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cDNA cloning of the bovine low density lipoprotein receptor: Feedback regulation of a receptor mRNA

(peptide sequence analysis/polysome immunopurification/oligonucleotide hybridization/adrenal cortex/cholesterol)

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ABSTRACT **The low density lipoprotein (LDL) receptor belongs to a class of migrant cell surface proteins that mediate endocytosis of macromolecular ligands. No cDNAs for this class of proteins have been isolated to date. In the current paper, we report the isolation of a cDNA clone for the LDL receptor from a bovine adrenal cDNA library. The library was constructed by the Okayama–Berg method from poly(A)$^+$ RNA that had been enriched in receptor mRNA by immunopurification of polysomes. Mixtures of synthetic oligonucleotides encoding the amino acid sequence of two neighboring regions of a single cyanogen bromide fragment were used as hybridization probes to identify a recombinant plasmid containing the LDL receptor cDNA. This plasmid, designated pLDLR-1, contains a 2.8-kilobase (kb) insert that includes a sequence which corresponds to the known amino acid sequence of a 36-residue cyanogen bromide fragment of the receptor. pLDLR-1 hybridized to a mRNA of ≈5.5 kb in the bovine adrenal gland. This mRNA, like the receptor protein, was 9-fold more abundant in bovine adrenal than in bovine liver. pLDLR-1 cross-hybridized to a mRNA of ≈5.5 kb in cultured human epidermoid carcinoma A-431 cells. This mRNA was markedly reduced in amount when sterols were added to the culture medium, an observation that explains the previously observed feedback regulation of LDL receptor protein. Southern blot analysis of bovine genomic DNA with ^{32}P-labeled pLDLR-1 revealed a simple pattern of hybridization, consistent with a single-copy gene containing introns.**

The low density lipoprotein (LDL) receptor is a cell surface protein that plays a central role in the metabolism of cholesterol in humans and animals (1). Current interest in this protein arises from several distinguishing characteristics. First, since its discovery 10 years ago, it has proven to be a model system for the study of receptor-mediated endocytosis, a process by which macromolecules enter cells after binding to specific receptors in coated pits on the cell surface (2). Second, the cholesterol liberated by catabolism of the internalized LDL particle regulates, via a feedback mechanism, the rate of synthesis of both the LDL receptor and the rate-controlling enzyme of cholesterol biosynthesis, 3-hydroxy-3-methylglutaryl-CoA reductase (1). Third, the increased demand for cholesterol in certain steroidogenic tissues such as the adrenal cortex and the ovarian corpus luteum is met by an increased number of LDL receptors (3). A fourth, and foremost distinguishing feature of the LDL receptor, is that mutations affecting its structure and function give rise to one of the most prevalent human genetic diseases, familial hypercholesterolemia (1).

The LDL receptor is a trace protein of cultured cells and animal tissues, constituting less than 0.01% of the total membrane protein (4, 5). Recent advances have rendered feasible an approach to the molecular genetics of the receptor. Thus, the LDL receptor has been purified to homogeneity and shown to be an acidic glycoprotein with an apparent M_r of 160,000 (4). A partial amino acid sequence has been obtained (6). Polyclonal (7, 8) and monoclonal (9) antibodies have been raised against the purified receptor and used to follow the biosynthesis and posttranslational processing of the protein in normal and mutant cells (5, 8). In the current experiments, we have used these tools, together with recombinant DNA techniques, to isolate a cDNA for the bovine LDL receptor.

