Apolipoprotein E Gene Mapping and Expression: Localization of the Structural Gene to Human Chromosome 19 and Expression of ApoE mRNA in Lipoprotein- and Non-Lipoprotein-Producing Tissues†

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ABSTRACT: Apolipoprotein E (apoE) binds to specific cell-surface receptors and appears to be an important determinant in lipoprotein metabolism in man. Cloned human apoE cDNA (pAE155) was used as a probe in chromosome mapping studies to detect the structural gene sequences in human–Chinese hamster cell hybrids. Southern blot analysis of *Hin*cII-digested DNAs from 13 hybrids localized the gene to human chromosome 19. This observation indicates that apoE is syntenic to at least two other genes related to lipid metabolism, those for the low-density lipoprotein (LDL) receptor (the *LDLR*) and apoC-II. The cloned apoE cDNA was further used to detect the presence of apoE mRNA in RNA extracts of various human and baboon tissues. Northern gel analysis using the ^{32}P-labeled pAE155 as a probe demonstrated the presence of hybridizable apoE mRNAs in human liver and in baboon liver, intestine, spleen, kidney, adrenal gland, and brain but not in baboon skeletal muscle. The apoE mRNAs appear to be intact and migrate on an agarose gel under denaturing conditions at approximately 18 S. To assay for the biological activity of the apoE mRNAs in these tissues, they were translated in a reticulocyte lysate system in vitro. Immunoprecipitation with an apoE-specific antiserum followed by sodium dodecyl sulfate gel electrophoresis and fluorography demonstrated that immunoreactive apoE with the expected apparent size was a product of translation of mRNAs from baboon liver, intestine, kidney, spleen, and brain but not that from baboon skeletal muscle. By quantitative slot–blot hybridization, taking baboon liver RNA as 100%, the various organs contained hybridizable apoE mRNA sequences at the following relative concentrations: adrenal gland, 59.6%; spleen, 11.8%; brain, 5.6%; kidney, 3.2%; small intestine, 0.8%; skeletal muscle, 0.5% or less. These findings indicate that apoE is synthesized in lipoprotein- and non-lipoprotein-producing tissues. The peripheral synthesis of apoE may play an important role in lipid transport and metabolism and/or some other as yet unidentified functions (especially in brain).

Apolipoprotein E (apoE)[1] is an apoprotein found in chylomicrons, chylomicron remnants, very low density lipoproteins, and high-density lipoproteins in man and in other mammalian species (Mahley & Innerarity, 1983; Brown & Goldstein, 1984). The complete amino acid sequence of apoE was reported by Rall et al. (1982b). It is a polypeptide of 299 amino acids. There are a series of amphipathic helices (Segrest et al., 1974) in the carboxyl-terminal third of the polypeptide chain that may represent the lipid-binding site(s) for apoE (Rall et al., 1982b). An important function of apoE appears to be the mediation of cellular uptake of lipoproteins through specific cell-surface receptors. ApoE binds to the low-density lipoprotein receptor of various cells and tissues. It also binds to a specific apoE receptor in the liver and mediates the hepatic uptake of chylomicron remnants (Mahley & Innerarity, 1983; Hui et al., 1981; Sherill et al., 1980).

Biochemical and genetic analyses have shown that apoE protein is polymorphic, with the existence of multiple isoforms. Type III hyperlipoproteinemia appears to be associated with homozygosity for a specific apoE allele (Utermann et al., 1979; Zannis & Breslow, 1981; Schneider et al., 1981). Studies of apoE mutants of known structure have indicated the importance of residues 142, 145, and 146 in mediating receptor binding (Weisgraber et al., 1982, 1984; Rall et al., 1982a, 1983); the positive charge of arginine-158 (which is substituted with a cysteine in the E2 isoform) also appears to be important in maintaining the correct conformation necessary for normal binding (Innerarity et al., 1984).

In experimental animals, plasma apoE is stimulated by high cholesterol feeding and carbohydrate ingestion (Swaney et al., 1977; Wong & Rubenstein, 1979; Lin-Lee et al., 1981b). Furthermore, the liver and, to a much lesser extent, the small intestine appear to be the major sources of plasma apoE in the rat (Wu & Windmueller, 1979).

To further understand the molecular basis of the regulation of apoE synthesis in primates, we have cloned the cDNA for human hepatic apoE mRNA. Using the cloned cDNA as a hybridization probe, we have localized the structural gene for apoE to human chromosome 19 by Southern blot analysis of DNAs from a panel of human–Chinese hamster somatic cell hybrids. To study the tissue-specific expression of apoE, we have performed Northern gel analysis of RNAs from a variety of primate tissues. We find that apoE mRNA sequences are

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[1] Abbreviations: apoE, apolipoprotein E; SDS, sodium dodecyl sulfate; CSF, cerebrospinal fluid; SSC, standard saline citrate (150 mM NaCl and 15 mM sodium citrate, pH 7.0); ds, double stranded; kb, kilobase; LDL, low-density lipoprotein.

