Apolipoprotein E receptor 2 is a recently identified receptor that resembles low and very low density lipoprotein receptors. Isolation and characterization of genomic clones encoding human apolipoprotein E receptor 2 revealed that the gene spans ~60 kilobases and contains 19 exons. The positions of the exon/intron boundaries of the gene are almost identical to those of low and very low density lipoprotein receptors. Fluorescent in situ hybridization of human chromosomes revealed that the gene is located on chromosome 1p34. Isolation of a cDNA encoding a variant receptor and reverse transcription-polymerase chain reaction chain reaction indicate the presence of multiple variants with different numbers of cysteine-rich repeats in the binding domain of the receptor. We also found a variant receptor lacking a 59-amino acid insertion in the cytoplasmic domain. The transcription start site was mapped to the position 236 base pairs upstream of the AUG translation initiator codon by primer extension analysis. Sequence inspection of the 5'-flanking region revealed potential DNA elements: AP-2, GC factor, PEA3, and Sp1. The minimal promoter region and a region required for nerve growth factor inducibility in PC12 cells were also determined.

Apolipoprotein E (apoE) is a 34-kDa lipophilic protein that circulates in the plasma primarily as a major component of various lipoproteins including chylomicron remnants, intermediate density lipoprotein, very low density lipoprotein (VLDL), β-migrating VLDL (β-VLDL), and high density lipoprotein (with apoE) (reviewed in Ref. 1). It is a key molecule responsible for the cellular recognition and internalization of these lipoproteins. Biochemical and genetic studies have demonstrated that apoE is involved in the hepatic clearance of chylomicron remnants and VLDL remnants from the plasma (re-viewed in Refs. 1 and 2).

ApoE also has functions in the central nervous system (reviewed in Refs. 3 and 4). Although the major site of apoE synthesis is the liver, the brain contains the second highest abundance of apoE mRNA (5). ApoE synthesis is dramatically increased after injury of the rat sciatic or optic nerves (6). In the brain, significant concentrations of apoE are detected in astrocytes, including Bergmann’s glia of the cerebellum, tanyocytes of the third ventricle, pitiucytes of the neurohypophysis, and Müller cells of the retina (7). These results indicate that apoE may be involved in the mobilization and utilization of lipid in the central nervous system. In humans, there are three major isoforms of apoE, designated E2 (Cys112 and Cys158), E3 (Cys112 and Arg158), and E4 (Arg112 and Arg158), which are products of three alleles at a single gene locus. Genetic data indicate that the e4 allele is present with increased frequency in patients with sporadic (8) and late-onset familial (9) Alzheimer’s disease.

In previous studies, we have isolated a human cDNA encoding a novel receptor that binds apoE-rich β-VLDL with high affinity and internalizes it into the cells (10). This new receptor, designated apoE receptor 2 (apoER2), consists of five domains that resemble those of the low density lipoprotein receptor (LDLR) (11) and the VLDL receptor (VLDLR) (12, 13): (i) an amino-terminal ligand-binding domain composed of multiple cysteine-rich repeats, (ii) an epidermal growth factor precursor homology domain, (iii) an O-linked sugar domain with clustered serine and threonine, (iv) a transmembrane domain, and (v) a cytoplasmic domain with an FDNPVY sequence (14). The structural features of each domain of apoER2 are highly similar to those of LDLR (11) and VLDLR (12, 13). A key structural difference among the three receptors is the number of cysteine-rich repeat sequences in their ligand-binding domains: apoER2 and LDLR contain a 7-fold repeat, whereas that of VLDLR is 8-fold. Although apoER2 and LDLR contain the same number of cysteine-rich repeat sequences, the ligand-binding domain structure of apoER2 is much more closely related to that of VLDLR: apoER2 and VLDLR contain a short linker sequence between repeats 5 and 6, whereas that of LDLR is located between repeats 4 and 5.

ApoER2 mRNA is detectable most intensely in brain and testis and, to a much lesser extent, in ovary, but is undetectable in other tissues in rabbit (10). In human tissues, apoER2 mRNA is abundant in brain and placenta and undetectable in other tissues. This pattern of tissue distribution and the relative abundance of apoER2 mRNA are completely different from those of LDLR and VLDLR: VLDLR mRNA is most highly expressed in heart and muscle (12), whereas LDLR mRNA is expressed in various tissues including liver (14). This pattern of tissue expression of apoER2 mRNA suggests that the receptor...
plays a role in the uptake of apoE containing high density lipoprotein secreted from astrocytes in the central nervous system.