METHODS

Materials. Bovine tissue was frozen in liquid nitrogen within 5 min of slaughter. Adrenal glands were powdered in liquid nitrogen in a Waring blender and stored at −70°C prior to polysome isolation. A polyclonal antibody against the bovine adrenal LDL receptor was raised in rabbits and purified on staphylococcal protein A-Sepharose (8). This antibody and its corresponding nonimmune rabbit IgG were free of gross RNase contamination as shown by their failure to alter the sedimentation behavior of polysomes on sucrose gradients. Human epidermoid carcinoma A-431 cells were grown in the absence or presence of sterols (25-hydroxycholesterol plus cholesterol) as described (10). Oligonucleotides were synthesized by the phosphoramidite method (11) and generously provided by Mark Zoller and Tom Atkinson (University of British Columbia, Vancouver, BC). The plasmids used for cDNA cloning (12) were kindly provided by H. Okayama and P. Berg (Stanford University, Stanford, CA). A chicken actin cDNA plasmid [cDNA insert of 1.8 kilobases (kb) cloned in pBR322] was kindly provided by Raymond J. MacDonald of our institution. Experiments involving recombinant DNA were performed in accordance with the National Institutes of Health guidelines for recombinant DNA research.

Protein Sequence of LDL Receptor. Homogeneous LDL receptor was isolated from bovine adrenal cortex (4). CNBr fragments were generated from two different preparations of reduced and [^{3}H]carboxymethylated receptor (1.6 and 1.8 mg of protein) and fractionated by reverse-phase HPLC on a Brownlee (Santa Clara, CA) RP 300 column (6). The CNBr peptide described here was subjected to two separate runs on an automated Beckman 890C sequencer using a 0.25 M Quadrol program and the nonprotein carrier Polybrene (6). Yields of the NH_2-terminal residue of the CNBr peptide were 400 and 1,100

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Abbreviations: bp, base pair(s); kb, kilobase(s); LDL, low density lipoprotein.

pmol for the two runs. Repetitive yields, calculated on the basis of recovery of the phenylthiohydantoin of [^{3}H]cysteine, averaged 91%.

Polysome Immunopurification of mRNA. Polysomes enriched in mRNA for the LDL receptor were prepared by modifications of several recently described procedures (13–15). Ten-gram aliquots of powdered adrenals were homogenized with a Brinkmann Polytron in 42 ml of 25 mM Tris·HCl, pH 7.5/25 mM NaCl/5 mM $MgCl_2$/2% (vol/vol) Triton X-100/0.3 mg of heparin per ml/1 μg of trichodermin per ml/60 μg of phenylmethylsulfonyl fluoride per ml. Polysomes were isolated from the homogenate by $MgCl_2$ precipitation (16) and stored at −70°C. Twenty-five A_{260} units of polysomes were obtained per gram of adrenal powder. On linear sucrose gradients approximately 70% of the A_{260} material sedimented as polysomes; the remaining absorbance was present in 80S monosomes. Polysomes (1,000 A_{260} units) were clarified with a 10-min centrifugation at 20,000 × *g*, then diluted to 15 A_{260}/ml in a buffer containing 25 mM Tris·HCl at pH 7.5, 150 mM NaCl, 5 mM $MgCl_2$, 0.1% Nonidet P-40, heparin at 0.2 mg/ml, and trichodermin at 1 μg/ml and incubated with 6.25 mg of anti-receptor IgG or nonimmune IgG for 1 hr with stirring at 4°C. The polysome/antibody slurry was then passed twice through a column of protein A-Sepharose (0.7 × 13 cm) equilibrated in the above dilution buffer at a flow rate of 8–10 ml/hr at 4°C. The column was washed overnight with 120 ml of dilution buffer. Bound polysomes were eluted at a maximal flow rate with 20 ml of 25 mM Tris·HCl, pH 7.5/20 mM EDTA. The eluted fraction was heated 5 min at 65°C, brought to 0.5 M NaCl and 0.2% $NaDodSO_4$, cooled to 24°C, and passed through a column of oligo(dT)-cellulose (0.8 × 2.3 cm) equilibrated in 10 mM Tris·HCl, pH 7.5/0.5 M NaCl. The column was washed with 20 ml of this buffer and poly(A)$^+$ RNA was eluted with 5 ml of 10 mM Tris·HCl, pH 7.5. Yeast carrier tRNA (50 μg) was added, and the RNA was precipitated twice with NaOAc and ethanol. This immunopurified poly(A)$^+$ RNA was resuspended in 20 μl of water and stored at −70°C.