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present in RNAs extracted from human liver and from baboon liver, intestine, spleen, kidney, adrenal gland, and brain tissue. Cell-free translation experiments confirmed that the apoE mRNAs in these tissues are functional and can be translated into immunoreactive apoE. The expression of apoE in non-lipoprotein-producing tissues such as brain is unexpected and raises the possibility of alternative functions for apoE in neural tissues.

Experimental Procedures

Human ApoE cDNA Cloning. A full-length human apoE cDNA was identified from a library of human liver cDNA clones by the technique of oligonucleotide hybridization. A mixture of eight oligonucleotides with the sequences 5′d[CC CAT (T/C)TC (T/C)TC CAT]3′ was custom synthesized by P-L Biochemicals. This sequence is complementary to the predicted mRNA sequence for amino acids 218–222 (Met-Glu-Glu-Met-Gly) (Rall et al., 1982b). The mixture of oligonucleotides was labeled at the 5′ end by transfer of ^{32}P from [γ-^{32}P]ATP by using bacteriophage T4 polynucleotide kinase as described (Maxam & Gilbert, 1980). Labeled oligonucleotides were purified by DE52 ion-exchange chromatography.

The human liver cDNA library was constructed from a mRNA preparation from adult liver tissue. The ds cDNA was inserted into the *Pst*1 site of pBR322 by the GC-tailing technique. The host was *Escherichia coli* K12 RR1.

The screening of bacterial colonies on Whatman 541 filters by colony hybridization was performed exactly as we have described previously (Cheung & Chan, 1983). The temperature of hybridization was 38 °C. The identity of the clones was confirmed by direct nucleotide sequencing by a combination of the chemical degradation method of Maxam & Gilbert (1980) and the dideoxynucleotide chain termination technique (Sanger et al., 1980) following subcloning of various restriction fragments of the DNA into the M13 vectors mp8 and mp9 (Messing & Vieira, 1982).

Localization of ApoE Structural Gene by Somatic Cell Hybrids. Mapping of the apoE structural gene on human chromosomes was performed by Southern blot analysis of DNAs isolated from a panel of human–Chinese hamster somatic cell hybrids (Kao et al., 1982). The conditions for culture of the parental cell lines (CHO-K1 and HT-1080), fusion between the auxotrophic mutants of CHO-K1 cells and human cells, and the characterization of the panel of somatic cell hybrids with respect to their chromosome content and isozyme complements have all been described previously (Kao, 1973; Kao et al., 1976, 1982; Moore et al., 1982; Cheung et al., 1984). The cell hybrids used in the present study are subpopulations derived from the respective original hybrids. These hybrid subpopulations have been characterized by cytogenetic and isozyme analyses at about the same time the DNAs were prepared. The cytogenetic analysis involved sequential staining of the same chromosome slides with trypsin-banding and Giemsa-11 differential-staining procedures (Kao et al., 1976; Morse et al., 1982). The human chromosome content in some hybrids has also been confirmed by isozyme analysis. Due to the continued growth in culture for some hybrids and thawing of the frozen cultures for other hybrids, some changes in the human chromosome content occurred in the subpopulations as compared to the original hybrids. The percentage of each human chromosome retained in the hybrid subpopulations generally ranged from 50 to 100% of the cells analyzed.

Preparation of DNA from Cultured Cells and Cell Hybrids. For DNA preparation, cells were grown in 150-mm dishes to confluency and harvested by trypsinization. They were treated with proteinase K after washing, and DNA was isolated as described (Kao et al., 1982; Cheung et al., 1984).

Digestion, Agarose Gel Electrophoresis, and Southern Blot Analysis of DNA. Digestion and agarose gel electrophoresis of somatic hybrid cell DNA were performed as described in Cheung et al. (1984). Pilot experiments indicated that digestion of human DNA from different individuals with the restriction enzyme *Hinc*II (Amersham) produces Southern blots with more than one pattern, suggesting that the *Hinc*II sites are polymorphic. However, DNA from the parental cell, HT-1080, consistently yielded an ~10-kb band, and DNA from CHO cells produced no detectable cross-hybridizing band (Figure 1). Using *Hinc*II, we performed Southern blot and hybridization analyses of the DNA from various cell hybrids in the presence of dextran sulfate as previously described (Cheung et al., 1984).

Northern Blot Hybridization of mRNA. Total RNA was prepared from human liver and from baboon liver, intestine, kidney, muscle, spleen, adrenals, and brain by the guanidine hydrochloride method as previously described (Lin-Lee et al., 1981a,b). Poly(A) RNA was isolated by two cycles of oligo(dT)–cellulose chromatography (Aviv & Leder, 1972). Poly(A) RNA was glyoxylated and separated by electrophoresis on 1.5% agarose gels and transferred to nitrocellulose paper by the method of Thomas (1980). ApoE mRNA sequences were detected by hybridization to the nick-translated (Chan et al., 1980) ^{32}P-labeled cloned apoE cDNA insert from pAE155.