Recently, Novak et al. (15) have identified a novel LDLR homologue with an 8-fold cysteine-rich repeat predominantly expressed in chicken brain. This chicken protein, designated LR8B, consists of five domains resembling those of LDLR, VLDLR, and apoER2. Comparison of the amino acid sequence of LR8B with those of human LDLR, VLDLR, and apoER2 reveals that it is a chicken homologue of apoER2: the two proteins have ~77% of their amino acids in common, and the identities extend throughout the proteins, excluding an extra cysteine-rich repeat present in LR8B and an insertion sequence present in human apoER2. The presence of apoER2 in chicken is striking because birds are not known to synthesize apoE.

To clarify the structural and functional relationships of apoER2 and as an initial approach to study the mechanisms regulating apoER2 gene expression, we have cloned and characterized the human gene encoding apoER2. In this paper, we describe the exon/intron organization, chromosome location, and transcription units of the human apoER2 gene. We also present evidence for the presence of multiple forms of variant receptor generated by alternative splicing.

EXPERIMENTAL PROCEDURES

Materials—Unless otherwise indicated, all restriction and DNA-modifying enzymes were from Takara Shuzo Corp. (Kyoto, Japan). [γ-32P]ATP (3000 Ci/mmol) and [α-32P]dCTP (3000 Ci/mmol) were from Amersham Corp. Oligonucleotides were synthesized with an automated DNA synthesizer (Applied Biosystems Inc., Model 381A).

General Methods—Standard molecular biology techniques were performed essentially as described by Sambrook et al. (18). cDNA and genomic clones were subcloned into pBluescript vectors in both orientations and manually or on an Applied Biosystems Model 373A DNA sequencer. Large DNA fragments were shortened successively by exonuclease III (20) and subcloned into pBluescript vectors.

Isolation of Genomic Clones—Recombinant bacteriophage clones were isolated by plaque hybridization from a library of human normal peripheral leukocytes in AEMBL3 vectors (13) using the entire coding region of the human apoER2 cDNA (10) labeled with [γ-32P]ATP. Five × 10^9 cpm of primer was coprecipitated with either 1 μg of poly(A) RNA or 15 μg of yeast tRNA and primer were resuspended in 100 μl of standard reverse transcription buffer and heated at 95 °C for 1 min. cDNA synthesis was carried out using 200 units of Superscript (Life Technologies, Inc.) and random hexamers in 20 mM Tris-HCl, pH 8.3, 10 mM MgCl2, 50 mM KCl, 3 mM dithiothreitol, 0.1% Nonidet P-40, and 0.45 mM dNTP at 45 °C for 1 h, 1-μl aliquots of the reaction mixture were then subjected to "hot start" PCR using AmpliTaq Gold (Perkin-Elmer) and a set of primers corresponding to the ligand-binding domain (sense primer: oligonucleotide 24, 5'-TCT CGG GCT TCT GGC GCT-3' (25 pmol); and antisense primer: oligonucleotide 1114, 5'-TCT GGG CCA GGA GCT G-GA A-3' (25 pmol)) in a total volume of 100 μl. After heating at 94 °C for 10 s, PCR was performed for 33 cycles, consisting of 30 s at 94 °C, annealing of primers for 1 min at 63 °C, and extension for 90 s at 72 °C. This was followed by a final extension step at 72 °C for 10 min. To amplify the region corresponding to the cytoplasmic domain, PCR was carried out under standard conditions for 33 cycles with primer annealing at 63 °C in a total volume of 100 μl (sense primer: oligonucleotide 2546, 5'-GAA ACT GGA AGC AGA AGA AC-3' (25 pmol); and antisense primer: oligonucleotide 2918, 5'-GAG GCA CGA AGG GGG TGA T-3' (25 pmol)). The PCR products were analyzed by electrophoresis on a 2% agarose gel.

