Communication

In the Absence of a Downstream Element, the Apolipoprotein E Gene Is Expressed at High Levels in Kidneys of Transgenic Mice*

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Human apolipoprotein (apo) E gene constructs with 30 or 5 kilobases of 5′-flanking and 1.5 kilobases of 3′-flanking regions were used to create transgenic mice. High levels of human apoE mRNA were present in the transgenic kidney, but none was detected in the liver, which is normally the major source of apoE. When a construct with 5 kilobases of 5′- and 23 kilobases of 3′-flanking regions was used, only trace levels of human apoE mRNA were detected in the kidney, whereas high levels were found in the liver. These results indicated that regulatory elements downstream of the human apoE gene interacted with the transcription initiation complex to stimulate gene expression in the liver while suppressing expression in the kidney. In each case, human apoE was secreted into the plasma. The source of human apoE in the transgenic kidney was the epithelial cells lining the proximal tubule and Bowman's capsule.

Apolipoprotein (apo) E is a major structural component of various classes of mammalian lipoproteins, including chylomicron remnants, very low density lipoproteins, intermediate density lipoproteins, and high density lipoproteins (1). Human apoE is a single-chain protein of 299 amino acids (M, = 34,200) (2). It can have an additional 3–5% of its mass in a carbohydrate moiety attached to threonine-194 that can exist in multiply sialylated forms (3, 4). Human apoE is encoded in many tissues (10). This same distribution of sites of apoE synthesis is found in several different species, although there are interspecies differences in relative production levels. The expression of most other apolipoproteins is restricted to many fewer tissues (7, 9).

The function of widespread extrahepatic apoE production may be to control the availability and redistribution of lipid for cell metabolic requirements in a variety of tissues. For example, it has been shown that accumulated macrophage-derived apoE participates in remyelination during peripheral nerve regeneration (16), and the use of cholesterol for steroid hormone synthesis by the adrenals may require the direct involvement of a specialized apoE-mediated delivery system.

To understand the role of apoE in extrahepatic lipid metabolism, a knowledge of the molecular signals that regulate apoE gene expression is required. An important first step would be the identification of tissue-specific control sequences associated with the gene. Since apoE appears to have a central role in linking extrahepatic lipid metabolism with that of the liver, the use of transgenic animals to examine the expression of modified constructs of the apoE gene is likely to be especially informative. In this study with transgenic mice, we have determined that in the absence of a downstream regulatory element, high levels of apoE are expressed in the kidney. In addition, a downstream element is required for expression in the liver.

MATERIALS AND METHODS

Preparation and Analysis of Transgenic Mice—DNA was purified by CsCl gradient centrifugation. DNA fragments intended for microinjection were purified further by gel electrophoresis (17), phenol extraction, and ethanol precipitation and then diluted to 1 ng/ml in 5 mM Tris, pH 7.4, 0.2 mM EDTA. Single cell embryos from ICR x ICR-bred mice were microinjected as described (18). The embryos surviving microinjection were transferred to the oviducts of pseudopregnant ICR female mice. Offspring were weaned at 3–4 weeks of age, and DNA was prepared from a small portion (1 cm) of their tails as described (18). Transgenic founder animals (F0) were identified by Southern blot analysis (19), and the founders were used to generate transgenic F1 mice. Approximately 35% of the founders were mosaic, and less than 50% of their F1 offspring were positive. Therefore, the animals used for all RNA and protein analysis were transgenic F1 male mice between 6 and 10 weeks of age.

Preparation and Analysis of Total RNA—Total cellular RNA was isolated as described (20). Antisense RNA probes for either human or mouse apoE mRNA were transcribed using bacteriophage T3 RNA polymerase in the presence of [32P]UTP (21) from cDNA fragments that had been cloned in Bluescribe vectors (Stratagene, San Diego, California 92241) (22). It can have an additional 3–5% of its mass in a carbohydrate moiety attached to threonine-194 that can exist in multiply sialylated forms (3, 4). Human apoE is encoded in many tissues (10). This same distribution of sites of apoE synthesis is found in several different species, although there are interspecies differences in relative production levels. The expression of most other apolipoproteins is restricted to many fewer tissues (7, 9).