Quantitative Slot–Blot Analysis of ApoE mRNA. Total RNA (0.5–20 μg) from various baboon organs was dissolved in 400 μL of 7.5 × SSC and 75% formaldehyde. The sample was heated to 65 °C for 15 min. It was blotted onto Zeta-Probe membrane (Bio-Rad) that had been prewetted with 10 × SSC in a slot–blotting apparatus (Minifold II, Schleicher & Schuell). The membrane was air-dried and baked at 80 °C for 2 h in a vacuum oven. It was washed in a solution of 0.1 × SSC and 0.5% SDS at 65 °C for 1 h. Prehybridization was performed in 5 × SSC, 20 mM sodium phosphate, pH 7.0, 4 × Denhardt's solution (Denhardt, 1966), 200 μg/mL sheared denatured salmon sperm DNA, and 50% formaldehyde at 42 °C for 16 h. Hybridization was performed for 24 h at 42 °C in the same buffer containing, in addition, 50 μg/mL *E. coli* tRNA and the ^{32}P-labeled nick-translated cloned human apoE cDNA probe (pAE155, 1 × 10^7 cpm/mL). The hybridization solution was discarded, and the membrane was washed first in 2 × SSC and 0.1% SDS at 21 °C for 2 h, then in 0.1 × SSC and 0.1% SDS at 21 °C for 30 min, and finally in 0.1 × SSC and 0.1% SDS at 55 °C for 30 min. The membrane was air-dried. Autoradiography was performed at −70 °C for 16 h with Kodak XAR-5 film. The radioactive bands were cut out from the membrane and counted in Scinti-Verse (Fisher Scientific Co.) in a Beckman scintillation counter.

In Vitro Cell-Free Translation of Human and Baboon RNAs. Cell-free translation of poly(A) RNA was performed in a reticulocyte lysate system as previously described (Pelham & Jackson, 1976). [^{35}S]Methionine was the radiolabeled amino acid precursor. The translation reaction was linear for 120 min. It was terminated for product analysis after 90 min by submersion of the reaction tubes in ice and addition of unlabeled methionine. Total translation activity was quantified by trichloroacetic acid precipitation (Lin-Lee et al., 1981b). Immunoprecipitation with a monospecific anti-human apoE serum and SDS–polyacrylamide slab gel analysis of the im-

Table I: Synteny Analysis of the Human ApoE Gene in 13 Human CHO-K1 Cell Hybrids by Molecular Hybridization Using cDNA Probe pAE155

Hybrids	Human Chromosomes[a]																							GPI[b]	ApoE[c]
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	X		
CP3-1	−	−	−	+	+	−	−	−	−	−	+	+	−	+	−	+	+	+	−	+	+	−	+	−	−
CP4-1	−	−	−	+	+	−	−	+	−	−	+	−	−	+	−	−	−	−	−	−	−	+	+	−	−
CP5-1	+	−	−	−	+	−	−	+	+	−	−	+	−	+	+	−	+	−	+	−	+	+	−	+	+
CP6-1	−	−	−	+	−	−	−	−	−	−	−	+	−	+	−	−	+	+	+	−	+	+	−	+	+
CP11-1	−	−	−	−	+	−	−	−	−	−	−	−	−	+	−	−	−	+	−	−	+	−	−	−	−
CP12-1	−	+	−	+	−	−	−	+	+	+	+	+	+	−	−	−	−	−	−	−	+	+	+	−	−
CP14-1	−	−	−	+	+	−	−	−	−	−	−	−	−	−	−	−	+	−	−	−	+	+	+	−	−
CP16-1	−	−	−	−	+	−	−	−	−	−	−	−	−	+	−	−	+	−	+	+	+	+	−	+	+
CP17-1	+	−	−	+	+	−	−	−	−	−	−	+	−	−	−	−	+	−	−	−	−	−	−	−	−
CP18-1	+	−	−	−	−	−	−	+	−	−	+	−	−	+	+	−	+	+	+	−	−	−	−	+	+
CP20-1	−	+	−	−	+	−	−	−	+	−	−	−	−	−	−	−	+	−	−	−	+	−	−	−	−
CP26-1	+	−	−	+	+	+	+	−	+	+	+	+	+	−	−	−	+	−	−	−	−	+	−	−	−
CP28-1	+	−	−	+	+	−	−	+	+	−	−	−	−	−	−	−	−	+	+	+	−	−	−	+	+
Number of Concordant Hybrids	9	6	8	4	4	7	7	9	7	6	5	6	6	9	10	7	7	9	13	9	5	7	4		
Percent of Concordance	69.2	46.2	61.5	30.8	30.8	53.8	53.8	69.2	53.8	46.2	38.5	46.2	46.2	69.2	76.9	53.8	53.8	69.2	100.0	69.2	38.5	53.8	30.8		

[a] Identified by trypsin banding and Giemsa-11 differential staining in sequential steps. [b] Identified by isozyme analysis for detecting the human chromosome 19 isozyme marker glucosephosphate isomerase (GPI). [c] Identified by Southern blot analysis using the apoE cDNA probe.

munoprecipitated product were performed as described previously by Lin-Lee et al. (1981a,b). The slab gels were processed for fluorography and exposed to Kodak XS-1 film as described (Lin-Lee et al., 1981a,b).