***In Vitro* Translation.** Aliquots of poly(A)$^+$ mRNA were incubated with 2.5 mM CH_3HgOH for 10 min at 4°C and then translated in rabbit reticulocyte lysates prepared as described by Pelham and Jackson (17) and supplemented with 80 mM KOAc, 1 mM $Mg(OAc)_2$, 19 amino acids (excluding methionine) at 16 μM each, and [^{35}S]methionine at 0.2 mCi/ml (1 Ci = 3.7 × 10^{10} Bq). The final concentration of CH_3HgOH in the translation reaction was 0.3 mM. Translation products were analyzed by electrophoresis on $NaDodSO_4$/7% polyacrylamide gels.

cDNA Cloning. A cDNA library was constructed by the method of Okayama and Berg (12) from immunoselected poly(A)$^+$ RNA derived from 2,000 A_{260} units of polysomes. In the cloning reactions, which employed enzymes obtained from Life Sciences and P-L Biochemicals, 1.4 μg of dT-tailed vector primer and 0.52 pmol of dG-tailed linker were used.

Identification of LDL Receptor cDNA Clones. Portions of the cDNA library were used to transform *Escherichia coli* RR1 to ampicillin resistance by the $CaCl_2$-shock procedure (ref. 18, p. 250). Colonies were plated at high density on nitrocellulose filters, and two replica filters were prepared for hybridization (ref. 18, p. 316). To reduce nonspecific background, baked filters were washed overnight in 50 mM Tris·HCl, pH 8/1 mM EDTA/1 M NaCl/0.1% $NaDodSO_4$ at 37 or 42°C and then incubated at 65°C for 3 hr in 4× NaCl/Cit (1× NaCl/Cit = 0.15 M NaCl/15 mM sodium citrate), 10× Denhardt's solution (ref. 18, p. 327), and sonicated and denatured *E. coli* DNA at 100 μg/ml. Hybridization was performed overnight in the latter solution containing ^{32}P-5′-end-labeled oligonucleotide mixtures (6 × 10^6 cpm/pmol) at 1 pmol/ml. Hybridization temperature for a given oligonucleotide probe corresponded to the minimum melting temperature, t_m, calculated from the empirical formula t_m = 2°C(no. of dA·dT bp) + 4°C(no. of dG·dC bp), in which bp is base pairs (19). Filters were washed three times in 4× NaCl/Cit at the hybridization temperature for 30 min per wash, dried at room temperature, and subjected to autoradiography. Positive clones were picked from the master plate and purified through several rounds of screening. Bacteriophage M13 cloning and sequencing procedures were as described (20–22).

Blot Hybridization of Poly(A)$^+$ RNA. Total RNA was isolated from the indicated source by treatment of tissues or cells with guanidinium thiocyanate (ref. 18, p. 196). Poly(A)$^+$ RNA was purified by oligo(dT)-cellulose chromatography, denatured with glyoxal, size-fractionated by electrophoresis (20 V, 16 hr) on 1.5% agarose gels containing 40 mM 3-*N*-morpholinopropanesulfonic acid (pH 7.0), and then transferred to Zeta Probe membranes (Bio-Rad) by capillary blotting in 20× NaCl/Cit. Prehybridization and hybridization were carried out as described (ref. 18, p. 326).

Blot Hybridization of DNA. DNA was isolated from bovine liver (ref. 18, p. 281), digested to completion with restriction enzymes, size-fractionated by agarose gel electrophoresis, and analyzed by the method of Southern (23).