Primer Extension Analysis—An oligonucleotide (oligonucleotide 180R, 5'-TCT CAG CCC TTC GAG TCC TTG-3') complementary to the 5'-end of the human apoER2 mRNA was end-labeled with [γ-32P]ATP. 5 × 10^6 cpm of primer was coprecipitated with either 1 μg of poly(A) RNA or 15 μg of yeast tRNA and primer were resuspended in 100 μl of standard reverse transcription buffer and heated at 95 °C for 1 min. cDNA synthesis was carried out using 200 units of Superscript at 45 °C for 1 h. Primer extension products were analyzed on 6% denaturing polyacrylamide gels adjacent to dideoxynucleotide chain termination sequencing ladders derived from double-stranded genomic DNA fragments. Primer extension products were analyzed using the same method. To confirm the results of primer extension analysis, RT-PCR (see above) was carried out with combinations of an antisense primer (oligonucleotide R, 5'-GGG CAC GCC GAG CAC ACA C-3' (25 pmol)) and three sense primers (oligonucleotide A, 5'-TGA GGG TGA GCA GGA GCA AAC AC-3' (25 pmol); oligonucleotide B, 5'-AAC CTG CTT GGA ATG CAG CCG AGG-3' (25 pmol); and oligonucleotide C, 5'-GCA AGG ACT GGG GGA GAA GAA-3' (25 pmol). P5 was carried out under standard conditions. The thermal profile used was 94 °C for 30 s, 60 °C for 1 min, and then 72 °C for 2 min.

Promoter-Luciferase Constructs—To test for promoter activity, various lengths of the 5'-upstream regions of exon 1 were fused to the luciferase gene present in pPGV-B (Toyonoki Inc., Tokyo). This plasmid contains no eukaryotic promoter or enhancer elements. DNA fragments containing nucleotides 437 to +8 or nucleotides -316 to +8 were generated by exonuclease III, blunt-ended with Klenow fragment, followed by digestion with NotI, and then inserted into the SmaI/NotI sites of pPGV-B to create reporter plasmids pLAER437 and pLAER316, respectively. A reporter plasmid containing nucleotides -148 to +8 (pLAER148) was created by insertion of the 156-bp PstI fragment of pLAER437 into the PstI/NotI sites of pPGV-B. The sequences of the inserts of these reporter plasmids were confirmed by nucleotide sequencing.

Transient Transfection Assays—HeLa cells were cultured in Dulbecco's modified Eagle's medium supplemented with 10% fetal calf serum, 100 units/ml penicillin, and 100 μg/ml streptomycin. PC12 cells were maintained in Dulbecco's modified Eagle's medium supplemented with 10% fetal bovine serum, 100 units/ml penicillin, and 100 μg/ml streptomycin. Cells were transfected with 1.5 μg of plasmid and 0.5 μg of the β-galactosidase expression plasmid pCMVβ (27) using LipofectAMINE (Life Technologies Inc.) reagent according to the manufacturer's recommendations. Cells were harvested 48 h after transfections for the measurement of luciferase activities. PC12 cells were cultured in the presence or absence of 50 ng/ml nerve growth factor.
factor (NGF) for 3 h before harvest for luciferase assay. In each transfection experiment, parallel plates of HepG2 and PC12 cells were transfected with pPGV-B and pPGV-C (ToyoInki Inc.), which serve as negative and positive controls, respectively. pPGV-B lacks a eukaryotic promoter, and apparently no luciferase activity was detected in both cell lines transfected with pPGV-B. The pPGV-C plasmid contains the SV40 early promoter and enhancer driving the expression of the luciferase mRNA transcript.

Luciferase Assay—Transfected cells were washed three times with phosphate-buffered saline; lysed in 500 μl of 25 mM Tris phosphate, pH 7.8, 2 mM dithiothreitol, 2 mM CDTA, 10% glycerol, and 0.1% Triton X-100; and centrifuged to remove cell debris. An aliquot of 20 μl of the cell extract was incubated in the presence of ATP, luciferin, and coenzyme A (28), and light emission was measured using a Berthold Lumat LB 9501 luminometer. The protein content of the cell extract was measured by the method of Lowry et al. (29).

β-Galactosidase Assay—An aliquot of the cell extract from lysed transfected cells was incubated at 37 °C for 1 h with 4.85 mg/ml chlorophenol red-β-D-galactopyranoside (Boehringer Mannheim), 62.3 mM MgCl₂, and 45 mM β-mercaptoethanol. The reaction was stopped with 0.5 ml of 1 x Na₂CO₃, and the amount of chlorophenol red formed was measured spectrophotometrically at 575 nm (30). To normalize the transfection efficiency for each individual transfection, the luciferase activity expressed as integrated light output values/mg of protein was divided by the β-galactosidase activity expressed as units/mg of protein.