Results

Human ApoE cDNA Clone. A partial apoE cDNA clone (pAE87) was first identified in a human liver cDNA library by the technique of oligonucleotide hybridization (Cheung & Chan, 1983). The insert of pAE87 was used as a probe, and another 50 apoE cDNA clones were identified after we screened an additional 50 000 colonies. One of these apoE clones, pAE155, contained an insert of 1100 nucleotides and was characterized by DNA sequencing. This cloned DNA includes the poly(A) tail, 3′ nontranslated region, all the coding region, and 110 nucleotides in the 5′ nontranslated region of apoE mRNA. The sequence is identical with the "normal" sequence published by McLean et al. (1984). We have used the cDNA insert of pAE155 as a hybridization probe in our subsequent studies.

Hybridization of pAE155 Probe with Somatic Cell Hybrid DNA. The somatic cell hybrid DNAs were digested with *Hin*cII, transferred to nitrocellulose paper, and hybridized to the ^{32}P-labeled human apoE cDNA probe, pAE155. As shown in Figure 1, the *Hin*cII-digested parental cell (HT-1080) DNA showed an ~10-kb band on Southern analysis. Chinese hamster DNA failed to show any hybridization signal under our conditions of hybridization. Table I summarizes the results of Southern blot analysis of a panel of 13 human–Chinese hamster somatic cell hybrid DNAs. Five of the 13 hybrid DNAs gave positive hybridization signals, and eight of them did not show any signal even on prolonged exposure of the X-ray film. In our synteny analysis, concordant hybrids are those that have the particular human chromosome and the apoE gene either present or absent together. Conversely, those

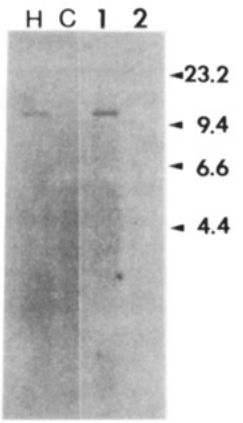

FIGURE 1: Hybridization of pAE155 to *Hin*cII-digested DNA from human–Chinese hamster somatic cell hybrids and their parental lines. Lanes: H, human cell HT-1080; C, Chinese hamster cell CHO-K1; 1, positive cell hybrid, showing presence of the ~10-kb human-specific apoE band; 2, negative cell hybrid. Size standards are in kilobases and are obtained from DNA fragments of *Hin*dIII-digested λ phage.

hybrids having either the chromosome or the apoE gene present singly are discordant hybrids. Concordant segregation frequency is expressed by dividing the number of concordant hybrids by the total number of hybrids analyzed (13 hybrids in this study). By such analyses, the presence or absence of the ~10-kb human apoE band correlates exclusively with human chromosome 19 (Table I). Furthermore, the apoE gene cosegregates with human chromosome 19 isozyme marker glucose phosphate isomerase in all the hybrids examined (Table I). These results allow assignment of the structural gene for human apoE to chromosome 19 and to no other chromosome.

Northern Blot Analysis of ApoE mRNA. Northern blot analysis of various poly(A) RNA preparations from human and baboon tissues using ^{32}P-labeled pAE155 as a probe indicates the presence of hybridizable apoE mRNA sequences

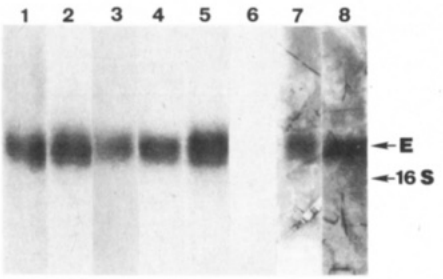

FIGURE 2: Northern blot hybridization of ^{32}P-labeled pAE155 to poly(A) RNA from various human and baboon tissues. Sources of RNA: lane 1, human liver (0.1 μg); lane 2, baboon liver (0.1 μg); lane 3, baboon intestine (10 μg); lane 4, baboon kidney (1.0 μg); lane 5, baboon spleen (1.5 μg); lane 6, baboon skeletal muscle (10 μg); lane 7, baboon cerebral cortex (2.0 μg); lane 8, baboon adrenal gland (1.0 μg). The filter was exposed to X-ray (Kodak XS-1) film at −70 °C for 72 h. A more prolonged exposure (for 10 days) did not reveal any detectable apoE band in lane 6 (skeletal muscle RNA). E, migration of apoE mRNA; 16S, migration of *E. coli* 16S RNA.