RESULTS

The strategy employed in cloning the LDL receptor cDNA is outlined in Fig. 1. The individual steps in the isolation and characterization of the cDNA were as follows:

Preparation of Two Oligonucleotide Families Corresponding to Amino Acid Sequence of LDL Receptor. The purified LDL receptor was digested with CNBr, an internal CNBr fragment was isolated by HPLC, and its partial amino acid sequence was determined by automated Edman degradation. Two families of synthetic oligonucleotide probes that corresponded to all possible codons specifying the sequence of amino acids in two neighboring regions of this CNBr fragment were synthesized. One family of oligonucleotides, designated A in Fig. 1, consisted of 32 tetradecamers encoding (Met)-Ala-Glu-Asn-Leu. The existence of a methionine residue at the amino ter-

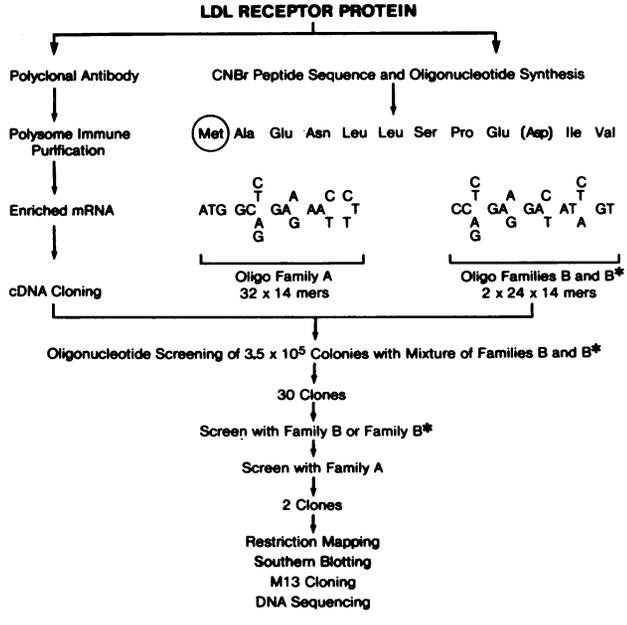

FIG. 1. Strategy for cloning a cDNA for the bovine LDL receptor. Individual steps are described in the text.

minus of this sequence was inferred from the fact that the peptide was generated by CNBr digestion. A second family of tetradecamers, designated B and B* in Fig. 1, encoded the sequence Pro-Glu-(Asp)-Ile-Val. The assignment of the Asp residue in this sequence was provisional because it was observed in only one of two sequenator runs. The B/B* oligonucleotide family consisted of a total of 48 members that were synthesized as two subfamilies of 24 each, differing only in the codons used to specify the Pro residue (CC^C_T in B and CC^A_G in B*).

Enrichment of LDL Receptor mRNA by Polysome Immunopurification. Polysomes were obtained from bovine adrenal glands by $MgCl_2$ precipitation and incubated with an anti-receptor IgG. The resulting polysome/IgG complexes were fractionated by adsorption to protein A-Sepharose and elution with EDTA. Poly(A)$^+$ RNA was isolated from the eluate by oligo(dT)-cellulose chromatography.

The immunoselected poly(A)$^+$ RNA was assayed for the presence of LDL receptor mRNA by *in vitro* translation in a reticulocyte lysate system (Fig. 2). Total adrenal gland poly(A)$^+$ RNA directed the synthesis of many proteins, as determined by $NaDodSO_4$ gel electrophoresis and fluorography of the synthesized products (lane 2). Poly(A)$^+$ RNA derived from the immunopurified polysomes directed synthesis of several of the same protein bands plus one clear addition: a protein that migrated with a M_r of ≈120,000 (arrow, lane 3). This protein was not demonstrable after translation of poly(A)$^+$ RNA selected from polysomes with nonimmune IgG (lane 4). Biosynthetic studies on the LDL receptor from humans (5), hamsters (24), and rabbits (25) have shown that the receptor is initially made as a 120,000 M_r precursor that undergoes a series of posttranslational glycosylation events during transport to the cell surface, resulting in a mature protein with an apparent M_r of

