The Lamin B Receptor of the Nuclear Envelope Inner Membrane: A Polytopic Protein with Eight Potential Transmembrane Domains

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Abstract. The lamin B receptor is a previously identified integral membrane protein in the nuclear envelope of turkey erythrocytes that associates with the nuclear intermediate filament protein lamin B (Worman, H. J., J. Yuan, G. Blobel, and S. D. Georgatos. 1988. *Proc. Natl. Acad. Sci. USA.* 85:8531–8534). In the present report, we use cell fractionation and antibodies against the lamin B receptor to localize it to an 8-M urea-extracted membrane fraction of chicken liver nuclei, supporting an inner nuclear membrane localization. We deduced the amino acid sequence of the chicken lamin B receptor from overlapping clones obtained by screening cDNA libraries with a probe generated by the polymerase chain reaction with primers based on the partial protein sequence of the isolated protein. The mature lamin B receptor has a calculated molecular mass of 73,375 D and eight segments of hydrophobic amino acids that could function as transmembrane domains as determined by hydropathy analysis. Preceding the first putative transmembrane segment is a highly charged 204-residue-long amino terminal region that contains two consensus sites for phosphorylation by protein kinase A. Since the lamin B receptor has been shown to be phosphorylated by protein kinase A in vitro and in vivo and this phosphorylation affects lamin B binding (Applebaum, J., G. Blobel, and S. D. Georgatos. 1990. *J. Biol. Chem.* 265:4181–4185), it is likely that this amino terminal region faces the nucleoplasm. The amino terminal region also contains three DNA-binding motifs that are found in gene regulatory proteins and histones, suggesting that the lamin B receptor may additionally play a role in gene regulation and/or chromatin organization.

THREE morphologically distinct membrane domains can be discerned in the nuclear envelope of eukaryotic cells. The first of these domains is the outer nuclear membrane, which contains ribosomes on its cytoplasmic side and resembles the rough endoplasmic reticulum (RER)[1] with which it is continuous at certain points (15, 18, 35). The second is the inner nuclear membrane, which is associated on its nucleoplasmic side with the nuclear lamina (12, 15, 18), a meshwork of intermediate filament proteins termed lamins (1, 14, 25, 32). The third distinct membrane is the nuclear pore membrane, which forms numerous annular transcisternal connections between the outer and inner nuclear membranes and is associated with the nuclear pore complexes (15, 18).

Because viral integral membrane proteins synthesized in the RER have access to the inner nuclear membrane presumably by lateral diffusion via the nuclear pore membrane (6, 41, 42), the distribution of integral membrane proteins between the RER and the three membrane domains of the nuclear envelope could be random. However, the unique association of specific structures (ribosomes, pore complexes, and lamina) with specific membrane domains of the nuclear envelope suggests a nonrandom distribution of at least those integral membrane proteins that may be involved in the anchorage of these structures. In support of this notion, several integral membrane proteins associated with these structures have been identified and localized to specific nuclear envelope membrane domains. An integral membrane glycoprotein (gp210) has been localized to the nuclear pore membrane where it presumably functions in the anchorage of the pore complexes (19, 44). Another integral membrane protein with an apparent molecular mass of 58 kD (p58), likely located in the inner nuclear membrane, has been identified as a lamin B receptor (43), which presumably functions in the anchorage of the lamina to this membrane. Three other proteins (p75, p68, and p55) that are recognized by a single monoclonal antibody have also been characterized as integral proteins of the inner nuclear membrane and proposed to be associated either directly or indirectly with the lamina (38). These three proteins and the lamin B receptor (whether or not they are related remains to be investigated) are all markers for the inner nuclear membrane, much as gp210 is

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1. *Abbreviations used in this paper*: PCR, polymerase chain reaction; RER, rough ER.

a marker for the nuclear pore membrane (19), and RER proteins are markers for the outer nuclear membrane (2, 35). Whether the outer nuclear membrane domain also possesses marker proteins that distinguish it from the RER proper is presently not known.

Except for gp210 of the nuclear pore membrane (44), the primary structures for integral inner nuclear membrane marker proteins and other pore complex membrane marker proteins have not been determined. Here we report the complete primary structure of the lamin B receptor, an inner nuclear membrane protein, deduced from the sequences of cDNA clones. We further discuss structural features of this protein with regards to its topology and its multiple potential functions.

Materials and Methods

Homogenization and Cellular Fractionation of Chicken Liver

Liver was removed from 3-d-old chicks and homogenized at 4°C in 250 mM sucrose, 50 mM triethanolamine (pH 7.5), 25 mM KCl, 5 mM $MgCl_2$, 1 mM DTT, and 0.2 mM PMSF. The homogenate was passed through two layers of cheesecloth and then centrifuged at 1,200 *g* for 10 min, yielding a pellet enriched in nuclei and a postnuclear supernatant. The latter was centrifuged at 10,000 *g* for 10 min yielding a pellet enriched in mitochondria and a postmitochondrial supernatant. The postmitochondrial supernatant was centrifuged at 100,000 *g* 60 min giving a pellet enriched in microsomes and a postmicrosomal supernatant.