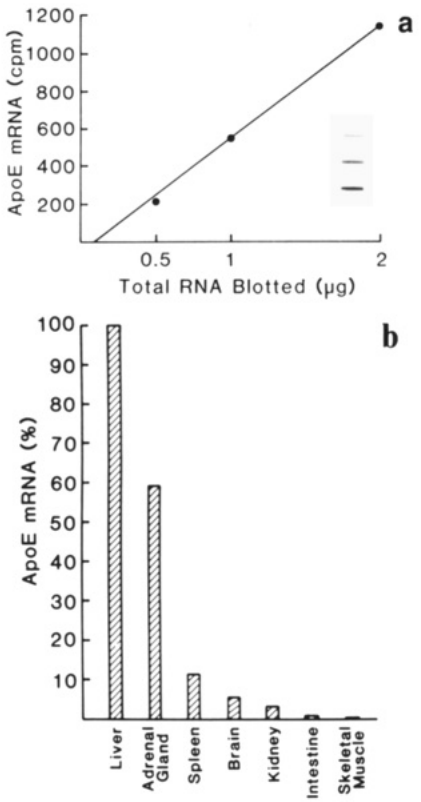

FIGURE 3: Quantitation of apoE mRNA sequences by slot–blot hybridization. (a) Three concentrations of baboon liver RNA were blotted onto Zeta-Probe membrane and hybridized to the nick-translated ^{32}P-labeled human apoE cDNA insert as described under Experimental Procedures. The blots were then cut out from the filter, and the radioactivity was determined by scintillation spectrophotometry. The inset is an autoradiograph of the filter from which the data on this curve were generated. (b) Concentrations of hybridizable apoE mRNA sequences in various baboon tissues as determined by quantitative slot–blot hybridization. The relative concentrations were computed from the slopes obtained from the hybridization assays shown in panel a. The concentrations of RNAs used in the individual assays all yielded linear plots as in panel a.

in human liver and in baboon liver, intestine, kidney, spleen, brain, and adrenal gland. The mRNA bands migrated with a relative mobility of approximately 18 S (Figure 2). Despite the use of large amounts of poly(A) RNA and a prolonged exposure of the X-ray film to the filter, apoE mRNA was not detectable in baboon skeletal muscle RNA.

Quantitation of ApoE mRNA Sequences by Slot–Blot Analysis. When increasing quantities of total RNA from the

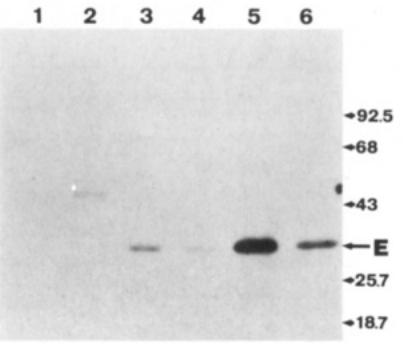

FIGURE 4: Fluorograph of translation products. Poly(A) RNA from various tissues was translated in vitro in a reticulocyte lysate system. ApoE immunoreactive product was precipitated by a specific antiserum, and the immunoprecipitated product was analyzed on a 10% polyacrylamide slab gel in SDS as described under Experimental Procedures. Translation products were pooled from multiple reactions. The amounts of mRNA used in these reactions are indicated below. Lanes: 1, baboon skeletal muscle (50 μg of RNA); 2, baboon jejunum (43 μg of RNA); 3, baboon kidney (11 μg of RNA); 4, baboon cerebral cortex (11 μg of RNA); 5, baboon spleen (3 μg of RNA); 6, baboon liver (2 μg of RNA). The gel was processed for fluorography as described previously (Lin-Lee et al., 1981a). It was exposed to Kodak XS-1 film for 40 h. On more prolonged exposures, the apoE band in lane 2 (baboon jejunum) was more intense. The higher molecular weight band in lane 2 was not authentic apoE since competition with excess apoE protein did not change its intensity while the apoE-specific band disappeared. Molecular weight standards used were the following: phosphorylase B, 92.5K; bovine serum albumin, 68K; ovalbumin, 43K; α-chymotrypsinogen, 25.7K; β-lactoglobulin, 18.4K. E signifies the position of migration of human apoE protein.

various organs and tissues were directly blotted on Zeta-Probe membrane, there was a linear relationship between the amount of RNA blotted and the radioactivity on the filter (Figure 3a). From the slopes of the curves generated by the various RNA samples, the relative concentrations of apoE mRNAs were determined. It is evident from Figure 3b that, of the various organs studied, the liver contained the highest concentration of hybridizable apoE mRNA sequences. Next to the liver, the following organs contained decreasing amounts of such sequences in the following order: adrenal gland, spleen, brain, kidney, and small intestine. Skeletal muscle RNA contained extremely low amounts of apoE mRNA sequences (approximately 0.5% that of liver) as determined by this assay.