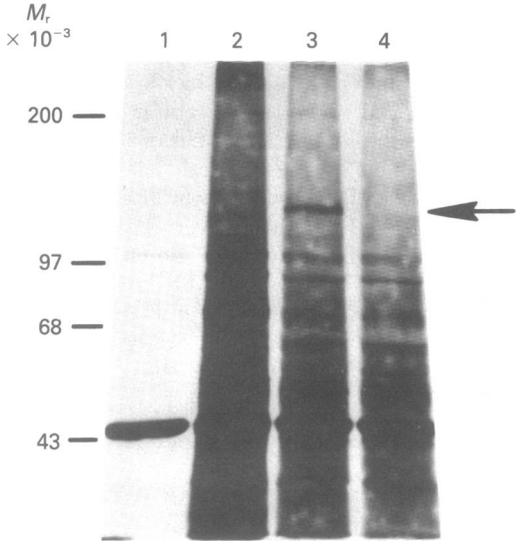

FIG. 2. *In vitro* translation of poly(A)$^+$ RNA selected with immune or nonimmune IgG. Polysomes from bovine adrenal gland were incubated with either anti-receptor IgG or nonimmune IgG as indicated below. After chromatography on protein A-Sepharose, the eluted polysomal RNA was fractionated by chromatography on oligo(dT)-cellulose. Aliquots of poly(A)$^+$ RNA were translated in a reticulocyte lysate in the presence of [^{35}S]methionine and subjected to electrophoresis on $NaDodSO_4$/7% polyacrylamide gels followed by fluorography. Additions to the lysates were lane 1, 2 μl of H_2O; lane 2, 0.1 μg of total poly(A)$^+$ RNA not subjected to immunoadsorption; lane 3, 2 μl of poly(A)$^+$ RNA adsorbed to anti-receptor IgG; lane 4, 2 μl of poly(A)$^+$ RNA adsorbed to nonimmune IgG. Gels were exposed to film for 23 hr at −70°C. The arrow marks the putative LDL receptor precursor described in the text.

160,000. Thus, the size of the enriched protein seen after translation of the immunoselected poly(A)$^+$ RNA was consistent with that of the LDL receptor precursor. However, despite numerous attempts with different anti-receptor antibodies, it was not possible to immunoprecipitate the putative precursor of the receptor translated *in vitro*.

Synthesis, Cloning, and Identification of LDL Receptor cDNA. The immunoselected poly(A)$^+$ RNA was used to construct a cDNA library by the method of Okayama and Berg (12). Transformation of competent *E. coli* RR1 cells resulted in a cDNA library containing more than 5×10^5 recombinants. The cDNA library was screened initially with a mixture of the oligonucleotide probes B and B* (Fig. 1), and 30 cDNA clones were identified. When these clones were probed separately with the subfamilies B or B*, 16 clones hybridized strongly with oligonucleotide mixture B, but not with B*. Twelve of the 30 clones were positive only with mixture B*. These 28 positive clones were then screened with oligonucleotide mixture A, and two plasmids, both from the latter group of 12, hybridized with this probe. These two clones were considered to contain cDNAs for the receptor and were chosen for further study.

Plasmid DNAs from the two clones that hybridized to both the B* and A oligonucleotide probes were subjected to restriction endonuclease mapping, and the results indicated that these two clones were identical. A partial restriction map for the 2.8-kb cDNA insert in these plasmids, designated pLDLR-1, is shown in Fig. 3*A*. Southern blotting experiments in which oligonucleotide mixtures A and B* were used as probes indicated that both of these sequences were present in a 432-bp *Pst* I fragment located at the 5′ end of the cDNA insert (Fig. 3*A*). This fragment was isolated from pLDLR-1 and cloned in both orientations in the bacteriophage M13 mp8 vector (26).