Electrophoresis and Immunoblotting

Electrophoresis on SDS-polyacrylamide slab gels was performed under reducing conditions as described by Laemmli (27). Immunoblotting was performed as previously described (43). For immunoblots with guinea pig antiserum that was raised against gel-purified turkey erythrocyte lamin B receptor (43), the serum was preabsorbed against human skin keratins (Sigma Chemical Co., St. Louis, MO) immobilized on nitrocellulose strips as described (20) to remove contaminating antikeratin antibodies. Guinea pig antiserum raised against gel-purified turkey lamin B (43) and rabbit antiserum raised against purified pig UDP-glucuronosyltransferase (antiserum supplied by Dr. A. Dannenberg, Cornell University Medical College, New York) were used without treatment or purification.

Isolation of Lamin B Receptor from Turkey Erythrocyte Nuclear Envelopes

Isolation of nuclei from turkey erythrocytes, preparation of nuclear envelopes, and extraction of nuclear envelopes with 1 M NaCl and 8 M urea to yield "urea-extracted nuclear envelopes" were performed as previously described (17, 43). Proteins of urea-extracted nuclear envelopes were separated by electrophoresis under reducing conditions on SDS-polyacrylamide slab gels and the Coomassie blue-stained band corresponding to the lamin B receptor (43) was isolated by electroelution as described by Hunkapiller et al. (23).

Proteolytic Cleavage of Lamin B Receptor Protein

V8 proteolytic cleavage of the lamin B receptor and separation of proteolytic fragments was performed as described by Cleveland et al. (10). Briefly, lamin B receptor protein isolated by electroelution as described above was resuspended at a concentration of 0.5 mg/ml in 0.5% SDS, 125 mM Tris-HCl (pH 6.8), and incubated with 75 μg/ml of V8 protease (Boehringer Mannheim Diagnostics, Inc., Houston, TX) at 37°C for 45 min. The V8 proteolytic cleavage peptides were separated by electrophoresis under reducing conditions on SDS-polyacrylamide gels (15% acrylamide).

Amino Acid Sequencing

Amino acid sequencing of the lamin B receptor was performed on both electroeluted lamin B receptor and on the protein migrating at 58 kD in urea-extracted turkey erythrocyte nuclear envelope fractions transferred to polyvinylidene difluoride membrane (Immobilon Transfer 0.45-μm pore size; Millipore Continental Water Systems, Bedford, MA). Electrophoretic transfer to polyvinylidene difluoride membrane, protein visualization and preparation for sequencing were performed according to Matsudaira (31). V8 cleavage peptides separated by electrophoresis on SDS-polyacrylamide slab gels were also electrophoretically transferred to polyvinylidene difluoride membrane for amino terminal sequencing. These samples were sequenced on a model 470A gas-phase sequenator (Applied Biosystems Inc., Foster City, CA) as described (22).

RNA Isolation and cDNA Preparation

RNA was isolated from livers of 2-d-old chicks by the method of Chirgwin et al. (9). Poly (A^+)-enriched RNA was isolated by one cycle of oligothymidylic acid-cellulose (Boehringer Mannheim Diagnostics, Inc.) chromatography according to the method of Aviv and Leder (4). Poly (A^+)-enriched RNA was reverse transcribed to cDNA using the Invitrogen (San Diego, CA) Reverse Transcription kit following the manufacturer's instructions.

Polymerase Chain Reaction (PCR)

The polymerase chain reaction was performed as previously described (36) using the Gene Amp Taq-polymerase kit (Perkin Elmer Corp./Cetus Corp., Emeryville, CA). The reaction was run for 25 cycles in a DNA thermal cycler (Perkin Elmer Corp./Cetus Corp.) with the following incubation conditions: denaturation at 94°C for 1 min, annealing at 45°C for 2 min, and extension at 72°C for 3 min. Primers (synthesized on a model 380B oligonucleotide synthesizer [Applied Biosystems Inc.] and purified by electrophoresis on 20% polyacrylamide gels), template (chicken liver cDNA), deoxynucleotides, and Taq polymerase were used at the concentrations recommended in the Gene Amp Kit instructions. The amplified reaction mixture was subjected to electrophoresis on agarose gels, and the DNA migrating at the expected size was eluted, phosphorylated, and subcloned into the Sma I site of PUC-mp19 by standard methods (30) for double-stranded sequencing.

cDNA Library Screening

A synthetic 25-mer oligonucleotide corresponding to a portion of the amplified PCR product was end labeled with [^{32}P]gamma-ATP (New England Nuclear, Boston, MA) using T4 polynucleotide kinase (New England Biolabs, Beverly, MA) as described (30). The ^{32}P-labeled probe was purified by electrophoresis on a 20% polyacrylamide gel and used to screen a previously characterized (28) chick embryo cDNA library (supplied by Dr. D. Johnson, University of California, San Francisco) constructed in lambda Zap (Stratagene, La Jolla, CA). Library screening, plaque purification, and DNA isolation by CsCl-gradient centrifugation were by standard methods (30). The cDNA insert of the largest positive clone from this library (termed DJ-5) was ^{32}P-labeled using the Boehringer Mannheim Random Primed DNA Labeling kit and used to isolate cross-hybridizing overlapping clones from a previously described (33) chick embryonal erythrocyte lambda gt11 cDNA library (supplied by Dr. R. Moon, University of Washington, Seattle, WA).