RESULTS AND DISCUSSION

Isolation of the Human ApoER2 Gene—The restriction map of a 60-kb region containing the human apoER2 gene was constructed by analysis of the four bacteria phage clones λNR7, λNR10, λNR19, and λNR22 (Fig. 1). Gaps between λNR7 and λNR10 and between λNR10 and λNR22 were estimated to be 3 kb and 1 kb, respectively, based on the analysis of genomic DNA by Southern blotting and PCR with exon-specific oligonucleotides. The exact exon/intron boundaries were determined by nucleotide sequencing. Analysis of these genomic clones revealed that the gene spans 60 kb and contains 19 exons (Table I) and is conformed to the GT/AG rule (31).

Genomic Organization of the Human ApoER2 Gene—Fig. 2 summarizes the genomic organization of human apoER2 and compares it with those of human LDLR and VLDLR. Exon 1 extends 236 bp upstream of the initial methionine codon (see below) and contains the signal sequence. Cysteine-rich repeats 1–3 and 7 of the ligand-binding domain are each encoded by individual exons. The other repeats are all contained in a single exon. Although the ligand-binding domain of human apoER2 consists of seven cysteine-rich repeats like that of LDLR, the exon/intron organization of this domain is much more closely related to that of the human VLDLR gene, which contains an extra exon encoding an additional cysteine-rich repeat. Like the human LDLR and VLDLR genes, the growth factor repeats in the apoER2 gene are each encoded by individual exons. Exons 9–13 encode the nonrepetitive sequences that are shared among LDLR, VLDLR, and epidermal growth factor precursor. Like the LDLR and VLDLR genes, the O-linked sugar domain of the apoER2 gene is encoded by a single exon of 225 bp. The transmembrane domain is interrupted by a single intron. Exon 17 contains the C-terminal half of the transmembrane domain and the first 44 amino acids of the cytoplasmic domain. Unlike LDLR and VLDLR, the cytoplasmic domain of apoER2 contains an insertion sequence of 59 amino acids. This insertion sequence is encoded by a single exon of 177 bp (exon 18). The last exon encodes the remaining 12 amino acids and the 3'-untranslated region of the mRNA.

Chromosome Location of the Human ApoER2 Gene—To localize the human apoER2 gene to a specific chromosome, two techniques were used. First, a PCR strategy was used with the National Institute of General Medical Sciences human/rodent somatic cell hybrid mapping panel 2. A set of PCR primers were designed specifically to detect apoER2 human genomic DNA and not to amplify the rodent (mouse or hamster) apoER2 gene. Analysis of DNA from human/rodent hybrid cells by human sequence-specific PCR revealed that the gene is located on chromosome 1 (data not shown). The apoER2 gene has 0% discordance only with chromosome 1, and we therefore conclude that the apoER2 gene is located within human chromosome 1.

The human apoER2 gene was more precisely located on chromosome 1 using color-labeled fluorescent in situ hybridization analyses. The entire region of the human apoER2 cDNA was nick-translated with biotin-16-dUTP and visualized. We consistently observed hybridization signals on chromosome 1 at band p34 (Fig. 3). Thus, the human apoER2 gene is on a chromosome different from those of LDLR and VLDLR: the genes for LDLR (32) and VLDLR (22, 33) are located on chromosomes 19p13 and 9p24, respectively.

Splicing Variants—In the course of cloning a cDNA encoding human apoER2 (pNR1), we obtained a novel cDNA (designated pNR2) with a deletion, which was presumably derived from an alternative splicing of the pre-mRNA. Nucleotide sequencing of this cDNA revealed that the cDNA encodes a variant apoER2 lacking repeat 4–7 in the ligand-binding domain as illustrated in Fig. 4A. This deletion corresponds to the skipping of exons 5.
and 6 during RNA processing. The variant receptor lacking binding repeats 4–7 was designated apoER2\textsuperscript{D4–7}. To test whether this variant indeed recognizes apoE, pNR2 was introduced into LDLR-deficient Chinese hamster ovary cells (26), and ligand binding was measured using \( ^{125}\text{I}-\text{b-VLDL} \). As shown in Fig. 4B, cells expressing the variant receptor bound apoE-rich b-VLDL with high affinity: the calculated \( K_d \) values of apoER2\textsuperscript{D4–7} (0.86 \( \mu \text{g/ml} \)) and apoER2 (0.86 \( \mu \text{g/ml} \)) for b-VLDL were exactly the same (Fig. 4B, inset). This result indicates that the deletion of binding repeats 4–7 in the ligand-binding domain of apoER2 has essentially no effect on b-VLDL binding.