Before proceeding further with pLDLR-1, we sought to confirm the homology with the oligonucleotide probes by determining the sequence of the relevant portion of the 432-bp *Pst* I fragment. However, obtaining a nucleotide sequence of this fragment in the region of oligonucleotide homology turned out to be difficult and required a novel approach. Preliminary sequence analysis of the M13 clones by using a universal primer suggested that the homologous sequences were located in the center of this fragment in regions of the sequencing gel that were difficult to read unambiguously, possibly due to the high G+C content of this DNA fragment. In an attempt to circumvent this problem, we used oligonucleotide mixture B* as a primer for dideoxynucleotide sequencing, using labeled nucleotides and the appropriate M13 clone. The autoradiograms generated in these experiments were unreadable, indicating that priming was occurring in more than one location on the M13 clone. In a final, successful, effort to obtain the sequence, we 5′-end-labeled the oligonucleotide B* mixture with [γ-^{32}P]-ATP and bacteriophage T4 polynucleotide kinase and extended this radiolabeled primer on the appropriate M13 clone by brief incubation with the Klenow fragment of *E. coli* DNA polymerase I in the presence of unlabeled nucleotides. The resulting partially double-stranded M13 DNA was cleaved with *Pst* I and denatured, and the mixture was run on a 7 M urea/polyacrylamide gel. A radiolabeled fragment ≈240 bp long was isolated and subjected to chemical sequence analysis (22). The results revealed the nucleotide sequence upstream of the oligonucleotide B* homology; this sequence included a region of DNA precisely complementary to one of the components of the A oligonucleotide mixture.

Fig. 3 shows the sequence of a 108-bp portion of the cDNA in the region of complementarity to the A and B* oligonucleotides. This DNA sequence was derived from a combination of the strategies described above. It encodes, in one of the three

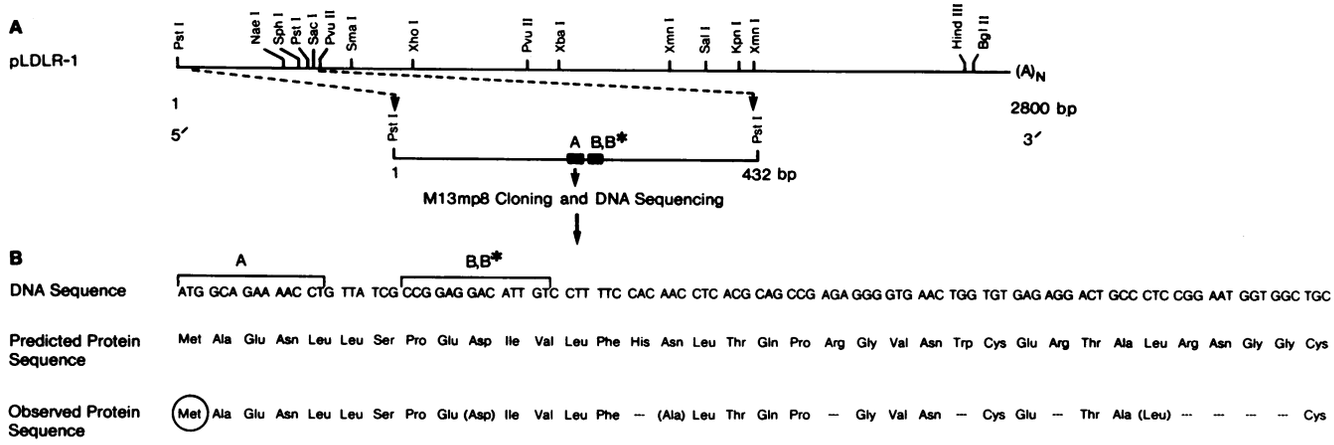

FIG. 3. (*A*) Restriction endonuclease map of cDNA insert in pLDLR-1. The numbers in bp indicate the size of the insert and of the *Pst* I DNA fragment containing regions of homology with oligonucleotide probes A and B/B*. (*B*) Comparison of the nucleotide sequence of a region of the pLDLR-1 cDNA and the amino acid sequence of a CNBr peptide of the bovine LDL receptor. The 108-bp nucleotide DNA sequence corresponds to the central portion of the 432-bp *Pst* I fragment. Overlines indicate the regions corresponding to the A and B/B* oligonucleotide probes. In the observed protein sequence, the amino acids in parentheses indicate tentative assignments based on data obtained in only one of the two sequenator runs. Dashes in the observed protein sequence correspond to amino acid residues whose identity was not determined in either of the sequenator runs. The circled methionine was inferred on the basis of the preference of CNBr to cleave after this residue.

possible reading frames, a protein sequence of 36 amino acids that agrees with one exception with that determined from the CNBr peptide (see *Discussion*). The correspondence of nucleotide and amino acid sequence was interpreted as strong evidence that pLDLR-1 contained a cDNA for the bovine LDL receptor.