Cell-Free Translation of ApoE mRNA from Various Tissues. To assess the functional state of the hybridizable apoE RNA sequences in some of the tissues, we have translated the poly(A) RNAs in a reticulocyte lysate translation assay. ApoE mRNA activity was detected by specific immunoprecipitation using a monospecific antiserum against human apoE. There was good cross-immunoreactivity between human and baboon apoE. The immunoprecipitated products were analyzed on SDS–polyacrylamide gels by fluorography. As we have reported previously, the apoEs synthesized in vitro have an apparent size identical with that of plasma apoE (Chan et al., 1983). It is evident that translatable apoE mRNAs are present in RNAs isolated from baboon liver, kidney, intestine, spleen, and brain but not baboon skeletal muscle, despite the use of large amounts of mRNA from the last tissue (Figure 4). In the translation of baboon intestine RNA, newly synthesized apoE accounts for a very small fraction of the total product (<0.01%). The very faint band in Figure 4 (lane 2) in the position of apoE became more intense following more prolonged exposure of the gel to X-ray film. The high molecular weight band in lane 2 is not authentic apoE since it was not abolished by the presence of excess unlabeled human apoE protein during the immunoprecipitation. In contrast, the

addition of excess apoE completely abolished all the radiolabeled bands (lanes 2–6) migrating with an apparent size similar to that of apoE (data not shown).

Discussion

Our laboratory has been interested in the biosynthesis of apoE in the mammal (Lin-Lee et al., 1981a,b; Tanaka et al., 1982; Chan et al., 1983). To further understand the expression of apoE at the molecular level in man and other primates, we have isolated a full-length human apoE cDNA. We have used the cloned cDNA as a hybridization probe to map the structural gene for apoE to specific human chromosomes by Southern blot analysis of human–Chinese hamster somatic cell hybrids. Our experiments have localized the apoE gene to human chromosome 19. This observation is in agreement with pedigree analysis, which shows linkage of the gene for apoE (*APOE*) with complement 3 (*C3*) on human chromosome 19 (Olaisen et al., 1982, 1984). It is interesting that at least two other lipoprotein-related genes have been mapped to chromosome 19: the genes for the LDL receptor (*LDLR*) (Francke et al., 1984) [or its clinical equivalent *FHC*, for the familial hypercholesterolemia locus (Berg & Heisberg, 1978)] and apoC-II (Jackson et al., 1984). On the basis of the linkage between *APOE* and *LDLR*, Franke et al. (1984) speculated on the possibility of an evolutionary link between a protein ligand (apoE) and its receptor (LDL receptor). Our study extends these previous observations and directly maps apoE structural sequences to the same chromosome to which the LDL receptor has been assigned.

To date, there are four apolipoprotein genes that have been assigned to specific human chromosomes: apoA-I to the long arm of chromosome 11 (Cheung et al., 1984; Law et al., 1984); apoA-II to chromosome 1 (Moore et al., 1984); apoC-II to chromosome 19 (Jackson et al., 1984; Fojo et al., 1984); and, now, apoE also to chromosome 19. The distance between the structural genes for apoE and apoC-II is unknown. Since the apoE cDNA probe does not hybridize to a number of apoC-II genomic fragments present in a cosmid clone isolated in our laboratory (C. F. Wei and L. Chan, unpublished results), we can conclude that they are not very closely linked. In the future, the determination of the exact chromosomal localization of the various apolipoprotein genes at the fine-banding level will be useful in our understanding of the structure, organization, and evolution of these genes.

Using the cloned apoE cDNA probe, we have performed additional studies to localize the sites of synthesis of the mRNA for apoE in various primate organs and tissues. Prior studies on rodents in this and other laboratories have shown that the liver and small intestine are the major sources of apolipoproteins (Wu & Windmueller, 1979; Lin-Lee et al., 1981b; Tanaka et al., 1982). Subsequently, apoE was found to be synthesized also in mouse macrophages (Basu et al., 1981) and cultured human monocytes (Basu et al., 1982), as well as in human kidney and adrenal gland (Blue et al., 1983). Using cloned apoE as a hybridization probe, we now show that apoE mRNA is present in human liver and in baboon liver, intestine, kidney, spleen, adrenal gland, and brain. To ensure that we were detecting intact mRNAs, we used Northern gel analysis, which also gave us the size distribution of apoE mRNAs in the various tissues and organs. We found that they all had a size of approximately 18 S, a size very similar to that of liver apoE mRNA. By Northern gel analysis, at the level of sensitivity of our assay, we did not detect any apoE mRNA in baboon skeletal muscle (Figure 2). In contrast, in our quantitative slot–blot analysis of skeletal muscle RNA, the radioactivity on the filter was slightly, but unequivocally, above background. By calculation, the hybridizable apoE RNA sequences were present in skeletal muscle at a concentration approximately 0.5% that in baboon liver RNA (Figure 3). We cannot be certain whether these sequences represent authentic apoE mRNA sequences present in such low concentrations that they were not detected in the Northern gel analysis or whether they represent some cross-hybridizing material not directly related to apoE mRNA sequences.

It is important to determine if the apoE mRNAs in the various organs were biologically active. We assayed for their activity in cell-free translation assays. Indeed, translation of the mRNAs in vitro produced immunoreactive apoE, which had the same apparent size as plasma apoE on SDS gel analysis, as we have previously reported for the liver apoE mRNA translation product (Chan et al., 1983) (Figure 4). These observations conclusively demonstrate the presence of biologically functional apoE mRNA in a number of organs and tissues in addition to the liver and intestine. Again, at the level of sensitivity of the assay, we did not detect translatable apoE mRNA in skeletal muscle.