RNA Analysis (Northern Blotting)

Northern blotting of chick liver poly(A^+)-enriched RNA, isolated as described above, was performed by the method of Thomas (40).

DNA Sequencing

DNA sequencing was performed by the dideoxy chain termination method (37) using the Sequenase kit (United States Biochemical Corp., Cleveland, OH) on either CsCl-gradient prified double-stranded DNA or single-stranded DNA subcloned into M13. Synthetic oligonucleotides were used as primers in the sequencing reactions.

Sequence Analysis

DNA sequence analysis was performed using the DNA Star software package (DNA Star, Inc., Madison, WI) on a Personal Computer AT (IBM). Hydropathy analysis was performed by the method of Kyte and Doolittle (26) using the program in the DNA Star package.

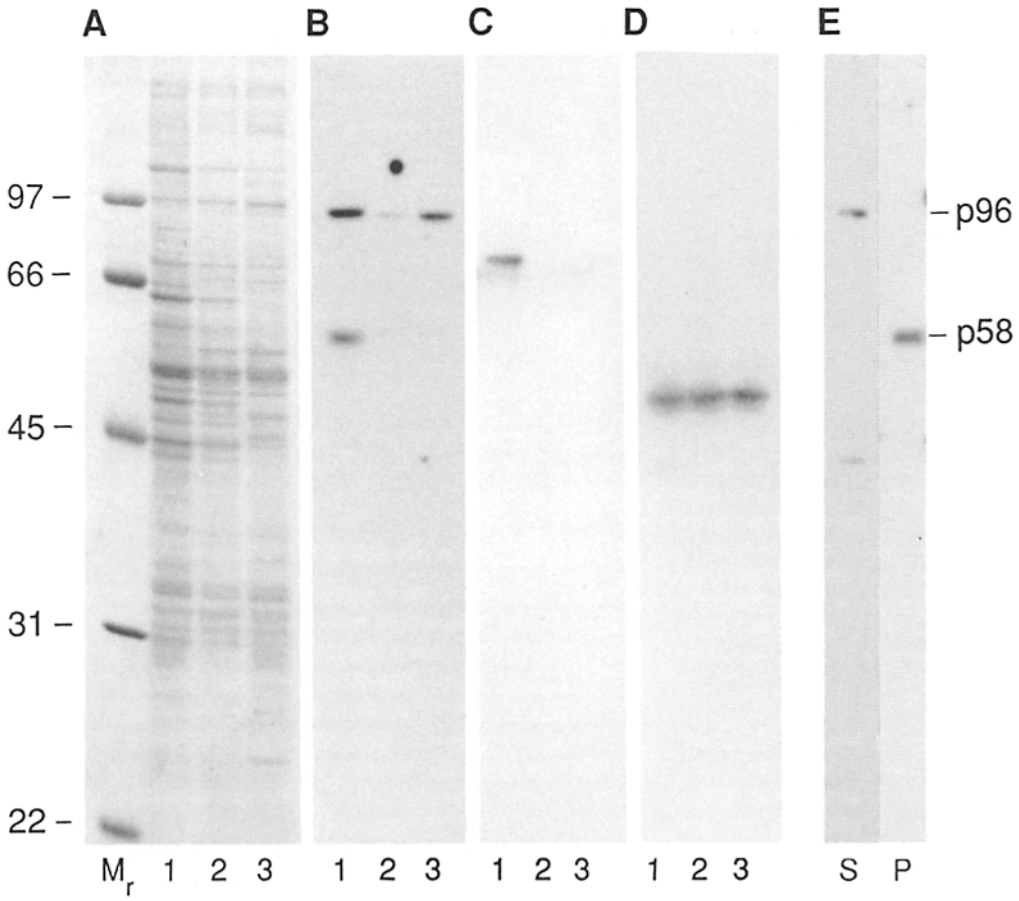

Figure 1. Subcellular fractionation of the lamin B receptor in chick liver cells. (*A*) Coomassie blue–stained SDS-polyacrylamide slab gel (10% acrylamide) of crude nuclear fraction (lane *1*), crude mitochondrial fraction (lane *2*), and microsomal fraction (lane *3*) obtained from homogenates of chicken liver. Migration of molecular mass standards are shown at left. (*B–D*) Autoradiograms of immunoblots of fractions identical to those shown in *A* probed with guinea pig serum containing antibodies against the lamin B receptor (*B*); guinea pig serum containing antibodies against turkey lamin B (*C*); and rabbit serum containing antibodies against UDP-glucuronosyltransferase (*D*). (*E*) Autoradiogram of immunoblot of proteins of chick liver nuclei that were in the supernatant fraction (*S*) or remained in the pellet (*P*) after extraction with 8 M urea. Nuclei were suspended in 100× volume excess of 8 M urea, 10 mM Tris-HCl (pH 8.0), 2 mM EDTA, 1 mM DTT, and 0.2 mM PMSF by bath sonication, incubated at room temperature for 40 min and centrifuged at 365,000 *g* 20 min to yield supernatant (*S*) and pellet (*P*) fractions. Bands corresponding to the lamin B receptor (p58) and cross-reactive 96 kD proteins (p96) are indicated at the right. Immunoblotting was performed using 1:500 dilutions of sera. Sera were used without purification; however, the guinea pig anti–lamin B receptor serum used in *B* and *E* was first preabsorbed against human skin keratins to remove contaminating antikeratin antibodies.

Materials