RNA Analysis by Blot Hybridization with pLDLR-1. To confirm the identity of pLDLR-1, total poly(A)$^+$ RNA was extracted from bovine adrenal glands and liver and analyzed in blotting experiments using nick-translated ^{32}P-labeled plasmid as a probe (Fig. 4). Increasing amounts of adrenal gland RNA (lanes 1 and 2) yielded a progressively stronger hybridization signal corresponding to a mRNA of ≈5.5 kb. Densitometric scanning showed that the signal obtained with a given amount of adrenal RNA was 9-fold more intense than that obtained with the same amount of liver RNA (lanes 3 and 4). Previous studies have shown that functional LDL receptor activity is about one order of magnitude more abundant in bovine adrenal than in bovine liver (3), a finding that coincides with the difference in the abundance of the mRNAs detected in Fig. 4.

The number of LDL receptors can be markedly reduced when cultured cells are grown in the presence of cholesterol or related sterols (1, 10). Poly(A)$^+$ RNA was isolated from human A-431 cells grown in the absence of sterols (receptor-induced) and presence of sterols (receptor-suppressed) and analyzed by blotting with pLDLR-1 (Fig. 4). A strong hybridization signal from a mRNA of ≈5.5 kb was detected in the induced RNA (lane 5), and this signal was reduced by more than 90% in the suppressed RNA (lane 6). When this same blot was reprobed with a ^{32}P-labeled plasmid containing a chicken actin cDNA insert of 1.8 kb, a signal corresponding to actin mRNA was detected in all lanes. Specifically, the intensities of the signal for actin mRNA in lanes 5 and 6 were identical (data not shown).

Analysis of Bovine DNA by Blot Hybridization with pLDLR-1. DNA was isolated from bovine liver, digested with restriction endonucleases, and analyzed by the method of Southern (23). Digestion of the DNA with *Eco*RI, *Xba* I, *Pst* I, *Bam*HI, and *Hin*dIII (lanes 1–5, respectively, in Fig. 5) and hybridiza-

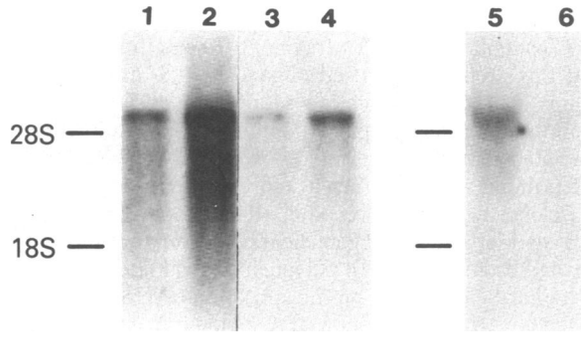

FIG. 4. Hybridization of ^{32}P-labeled pLDLR-1 to poly(A)$^+$ RNA from bovine tissues and human cells. Poly(A)$^+$ RNA was subjected to electrophoresis in agarose, transferred to Zeta Probe membranes, and hybridized at 42°C for 16 hr with ^{32}P-labeled pLDLR-1 (10^8 cpm/μg) at 10^6 cpm/ml in 50% (vol/vol) formamide/5× Denhardt's solution/5× NaCl/Cit/0.1% NaDodSO$_4$/100 μg of denatured salmon sperm DNA per ml/1 μg of poly(A) per ml. Amounts and sources of poly(A)$^+$ RNA were as follows: Lanes 1 and 2, 2 and 5 μg from bovine adrenal; lanes 3 and 4, 5 and 10 μg from bovine liver; lane 5, 20 μg from human A-431 cells induced for LDL receptor expression; and lane 6, 20 μg from human A-431 cells suppressed for LDL receptor expression. Gels were exposed to film for 24 hr (lanes 1–4) or 48 hr (lanes 5 and 6) at −70°C. The positions to which bovine 18S and 28S ribosomal RNAs migrated are indicated.