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CA). Human and mouse apoE mRNA protected 188 and 127 nucleotides of their respective probes. Ribonuclease protection analysis was performed as described previously (21). Protected fragments were analyzed by electrophoresis in 6% polyacrylamide gels containing 7 M urea, followed by autoradiography of dried gels.

Analysis of Human Apolipoprotein E and Apolipoprotein C-I Expression in Transgenic Mice—Whole plasma from transgenic and non-transgenic mice was examined by Western blot analysis as previously described (22).

Immunocytochemistry—Transgenic and control mouse tissues were immersion-fixed for 4 h at 4°C in 4% formaldehyde (freshly prepared from paraformaldehyde) in 0.15 M phosphate buffer (pH 7.4) and cryoprotected by incubation overnight with 18% sucrose in phosphate-buffered saline. Small pieces of tissue were then quickly frozen in liquid nitrogen-cooled 2-methylbutane (11). Cryosections (10 μm) were dried onto gelatin-coated slides and treated with rabbit anti-human apoE immunoglobulin G (0.5 μg/ml), followed by an immunoperoxidase detection assay (Vector Laboratories, ABC kit, Burlingame, CA) as described (16). The tissue was counterstained lightly with methyl green, and brightfield images were photographed.

RESULTS AND DISCUSSION

Apolipoprotein E gene constructs prepared from cloned human genomic DNA having different lengths of 5'- and 3'-flanking regions were used to create transgenic mice (Fig. 1). One apoE construct, CLE1 (23), consisted of recombinant cosmid DNA that included 30 kilobases (kb) of 5'-flanking sequence and 1.5 kb of 3'-flanking sequence. The portion of the cosmid vector sequence that included SV40 regulatory regions (24) was deleted before use in these studies. The second apoE construct, HEG1, was a 10-kb DNA fragment that included only the proximal 5 kb of 5'-flanking sequence but the same 1.5 kb of 3'-flanking sequence as in the cosmid. The third apoE construct, CI.361 (25), was a recombinant cosmid that included 5 kb of proximal 5'-flanking sequence and 23 kb of 3'-flanking sequence. In this latter construct, the apoC-I gene was located 5 kb downstream of the apoE gene in the same transcriptional orientation, and an apoC-I pseudogene was located 7.5 kb downstream from the normal apoC-I gene, also in the same orientation (25).

Transgenic ICR mice were prepared essentially as described (18), and the number of founder (F0) animals for the constructs used in these studies is indicated in the legend to Fig. 1. Founder animals were identified by Southern blot screening (26) of genomic DNA (data not shown). Serial dilutions of transgenic mouse DNA followed by Southern blot analysis indicated that the number of integrated copies of the human apoE gene in the founder animals varied between 1 and 50. Additional Southern blot mapping confirmed the absence of any gross rearrangement in the human apoE gene constructs transferred to the transgenic mice.

Total cellular RNA was isolated from various tissues of F1 transgenic mice and examined by RNAse protection analysis with a human apoE 32P-labeled antisense RNA probe (Fig. 2). Human apoE mRNA in both CLE1 and HEG1 mice was abundant in the kidneys. Low levels of human apoE mRNA were present in the testes and tail skin of the CLE1 mice. Longer exposures of autoradiograms detected human apoE mRNA in the testes and tail skin of the HEG1 mice (data not shown). No human apoE mRNA was detected in other tissues of these transgenic mice, including the liver. These results suggested that a strong transcriptional element that determined expression of the apoE gene in the kidneys was in close proximity to or within the apoE gene. Although cell-free transcription experiments using hepatic cell nuclear extracts have demonstrated that the apoE promoter contains strong transcriptional control sequences (19), no expression of these transgenic mouse apoE mRNAs produced no ribonuclease-protected fragments from cross-hybridization longer than about 40 nucleotides (data not shown).