Unless otherwise specified, routine chemical reagents were obtained from Sigma Chemical Co. or Fisher Scientific Co. (Pittsburgh, PA). Enzymes other than those in the kits described above were obtained from either Boehringer Mannheim Diagnostics, Inc. or New England Biolabs. Unless otherwise indicated, bacteria, cloning vectors, and plasmids were obtained from either Stratagene, Bethesda Research Laboratories (Gaithersburg, MD), Pharmacia Fine Chemicals (Piscataway, NJ), or Clonetech Laboratories, Inc. (La Jolla, CA).

Results

Localization of the Lamin B Receptor to the Inner Nuclear Membrane

The lamin B receptor (p58) has been previously identified in turkey erythrocytes using urea-extracted nuclear envelopes and ^{125}I-labeled lamin B in both solution binding and solid-phase ligand blotting assays (43). Antibodies raised against p58 yielded a smooth nuclear rim staining on immunofluorescence microscopy of avian erythrocytes identical to that obtained with antibodies against lamin B, consistent with a localization of p58 in the inner nuclear membrane (43). Because avian erythrocytes contain few other intracellular membranes, it could not be ruled out that, in cells with a more complex network of intracellular membranes, p58 might also be located elsewhere than in the inner nuclear membrane. To address this question, we fractionated a chicken liver homogenate by differential centrifugation (Fig. 1) into nuclear (lanes *1*), mitochondrial (lanes *2*), and microsomal (lanes *3*) fractions; resolved their proteins by SDS-PAGE (Fig. 1 *A*); and probed corresponding protein blots with antibodies against p58 (Fig. 1 *B*), lamin B (Fig. 1 *C*), and UDP-glucuronosyltransferase (Fig. 1 *D*), a marker for the ER and outer nuclear membrane (2, 16). The lamin B receptor (p58) fractionated exclusively into the nuclear fraction (Fig. 1 *B*, lane *1*) and not into the mitochondrial or

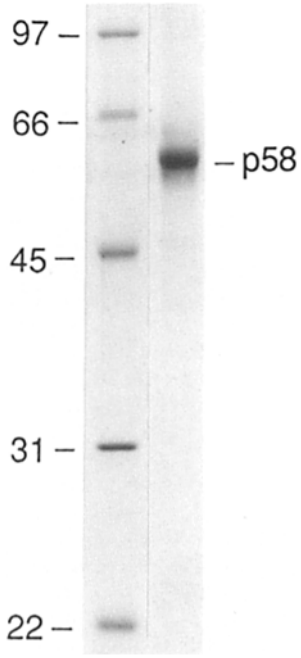

Figure 2. Isolation of lamin B receptor (p58). A Coomassie blue-stained SDS-polyacrylamide slab gel (10% acrylamide) of the lamin B receptor (p58) isolated by electroelution from urea-extracted turkey erythrocyte nuclear envelopes is shown. Molecular mass standards are shown in the left lane.

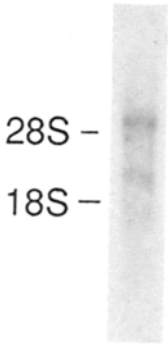

Figure 4. Northern blot of poly(A$^+$)-enriched RNA from chicken liver probed with the ^{32}P-labeled cDNA insert of clone DJ-5. 2 μg of poly(A$^+$) RNA were loaded on the gel. Hybridization was performed at 42°C for 24 h in 6 × SSC, 2 × Denhardt's solution, 50% formamide, 0.1% SDS, 20 mM NaPhosphate (pH 7.4), and 0.1 mg/ml of denatured salmon sperm DNA. Nitrocellulose sheets were then washed three times (10 min each time) at room temperature in 2 × SSC and then at 50°C with 1 × SSC for 30 min each wash. Migration of 28S and 18S rRNA in parallel lane of total RNA is indicated.

