and M. W. Kirschner. 1987. Nuclear lamin LI of *Xenopus laevis*: cDNA cloning, amino acid sequence and binding specificity of a member of the lamin B subfamily. *EMBO (Eur. Mol. Biol. Organ.) J.* 6:3801–3808.
21. Laemmli, U. K. 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature (Lond.)* 227:680–685.
22. Lehner, C. F., R. Stick, H. M. Eppenberger, and E. Nigg. 1987. Differential expression of nuclear lamin proteins during chicken development. *J. Cell Biol.* 105:577–587.
23. Magdolen, V., U. Oechsner, G. Muller, and W. Bandlow. 1988. The intron-containing gene for yeast profilin (PFY) encodes a vital function. *Mol. Cell. Biol.* 8:5108–5115.
24. McKeon, F., M. Kirschner, and D. Caput. 1986. Homologies in both the primary and secondary structure between nuclear envelope and intermediate filament proteins. *Nature (Lond.).* 319:463–468.
25. Newport, J. 1987. Nuclear reconstitution in vitro. Stages of assembly around protein-free DNA. *Cell.* 48:205–217.
26. O'Farrell, P. Z., H. M. Goodman, and P. H. O'Farrell. 1977. High resolution two-dimensional electrophoresis of basic as well as acidic proteins. *Cell.* 12:1133–1142.
27. Peterson, J. B., and H. Ris. 1976. Electron-microscopic study of the spindle and chromosome movement in yeast *Saccharomyces cerevisiae. J. Cell Sci.* 22:219–242.
28. Water, R. D., J. R. Pringle, and L. J. Kleinsmith. 1980. Identification of an actin-like protein and of its messenger ribonucleic acid in *Saccharomyces cerevisiae. J. Bacteriol.* 144:1143–1151.
29. Watts, F. Z., D. M. Miller, and E. Orr. 1985. Identification of myosin heavy chain in *Saccharomyces cerevisiae. Nature (Lond.).* 316:83–85.
30. Wolin, S. L., G. Krohne, and M. W. Kirschner. 1987. A new lamin in Xenopus somatic tissues displays strong homology to human lamin A. *EMBO (Eur. Mol. Biol. Organ.) J.* 6:3809–3818.
31. Worman, H. J., I. Lazaridis, and S. D. Georgatos. 1988. Nuclear lamina heterogeneity in mammalian cells. Differential expression of the major lamins and variations in lamin B phosphorylation. *J. Biol. Chem.* 263: 12135–12141.
32. Worman, H. J., J. Yuan, G. Blobel, and S. D. Georgatos. 1988. A lamin B receptor in the nuclear envelope. *Proc. Natl. Acad. Sci. USA.* 85: 8531–8534.