The recently identified chicken protein LR8B, the chicken homologue of apoER2, contains an 8-fold cysteine-rich repeat in the ligand-binding domain (15). Together with our demonstration of a variant lacking repeats 4–7, this suggests the presence of multiple variants with different numbers of cysteine-rich repeats in the ligand-binding domain of the receptor. To test this possibility, RT-PCR was carried out using poly(A) RNA from human brain and placenta and a pair of oligonucleotide primers that flank the region corresponding to the ligand-binding domain of the human receptor. Under standard conditions, we obtained multiple PCR products of unexpected length, presumably because of pre-PCR mispriming. To prevent possible pre-PCR mispriming (34), a hot start PCR was carried out. The RT-PCR RNA gave three major bands with 581, 704, and

\begin{table}
\centering
\caption{Exon/intron organization of the human apoER2 gene}
\begin{tabular}{|c|c|c|c|c|}
\hline
Exon No. & Exon size & Sequence at exon/intron junction & Amino acid interrupted & Intron length \text{kb} \\
\hline
1 & 360 & G Q G & \text{Amino acid} & 0.8 \\
2 & 120 & D C P & \text{Amino acid} & 13.0 \\
3 & 123 & G A C T G G C C & \text{Amino acid} & 3.2 \\
4 & 129 & G C T A C T C & \text{Amino acid} & 1.0 \\
5 & 387 & G A C T G G C C & \text{Amino acid} & 3.7 \\
6 & 123 & C T A C A G G & \text{Amino acid} & 0.1 \\
7 & 120 & T G T G G G G & \text{Amino acid} & 3.6 \\
8 & 126 & G G C T C G T G & \text{Amino acid} & 2.0 \\
9 & 175 & A T C T A T A G & \text{Amino acid} & 1.6 \\
10 & 228 & C T G C S A G G & \text{Amino acid} & 0.3 \\
11 & 119 & A C C C T G G & \text{Amino acid} & 1.6 \\
12 & 140 & G T G T T G T T G & \text{Amino acid} & 2.1 \\
13 & 142 & C C A A G G & \text{Amino acid} & 0.8 \\
14 & 153 & T A C C A G G & \text{Amino acid} & 1.7 \\
15 & 225 & C A G C A C T & \text{Amino acid} & 3.3 \\
16 & 69 & C C C C A T A G & \text{Amino acid} & 0.9 \\
17 & 173 & T A T C C T G C A & \text{Amino acid} & 1.7 \\
18 & 177 & A A A T C C A A G & \text{Amino acid} & 0.9 \\
19 & 1512 & & & \\
\hline
\end{tabular}
\label{table1}
\end{table}

\textbf{FIG. 2. Comparison of exon organization and protein domains of the human apoER2 gene with those of the LDLR and VLDLR genes.} The six functional domains of the three proteins are labeled in the lower portion of the figure. The 40-amino acid repeats in the binding domain of the three proteins are numbered. The growth factor repeats are lettered A–C. 5′- and 3′-untranslated regions are indicated by solid lines. The positions at which introns interrupt the coding region are indicated by arrowheads. Exon numbers are shown between the arrowheads. Chr., chromosome; EGF, epidermal growth factor.
1091 nucleotides (Fig. 5A). The three PCR products were subcloned into T-vectors and sequenced. The sequences of the 581- and 1091-nucleotide fragments fully matched those of the corresponding regions in pNR2 and pNR1, respectively. The 704-nucleotide fragment lacked 387 nucleotides corresponding to repeats 4–6 of the ligand-binding domain. This result agrees with our isolation of two cDNAs, which together indicate that human tissues express multiple forms of the receptor. We did not detect a variant with an 8-fold cysteine-rich repeat like chicken LR8B in the human tissues.

Comparison of the human apoER2 cDNA with chicken LR8B also revealed that the chicken homologue lacks the 59-amino acid insertion sequence in the cytoplasmic domain. To analyze the region corresponding to the cytoplasmic domain of the human receptor, RT-PCR was carried out using a pair of specific primers that span the relevant region. As shown in Fig. 5B, the variant lacking the insertion sequence was also expressed in human tissues. Although the function of the cytoplasmic insertion sequence of apoER2 is currently unknown, it may play a unique role in mammals: LR8B transcripts with the 59-amino acid insertion sequence in the cytoplasmic domain are not detected in chicken brain. Further functional analysis is required to elucidate the exact role of this domain in mammals.