microsomal fractions (Fig. 1 *B*, lanes *2* and *3*). Identical fractionation was seen for lamin B (Fig. 1 *C*, lanes *1–3*). These data strongly suggest that p58 is located in the inner rather than the outer nuclear membrane. If p58 were located in the outer nuclear membrane, it would be expected to be present also in the RER and therefore would be found not only in the nuclear fraction but also in the mitochondrial and microsomal fractions. All of these fractions contain the ER/outer nuclear membrane marker enzyme UDP-glucuronosyltransferase (Fig. 1 *D*, lanes *1–3*) and have also been shown previously to contain several other proteins of the ER and outer nuclear membrane (2).

It should be noted that the anti-p58 antibodies are apparently not monospecific. Although they reacted only with p58 when used to probe subcellular fractions of turkey erythrocytes (43), they also cross-reacted with a protein with an apparent molecular mass of 96 kD (p96) when chicken liver fractions were probed (Fig. 1 *B*). However, p96 is not an integral membrane protein since it can be extracted by 8 M urea (Fig. 1 *E*, lane *S*), whereas p58 remains associated with membranes after 8 M urea extraction (Fig. 1 *E*, lane *P*). The nature of this cross-reactive 96 kD protein remains to be investigated.

cDNA Cloning of the Lamin B Receptor

Nuclear envelopes from turkey erythrocytes were extracted with 1 M NaCl and 8 M urea and subjected to SDS-PAGE. The polypeptide band corresponding to p58 was electroeluted from the gels. The electroeluted p58 migrated as a single Coomassie blue-stained band on SDS-polyacrylamide gels (Fig. 2), and was recognized by anti-lamin B receptor antibodies (data not shown).

The amino acid sequences of the amino terminus (Fig. 3 *A*) and also three V8 proteolytic fragments (see Fig. 5) derived from electroeluted p58 were determined by gas-phase microsequencing. A pair of partially degenerate oligonucleotides for use as PCR primers was synthesized based on the amino terminal sequence of p58 (Fig. 3 *B*, *P1* and *P2*). Chicken liver cDNA was subjected to 25 cycles of PCR amplification using primers P1 and P2, and a product of the expected size was obtained. This product encoded the turkey p58 amino acid sequence situated between the two primers determined by protein sequencing, except for the Arg at position 15, which was deduced to be a Trp based on the cDNA coding sequence (Fig. 3 *C*). This may represent an interspecies difference between turkeys (protein sequence) and chickens (cDNA sequence). A 25-mer oligonucleotide (P3) corresponding to a portion of the sense strand of the PCR product was synthesized (Fig. 3 *D*) and used in subsequent chicken library screening and Northern blotting.

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    1                    10                    20                  27
A.  ProAsnArgLysTyrAlaAspGlyGluValValMetGlyArgArgProGlySerValLeuTyrTyrGluValGlnValThr

B.5'CCCAACCGAAAATATGC 3'                                3'ATAATACTCCAGGTTCA 5'
        TA G  G  C     (P1)                       (P2)     G  G  T  C  C
           C                                                          A
           T

C.                5'CGATGGCGAGGTGGTGATGGGTCGTTGGCCAGGAAGCGTCCTG 3'
                  3'GCTACCGCTCCACCACTACCCAGCAACCGGTCCTTCGCAGGAC 5'

D.                5'CGATGGCGAGGTGGTGATGGGTCGT 3'    (P3)
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Figure 3. Strategy for the synthesis of a specific PCR amplification probe for the lamin B receptor (p58). (*A*) Amino terminal amino acid sequence of the lamin B receptor determined by gas-phase microsequencing either of electroeluted protein (see Fig. 2) or of p58 transferred to polyvinylidene difluoride membrane from SDS-PAGE preparations of urea-extracted turkey erythrocyte nuclear envelopes. The underlined Arg (position 15) is the only amino acid residue that is different than those deduced from the cDNA sequence (see *C*, below) that encodes for a Trp at this position. (*B*) The amino acid sequence in *A* was used to synthesize partially degenerate sense (*P1*) and antisense (*P2*) primers for PCR. (*C*) Nucleotide sequence of the PCR amplification product using primers P1 and P2 and cDNA reverse transcribed from chicken liver poly(A$^+$)-enriched RNA as template. (*D*) Sense strand oligonucleotide (*P3*) used to screen cDNA library. These sequence data are available from EMBL/GenBank/DDBJ under accession number Y0082.