The approximate relative concentrations of apoE mRNA in the various baboon tissues can be estimated from the quantitative slot–blot analysis of RNA isolated from these tissues (Figure 3b). It appears that apoE mRNA is present at the highest concentrations in the liver; it is present in decreasing concentrations in the adrenal gland, spleen, brain, kidney, and small intestine. As discussed above, apoE mRNA is present, if at all, in extremely low concentration in skeletal muscle. Even though the small intestine has been known to be a source of apoE protein (Wu & Windmueller, 1979), the concentration of apoE mRNA in this organ is also very low (approximately 0.8% that in liver). Similar low concentrations of apoE mRNA have been observed previously in the rat intestine (Tanaka et al., 1982).

It is thus apparent that the potential for various peripheral tissues to synthesize apoE is considerable. Blue et al. (1983) have suggested that apoE synthesis might represent a mechanism for the removal of cholesterol from cells and/or the movement of cholesterol to the vascular compartment for delivery to the liver. One might speculate whether apoE is synthesized in all normal cells as part of "normal cellular function". This is probably not the case since baboon skeletal muscle produces little, if any, apoE mRNA. Preliminary studies in our laboratory using immunohistochemical techniques also indicate that only specific cells in various organs (e.g., tubular epithelial cells in the kidney; C. T Lin and L. Chan, unpublished data) harbor apoE protein.

The most unexpected observation was the presence of apoE mRNA sequences in brain. In a pioneering study, Roheim et al. (1979) reported the presence of apoE in human cerebrospinal fluid (CSF). Our present observation suggests that the source of CSF apoE is probably neural tissues. Being a large macromolecule, apoE probably does not cross the blood–brain barrier. If cholesterol efflux is the postulated function for the peripheral synthesis of apoE, it is highly unlikely that such a function exists for apoE synthesis in brain, again because of the existence of the blood–brain barrier. Thus, apoE probably serves some alternative function within the nervous system, possibly through its lipid-binding properties as transport molecules or through other (receptor-binding or as yet unrecognized) properties of the protein. Any hypothesis explaining this phenomenon must await the detailed topographical description of apoE protein in various neural cells and tissues and thorough neurophysiological studies of the possible involvement of apoE in various neural functions.

References

Aviv, H., & Leder, P. (1972) *Proc. Natl. Acad. Sci. U.S.A. 69*, 1408–1412.

Basu, S. K., Brown, M. S., Ho, Y. K., Havel, R. J., & Goldstein, J. L. (1981) *Proc. Natl. Acad. Sci. U.S.A. 78*, 7545–7549.

Basu, S. K., Ho, Y. K., Brown, M. S., Bilheimer, D. W., Anderson, R. G. W., & Goldstein, J. L. (1982) *J. Biol. Chem. 257*, 9788–9795.

Berg, K., & Heisberg, A. (1978) *Cytogenet. Cell Genet. 22*, 621–623.

Blue, M. L., Williams, D. L., Zucker, S., Khan, S. A., & Blum, C. B. (1983) *Proc. Natl. Acad. Sci. U.S.A. 80*, 283–287.

Brown, M. S., & Goldstein, J. L. (1983) *J. Clin. Invest. 72*, 743–747.

Chan, L., Dugaiczyk, A., & Means, A. R. (1980) *Biochemistry 19*, 5631–5637.

Chan, L., Antalis, T., Lin-Su, M. H., Cheung, P., & Lin-Lee, Y.-C. (1983) *Atherosclerosis (Berlin) 6*, 598–601.

Cheung, P., & Chan, L. (1983) *Nucleic Acids Res. 11*, 3703–3715.

Cheung, P., Kao, F.-T., Law, M. L., Jones, C., Puck, T. T., & Chan, L. (1984) *Proc. Natl. Acad. Sci. U.S.A. 81*, 508–511.

Denhardt, D. (1966) *Biochem. Biophys. Res. Commun. 23*, 641–646.

Fojo, S. S., Law, S. W., Brewer, H. B., Jr., Sakaguchi, A. Y., & Naylor, S. L. (1984) *Biochem. Biophys. Res. Commun. 122*, 687–693.

Francke, U., Brown, M. S., & Goldstein, J. L. (1984) *Proc. Natl. Acad. Sci. U.S.A. 81*, 2826–2830.

Hui, D. Y., Innerarity, T. L., & Mahley, R. W. (1981) *J. Biol. Chem. 256*, 5646–5655.

Innerarity, T. L., Weisgraber, K. H., Arnold, K. S., Rall, S. C., Jr., & Mahley, R. W. (1984) *J. Biol. Chem. 259*, 7261–7267.

Jackson, C. L., Bruns, G. A. P., & Breslow, J. L. (1984) *Proc. Natl. Acad. Sci. U.S.A. 81*, 2945–2949.

Kao, F.-T. (1973) *Proc. Natl. Acad. Sci. U.S.A. 70*, 2893–2898.