Characteristics of the 5′-Flanking Region of the Human ApoER2 Gene—The transcription start site was determined by primer extension analysis using poly(A) RNA from human placenta and brain. For primer extension analysis, we used a 21-mer oligonucleotide (oligonucleotide 180R) labeled with 32P at the 5′-end. In the primer extension analysis, we detected a major band corresponding to the position 236 bp upstream of the AUG translation initiator codon (Fig. 6A). To confirm these human tissues express multiple forms of the receptor. We did not detect a variant with an 8-fold cysteine-rich repeat like chicken LR8B in the human tissues.

Comparison of the human apoER2 cDNA with chicken LR8B also revealed that the chicken homologue lacks the 59-amino acid insertion sequence in the cytoplasmic domain. To analyze the region corresponding to the cytoplasmic domain of the human receptor, RT-PCR was carried out using a pair of specific primers that span the relevant region. As shown in Fig. 5B, the variant lacking the insertion sequence was also expressed in human tissues. Although the function of the cytoplasmic insertion sequence of apoER2 is currently unknown, it may play a unique role in mammals: LR8B transcripts with the 59-amino acid insertion sequence in the cytoplasmic domain are not detected in chicken brain. Further functional analysis is required to elucidate the exact role of this domain in mammals.

2 W. J. Schneider, personal communication.
results, RT-PCR was carried out using four pairs of PCR primers. The first cDNA synthesis was primed with random hexamers and then amplified with three sets of oligonucleotides, an antisense primer (oligonucleotide R) and three sense primers (oligonucleotides A–C) (Fig. 6B). In combinations of oligonucleotide R with oligonucleotides B and C, amplified cDNA fragments of the expected sizes (198 and 286 bp) were detected, but no amplification occurred with oligonucleotides R and A (Fig. 6B). These data indicate that the most upstream transcription initiation site is located between nucleotides 273 and 169. Based on the primer extension analysis, the transcription site was defined as the G 236 nucleotides upstream of the initiator methionine.

Fig. 7 shows the nucleotide sequence of the 5'-flanking region of human apoER2. Neither a typical TATA box sequence (35), its homologue, nor a typical CCAAT box was found in the 5'-flanking region. Potential sites for Sp1 (37, 38) are present at nucleotides −331, −276, and −32. The flanking region contains DNA motifs for AP-2 (39, 40) and a potential site for the GC factor (GCF) (41), PE3 (42), and Sp1 (37, 38) are boxed.

Fig. 8. Analysis of the promoter activity of the 5'-flanking region of the human apoER2 gene fused to the luciferase reporter gene. Varying lengths of the 5'-flanking region of the gene were fused with the luciferase gene (Luc) in pPGV-B (see “Experimental Procedures”). Each chimeric gene was cotransfected with a β-galactosidase expression plasmid (pCMVβ) into PC12 cells or HepG2 cells and assayed for luciferase and β-galactosidase activities as described under “Experimental Procedures.” PC12 cells were cultured in the presence or absence of 50 ng/ml NGF for 3 h before harvest. Luciferase activity in an individual experiment was corrected for variation in transfection efficiency by normalizing the value to the β-galactosidase activity in the same extract. The normalized activity of each promoter was then expressed relative to that of pPGV-B, with pPGV-B assigned a relative activity of 1.0. The data represent the mean of triplicate transfection experiments for each plasmid.

3 D.-H. Kim and T. T. Yamamoto, unpublished observations.
confers promoter activity and to identify the element(s) that respond to NGF, different lengths of 5'-upstream region of the first exon were fused to the luciferase gene. Following transfection into neuronal PC12 and non-neuronal HepG2 cells, the reporter constructs pLAER437 and pLAER316, containing nucleotides −437 to +8 and nucleotides −316 to +8, respectively, produced significant luciferase activities in both cell lines compared with a promoterless vector (pPGV-B) (Fig. 8). Consistent with the accumulation of the mRNA in PC12 cells by NGF, nucleotides 316 bp relative to the transcription site contain the minimal promoter that functions in both PC12 and HepG2 cells. Our data also indicate that the region containing nucleotides −316 to −148 is required for NGF inducibility in PC12 cells. This region contains potential binding sites for Sp1, AP-2, and PEA3 (Fig. 7). Of particular interest are the AP-2 and PEA3 sites. AP-2 is a factor known to mediate induction by 12-O-tetradecanoylphorbol-13-acetate (42). Whether the above elements are involved in transcriptional regulation of the apoER2 gene is currently under investigation.

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