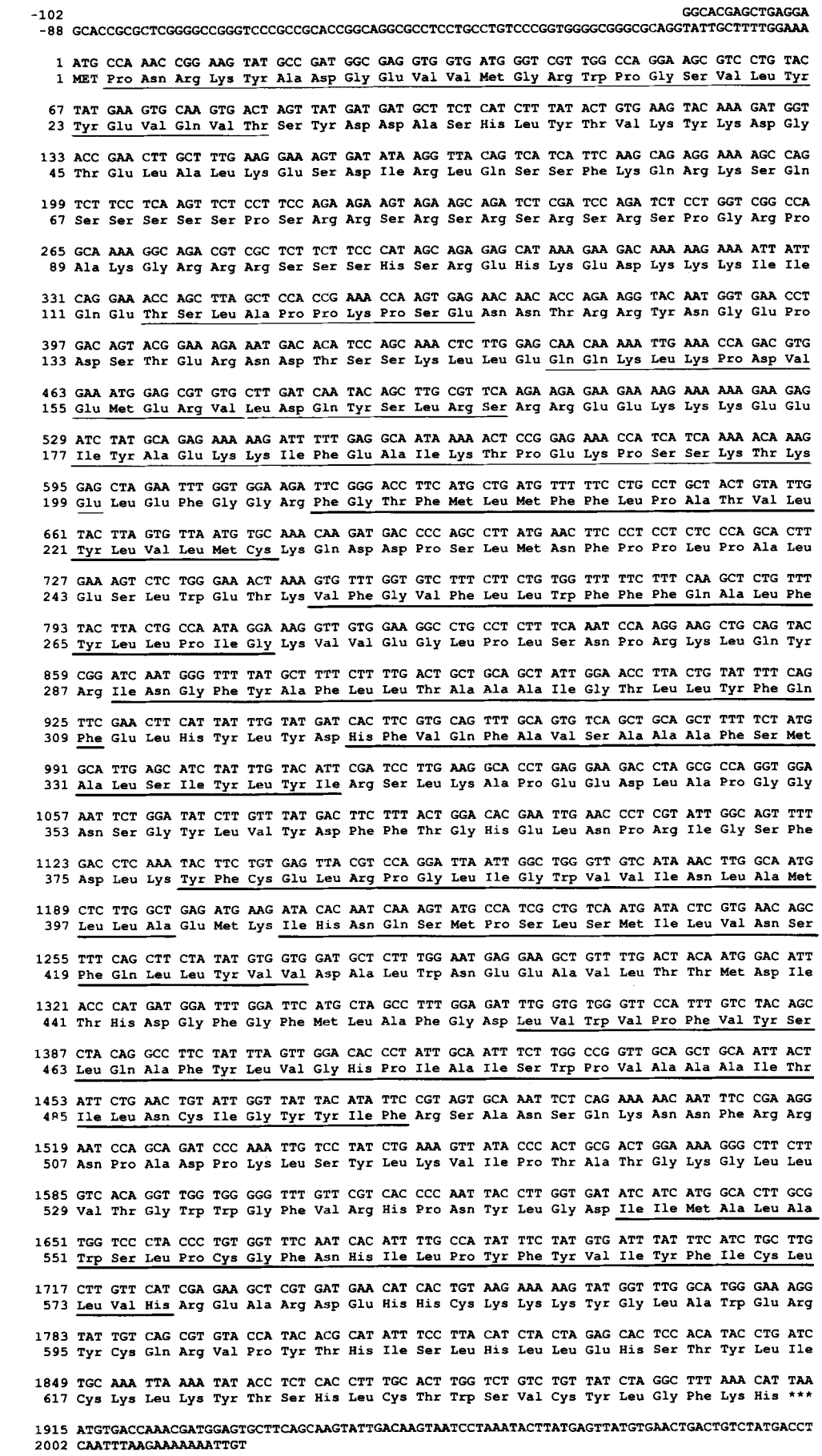

Figure 5. Nucleotide sequence of the lamin B receptor cDNA and the deduced amino acid sequence. The amino-terminal methionine residue is indicated as $^{+}1$ of the amino acid sequence and A of the initiator ATG codon as $^{+}1$ of the cDNA nucleotide sequence. Regions of the deduced amino acid sequence corresponding to the amino-terminal amino acid sequence and V8 peptide sequences determined in protein sequencing experiments are underlined with thin lines. Hydrophobic amino acid stretches that can form potential membrane spanning domains are underlined with thicker lines. The nucleotide sequence was determined by sequencing four overlapping cDNA clones. Clone DJ-5 was obtained by screening a chick embryonal cDNA library with ^{32}P-labeled oligonucleotide P3 (shown in Fig. 3). The cDNA insert of clone DJ-5 contained nucleotides $^{-}102$ through $^{+}1760$ of the sequence shown. The cDNA insert of clone DJ-5 was ^{32}P-labeled by random priming and used to screen a chick embryonal erythrocyte cDNA library in lambda gt11, and three shorter overlapping clones (M-3, M-3′, and M-7) were characterized. The cDNA inserts of M-3 and M-3′ contained nucleotides $^{+}1148$ to $^{+}1987$ and that of M-7 contained nucleotides $^{+}1655$ to $^{+}2021$. Double-stranded DNA sequencing of both stands of the insert of clone DJ-5 was performed by the dideoxy chain termination method using the Sequenase kit (United States Biochemical Corp.) with both dITP and dGTP. The cDNA inserts of lambda gt11 clones M-3, M-3′ (opposite strand of clone M-3), and M-7 were subcloned into the Eco RI site of M13-mp18 and single-stranded sequencing was performed also using the Sequenase kit. Synthetic oligonucleotide primers were used for sequencing. These sequence data are available from EMBL/GenBank/DDBJ under accession number Y0082.