Fig. 1. DNA constructs containing the human apoE gene. Flanking sequences of constructs are described in the text. Exons of the apoE gene are indicated by the solid boxes and introns by Roman numerals. For the CLE1 construct, three independent transgenic founders (F0) were obtained from 25 animals. (F0) were screened (i.e. 3 F0/25); for the HEG1 construct, 6 F0/9 animals; for the CI.361 construct, 9 F0/23 animals.
located more than 1.5 kb downstream from the apoE gene and interacts with the transcription initiation complex to specify expression in the liver. In addition to a liver-specific element, there may be a silencer element located downstream of the apoE gene that decreases apoE gene transcription in the kidney and thus determines the final pattern of apoE gene expression. In the absence of these downstream sequences, a strong upstream control element drives expression of the apoE gene in the kidneys. The downstream elements may be located in the region between the apoE and the apoC-I gene. Such elements might play a role in regulating both genes. Although downstream regulatory elements are not unique to the apoE gene, the finding that the principal tissue-specific element necessary for apoE gene expression in the liver is located 3' of the gene is unusual.

It is of interest to note that the apoA-I gene, linked closely to the genes for apoC-III and apoA-IV on chromosome 11 (27), requires a far distal element for expression in the intestine (28). However, the relative location of the intestine element with respect to this gene cluster is unclear. These findings with the apoE and apoA-I genes in transgenic mice suggest the possibility that common regulatory elements in each apolipoprotein gene cluster may determine expression in specific tissues.

Human apoE mRNA and plasma protein from the transgenic mice were examined to characterize the expression of the transgene. Northern blot analysis (29) (Fig. 3, upper panel) showed that the transgenic mice produced human apoE mRNA of normal size in both the liver and kidneys. It was indistinguishable in size from the apoE mRNA of cultured human hepatoma cells and normal mouse tissues. Transgenic mice harboring the CI.361 genomic fragment also expressed human apoC-I mRNA in the liver, but no apoC-I mRNA was detectable in the kidney (Fig. 3, upper panel).

Plasma was collected from the tails of F0 or F1 animals and examined by Western blot analysis (22) (Fig. 3, lower panel). For plasma apoE analysis, a polyclonal antibody was employed that had a partial cross-reaction with mouse apoE, which was about 0.3 kDa smaller than human apoE. Most of the human apoE in the plasma of liver-expressing CI.361 transgenic mice migrated at a mobility similar to that of human plasma apoE (3). In the plasma of the kidney-expressing HEG1 mouse, human apoE migrated with an electrophoretic mobility slower than that of normal human plasma apoE. Neuraminidase treatment did not alter the electrophoretic mobility of the human apoE produced by transgenic mouse kidneys (data not shown). This latter finding suggests that sialic acid heterogeneity, which normally accounts for some polymorphism in human plasma apoE (1, 3, 4), was not responsible for the slow electrophoretic mobility of the human apoE produced by the transgenic kidney. Therefore, discovering the basis of the differences in mobility of human apoE in transgenic mouse plasma requires further analysis beyond the scope.
of this communication. A likely possibility is differences in post-translational processing of human apoE among mouse tissues, since differences in apoE processing in the tissues of other species, such as sulfation of rat cerebrospinal fluid apoE (30), have been reported (1, 16, 30).

The cellular distribution of apoE in the HEG1 transgenic mouse kidney was investigated by immunocytochemistry with human apoE antibodies. The results shown in Fig. 4 indicate that human apoE was found in the epithelial cells that line Bowman's capsule and the proximal convoluted tubule. No human apoE was detectable in other tubular structures or in the medullary region of the kidney. These observations are consistent with a previous report that rat apoE synthesis in the kidney was limited to the epithelial cells of the proximal convoluted tubule (10). Thus, even when high levels of human apoE mRNA are expressed in the kidney, the cellular distribution of apoE protein production in this organ remains normal. Future studies will examine the mechanism of action of the downstream elements controlling kidney and liver expression and how the interaction of these elements with the apoE promoter may dictate the normal pattern of human apoE gene expression.

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