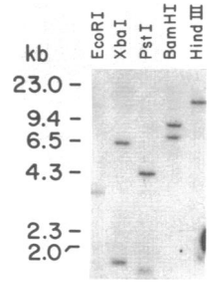

FIG. 5. Hybridization of ^{32}P-labeled pLDLR-1 to bovine genomic DNA. Ten-microgram samples of DNA from bovine liver were digested with the indicated restriction endonuclease, fractionated on 1% agarose gels, transferred to nitrocellulose, and hybridized at 42°C for 16 hr with the 432-bp *Pst* I fragment of pLDLR-1 at 3×10^6 cpm/ml (10^7 cpm/μg) as described in the legend to Fig. 4. The gel was exposed to film for 48 hr at −70°C. Molecular size standards were generated by *Hin*dIII cleavage of bacteriophage λ DNA.

tion with ^{32}P-labeled pLDLR-1 yielded results consistent with the presence of a single-copy gene containing introns.

DISCUSSION

Using a combination of polysome immunopurification and oligonucleotide hybridization (Fig. 1), we have isolated a cDNA clone (pLDLR-1) for the bovine LDL receptor. Several lines of evidence support this conclusion. The nucleotide sequence of a region of the cDNA insert in pLDLR-1 encodes, with the exception of one amino acid, a protein sequence identical to that obtained from a CNBr fragment of the LDL receptor (Fig. 3). This 108-bp nucleotide sequence encodes 26 amino acid residues that were not represented in the two families of oligonucleotides used as hybridization probes to select the cDNA. In one position of the amino acid sequence, an Ala residue was observed on a single sequenator run, whereas the DNA sequence predicts an Asn. This discrepancy may either be technical or due to polymorphism in the LDL receptor gene of different animals.

RNA blotting analysis was used to confirm the identity of pLDLR-1 (Fig. 4). When this plasmid was used as a probe, a mRNA of approximately 5.5 kb was detected in the bovine adrenal gland. This RNA was about 9-fold more abundant in the adrenal gland than in the liver, a result that agrees well with the relative number of LDL receptors in these two tissues (3). When the bovine cDNA was hybridized to RNA isolated from human A-431 cells, a mRNA of approximately 5.5 kb was detected in cells grown in the absence of sterols (receptor-induced). The amount of this mRNA was reduced by more than 90% when the human cells were grown in the presence of sterols to repress the synthesis of LDL receptors.

Several conclusions may be drawn from the RNA blotting results. First, the 2.8-kb cDNA insert in pLDLR-1 represents approximately one-half of the 5.5-kb bovine LDL receptor mRNA. Second, the bovine cDNA cross-hybridizes with a similar-sized mRNA in human A-431 cells. Third, the previously observed suppression of LDL receptor activity in cultured cells grown in the presence of sterols (sometimes termed "down regulation") can now be attributed to a reduction in the mRNA level.

The LDL receptor is one member of a class of cell surface proteins that bind ligands, carry them into cells via coated pits, and thereafter return to the surface (2, 27). Many of these receptors, like the LDL receptor, are subjected to down regulation. The current findings suggest that one mechanism for receptor down regulation in general may involve feedback regulation of the mRNA for the receptor. The availability of a cDNA probe to the LDL receptor should allow other questions to be answered regarding the structure, mechanism of action, and genetics of this important class of cell surface proteins. The ability of the bovine cDNA to cross-hybridize with the human mRNA should allow the isolation of the human gene, and hence dissection of the molecular events giving rise to the prevalent genetic disease familial hypercholesterolemia. Finally, inasmuch as the bovine cDNA cross-hybridizes with the rabbit LDL receptor mRNA (data not shown), it should be possible to isolate the rabbit gene for use in characterizing the molecular lesion in the WHHL rabbit, an animal counterpart of familial hypercholesterolemia (28).

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