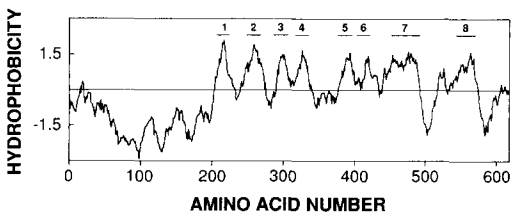

Figure 6. Kyte-Doolittle hydropathy plot for the lamin B receptor. Hydrophobic stretches at least 19 amino acids residues in length with maximal hydrophobic moments of 1.5 or greater are overlined and labeled 1–8. Analysis was performed using the DNA STAR program on an IBM PC-AT computer. The hydrophobic window was set at 19.

Three cross-hybridizing clones were isolated from 400,000 plaques of a lambda Zap chick embryonal cDNA library by screening with ^{32}P-labeled oligonucleotide P3. The largest of these clones (DJ-5) contained a cDNA insert of 1,862 bp with an open reading frame 1,760-bp long that coded for the lamin B receptor amino terminal sequence and all three V8 peptide fragments. The insert of clone DJ-5 did not contain an in-frame stop codon and was therefore used to screen a chick embryonal erythrocyte cDNA library. Three cross-hybridizing clones were characterized that overlapped DJ-5 and each contained an in-frame stop codon as well as 3′ untranslated sequences.

Northern blotting of poly(A$^+$)-enriched RNA showed two cross-reactive species of 2.3 and 4.4 kb when probed with the ^{32}P-labeled cDNA insert of clone DJ-5 (Fig. 4) or oligonucleotide P3 (data not shown). The 2.3 kb RNA is of adequate size to be the mRNA coding for the mature lamin B receptor protein. The 4.4-kb species may represent a differentially polyadenylated mRNA coding for the same protein, or may code for a related protein, perhaps the 96-kD protein that cross-reacts with anti-p58 antibodies (see Fig. 1, *B* and *E*).

Primary Structure of the Lamin B Receptor

A total cDNA sequence of 2,133 bp was obtained from clone DJ-5 and the overlapping clones (Fig. 5). The total sequence contained an open reading frame coding for 637 amino acids containing the amino terminal sequence (starting one amino acid downstream from the deduced initiator methionine) and the sequences of the three V8 protease fragments (Fig. 5, *thin underscoring*). No potential initiation codon is found upstream of the codon for Met (+1) and an in-frame stop codon is located 92 bases upstream from the codon for Met ($^+$1). Thus, the lamin B receptor is synthesized without a cleavable amino terminal signal sequence.

The calculated molecular mass of the lamin B receptor is 73,375 D, which is larger than the apparent molecular mass of 58,000 D estimated from SDS-polyacrylamide gels. This discrepancy is probably due to the known aberrant migration of membrane proteins subjected to SDS-PAGE. However, it cannot be ruled out that the primary translation product is processed in vivo or is degraded during cell fractionation. Both processes would have to be limited to the carboxy terminal region.

Hydropathy analysis (26) of the deduced amino acid sequence shows that the lamin B receptor contains eight hydrophobic segments with membrane spanning potential (Fig. 6). The domains labeled *1–6* in Fig. 6 are typical for transmembrane domains in that they are 19–23 amino acids long, have maximal hydropathic indices of 1.5 or greater and are flanked by charged amino acids. The domains labeled *7* and *8* are longer hydrophobic stretches than those typical for membrane spanning domains, and may be perimembranous hydrophobic pockets or could also potentially span the membrane more than once. The finding of multiple potential membrane spanning domains is consistent with the finding that the lamin B receptor is only extractable from membranes with detergents and resistant to extraction with 8 M urea or alkali (Fig. 1 *E*; also reference 43). The lamin B receptor of turkey erythrocytes does not bind concanavalin A (Worman, H. J., unpublished observation) and therefore does not likely contain Asn-linked oligosaccharides, even though the sequence contains three potential consensus sites for Asn-linked glycosylation at Asn ($^+$123), ($^+$139), and ($^+$405).