Kao, F.-T., Jones, C., & Puck, T. T. (1976) *Proc. Natl. Acad. Sci. U.S.A. 73*, 193–197.

Kao, F.-T., Hartz, J. A., Law, M. L., & Davidson, J. N. (1982) *Proc. Natl. Acad. Sci. U.S.A. 79*, 865–869.

Law, S. W., Gray, G., Brewer, H. B., Jr., Sakaguchi, A. Y., & Naylor, S. L. (1984) *Biochem. Biophys. Res. Commun. 118*, 934–942.

Lin-Lee, Y.-C., Bradley, B. A., & Chan, L. (1981a) *Biochem. Biophys. Res. Commun. 99*, 654–661.

Lin-Lee, Y.-C., Tanaka, Y., Lin, C. T., & Chan, L. (1981b) *Biochemistry 20*, 6474–6480.

Mahley, R. W., & Innerarity, T. L. (1983) *Biochim. Biophys. Acta 737*, 197–222.

Maxam, A. M., & Gilbert, W. (1980) *Methods Enzymol. 65*, 499–560.

McLean, J. W., Elshourbagy, N. A., Chang, D. J., Mahley, R. W., & Taylor, J. M. (1984) *J. Biol. Chem. 259*, 6498–6504.

Messing, J., & Vieira, J. (1982) *Gene 19*, 269–276.

Moore, M. N., Kao, F.-T., Tsao, Y. K., & Chan, L. (1984) *Biochem. Biophys. Res. Commun. 123*, 1–7.

Morse, H. G., Patterson, D., & Jones, C. (1982) *Mamm. Chromosome Newsl. 232*, 127–133.

Olaisen, B., Teisberg, P., & Gedde-Dahl, T., Jr. (1982) *Hum. Genet. 62*, 233–236.

Olaisen, B., Teisberg, P., Gedde-Dahl, T., Jr., Wihelmy, M., Mevag, B., & Helland, R. (1984) *Cytogenet. Cell Genet. 37*, 559.

Pelham, H. R. B., & Jackson, R. J. (1976) *Eur. J. Biochem. 67*, 247–256.

Rall, S. C., Jr., Weisgraber, K. H., Innerarity, T. L., & Mahley, R. W. (1982a) *Proc. Natl. Acad. Sci. U.S.A. 79*, 4696–4700.

Rall, S. C., Jr., Weisgraber, K. H., & Mahley, R. W. (1982b) *J. Biol. Chem. 257*, 4171–4178.

Rall, S. C., Jr., Weisgraber, K. H., Innerarity, T. L., Bersot, T. P., Mahley, R. W., & Blum, C. B. (1983) *J. Clin. Invest. 72*, 1288–1297.

Roheim, P. S., Carey, M., Forte, T., & Vega, G. L. (1979) *Proc. Natl. Acad. Sci. U.S.A. 76*, 4646–4649.

Sanger, F., Coulson, A., Barrell, B., Smith, A., & Roe, B. (1980) *J. Mol. Biol. 143*, 161–178.

Schneider, W. J., Kovanen, P. T., Brown, M. S., Goldstein, J. L., Utermann, G., Weber, W., Havel, R. J., Kotite, L., Kane, J. L., Innerarity, T. L., & Mahley, R. W. (1981) *J. Clin. Invest. 68*, 1075–1085.

Segrest, J. P., Jackson, R. L., Morrisett, J. D., & Gotto, A. M., Jr. (1974) *FEBS Lett. 38*, 247–253.

Sherrill, B. C., Innerarity, T. L., & Mahley, R. W. (1980) *J. Biol. Chem. 255*, 1804–1807.

Swaney, J. B., Braithwaite, F., & Eder, H. A. (1977) *Biochemistry 16*, 271–278.

Tanaka, Y., Lin-Lee, Y.-C., Lin-Su, M. H., & Chan, L. (1982) *Metab., Clin. Exp. 31*, 861–865.

Thomas, P. (1980) *Proc. Natl. Acad. Sci. U.S.A. 77*, 5201–5205.

Utermann, G., Vogelberg, K. H., Steinmetz, A., Schoenborn, W., Pruin, N., Jaeschke, M., Ilees, M., & Canzler, H. (1979) *Clin. Genet. 15*, 37–62.

Weisgraber, K. H., Innerarity, T. L., & Mahley, R. W. (1982) *J. Biol. Chem. 257*, 2518–2521.

Weisgraber, K. H., Rall, S. C., Jr., Innerarity, T. L., Mahley, R. W., Kuusi, T., & Ehnholm, C. (1984) *J. Clin. Invest. 73*, 1024–1033.

Wong, L., & Rubenstein, D. (1979) *Atherosclerosis (Shannon, Irel.) 34*, 249–258.

Wu, A. L., & Windmueller, H. G. (1979) *J. Biol. Chem. 254*, 7316–7322.

Zannis, V. I., & Breslow, J. L. (1981) *Biochemistry 20*, 1033–1041.