Another structural feature of the lamin B receptor sequence is a 204-residue-long amino terminal region from Pro ($^+$2) to Arg ($^+$205) that precedes the first potential transmembrane segment (Figs. 5 and 6). This region contains 79 charged residues and has a calculated pI of 9.89. It also contains two consensus sites (Arg-Arg-X-Ser) for phosphorylation by protein kinase A (13) at Ser ($^+$95) and Ser ($^+$96). Previous studies have shown that the turkey erythrocyte lamin B receptor is phosphorylated by protein kinase A both in vivo and in vitro (3). This amino terminal domain also contains the motif of Ser/Thr-Pro-X-X (at Ser [$^+$71], Ser [$^+$84], and Thr [$^+$190]) that has been proposed to be a DNA-binding unit common to several gene regulatory proteins and histones (39). Two of these Ser/Thr-Pro-X-X sequences (at Ser [$^+$71] and Thr [$^+$190]) also conform to the proposed consensus site for phosphorylation by p34^{cdc2} protein kinase (34); however, it remains to be shown that the lamin B receptor is a substrate for this enzyme. No significant overall sequence identity was seen between the lamin B receptor and other proteins listed in the Dayhoff and translated Genebank protein sequence data bases.

Discussion

We report here the cDNA sequence and deduced primary structure of the chicken lamin B receptor (calculated molecular mass of 73,375 D) and provide further evidence that this protein is located in the inner nuclear membrane. The deduced molecular mass is larger than that previously estimated by electrophoresis (58 kD). In future experiments, a full-length cDNA can be constructed from the overlapping clones isolated in this study and used in cell-free translation assays to determine if this discrepancy is a result of post-translational processing or aberrant migration on gels. Hydropathy analysis suggests that the lamin B receptor is likely to have several (up to eight) transmembrane segments. The polytopic (7) nature of the lamin B receptor was unexpected as a single transmembrane segment with a single lamin B binding domain exposed to the nucleoplasm would be sufficient to anchor lamin B to the inner nuclear membrane. It therefore remains possible that the lamin B receptor serves an additional function or functions. An example of a multifunctional polytopic integral membrane protein is band 3 of the erythrocyte plasma membrane (24). This protein functions as a Cl^-/HCO_3^- exchanger (8) and also has a cytoplasmically exposed segment that serves as a binding site

for the protein ankyrin (5). Whether the lamin B receptor functions in ion or solute transport remains to be determined. Besides functioning as membrane transport proteins, polytopic integral membrane proteins have also been shown to function in signal transduction mediated by GTP-binding proteins (11) and as the enzyme catalyzing the rate-limiting reaction of cholesterol biosynthesis (21, 29).

Besides its polytopic nature, the lamin B receptor contains a 204–amino acid residue long amino terminal region that precedes the first potential transmembrane segment. This region is particularly charged and contains two sequence motifs with potentially important implications and functions. The first motif is the consensus site for phosphorylation by the cAMP-dependent protein kinase A (Arg-Arg-X-Ser) (13), and two such sites overlap each other in this domain of the molecule. As the lamin B receptor has previously been shown to be phosphorylated by protein kinase A in vitro and in vivo (3), it is likely that this entire amino terminal domain is exposed on the nucleoplasmic side (rather than the perinuclear cisternal side) of the inner nuclear membrane where it would be accessible to protein kinase A. Furthermore, the protein kinase A catalyzed phosphorylation of the lamin B receptor has been shown to affect lamin B binding (3), also making it more likely that these potential phosphorylation sites are located on the nucleoplasmic side of the inner membrane in the vicinity of lamin B. If the lamin B receptor has an even number of transmembrane domains (such as the eight predicted), the carboxy terminal segment of the protein would also be located on the nucleoplasmic side of the inner nuclear membrane.

The second functional motif found in the 204–amino acid amino terminal domain of the lamin B receptor is the sequence Ser/Thr-Pro-X-X, which occurs three times. This motif has been proposed to be a DNA-binding unit and occurs in histones and more frequently in gene regulatory proteins that bind DNA (39). When the first X is polar and the second X basic, as occurs twice in the lamin B receptor, this motif may also serve as a consensus site for phosphorylation by $p34^{cdc2}$ protein kinase, which is involved in the initiation of mitosis (34). Thus, because of these potential properties as well as its ability to bind to the lamina, the lamin B receptor may function in both the regulation of gene expression and cell division.

We are indebted to Dr. D. Atherton of The Rockefeller University/Howard Hughes Medical Institute Biopolymer Facility (New York) for protein sequencing and oligonucleotide synthesis. We also thank C. Bennett, J. Newman, and C. Rosen for technical assistance; Dr. N. Chaudhary (Rockefeller University) for considerable help and advice; and Drs. D. Johnson, R. Moon, and A. Dannenberg for supplying essential reagents. The help of Drs. J. Aris, J.-C. Courvalin, S. Georgatos, R. Henriquez, M. Schindler, D. Schnell, G. Shelness, J. Sukagawa, and R. Wozniak (Rockefeller University) is also greatly appreciated.

Dr. H. J. Worman is a recipient of a Physician-Scientist Award (grant No. DK01790) from the National Institutes of Health.

Received for publication 30 April 1990 and in revised form 15 June 1990.

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