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Accelerated Publications

Structure of the Human Hepatic Triglyceride Lipase Gene[†,‡]

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ABSTRACT: The structure of the human hepatic triglyceride lipase gene was determined from multiple cosmid clones. All the exons, exon–intron junctions, and 845 bp of the 5′ and 254 bp of the 3′ flanking DNA were sequenced. Comparison of the exon sequences to three previously published cDNA sequences revealed differences in the sequence of the codons for residues 133, 193, 202, and 234 that may represent sequence polymorphisms. By primer extension, hepatic lipase mRNA initiates at an adenine 77 bases upstream of the translation initiation site. The hepatic lipase gene spans over 60 kb containing 9 exons and 8 introns, the latter being all located within the region encoding the mature protein. The exons are all of average size (118–234 bp). Exon 1 encodes the signal peptide, exon 4, a region that binds to the lipoprotein substrate, and exon 5, an evolutionarily highly conserved region of potential catalytic function, and exons 6 and 9 encode sequences rich in basic amino acids thought to be important in anchoring the enzyme to the endothelial surface by interacting with acidic domains of the surface glycosaminoglycans. The human lipoprotein lipase gene has been recently reported to have an identical exon–intron organization containing the analogous structural domains [Deeb & Peng (1989) *Biochemistry 28*, 4131–4135]. Our observations strongly support the common evolutionary origin of these two lipolytic enzymes.

Hepatic triglyceride lipase is a lipolytic enzyme synthesized by the liver parenchymal cells and is localized primarily on the sinusoidal surface of the liver. It catalyzes the hydrolysis of tri-, di-, and monoglycerides, acyl-CoA thioesters, and phospholipids (Kuusi et al., 1982; Jensen et al., 1982; Jackson, 1983; Laboda et al., 1986). The enzyme hydrolyzes the triglycerides of intermediate-density lipoproteins to produce low-density lipoproteins and triglycerides and phospholipids of high-density lipoproteins ($HDL)_2$ to produce HDL_3 (Rao et al., 1982; Kinnunen et al., 1984). There is an inverse relationship between hepatic lipase activity and plasma HDL levels (Kuusi et al., 1983, 1987). Patients with familial hepatic triglyceride lipase deficiency accumulate high levels of HDL_2 in plasma, leading to hyper-α-triglyceridemia (Breckenridge et al., 1982; Little & Connelly, 1986; Carlson et al., 1986). Furthermore, studies in vivo and in vitro suggest that hepatic lipase is involved in the delivery of HDL phospholipid and cholesterol to the liver (Kussi et al., 1979; Jansen et al., 1980; Bamberger et al., 1983, 1985). The observation that high HDL levels protect against the development of atherosclerosis (Barr et al., 1951; Miller & Miller, 1975) has stimulated considerable interest in the potential role of hepatic lipase in HDL regulation and atherogenesis.

Cloned cDNAs of rat and human hepatic lipase have been isolated in a number of laboratories (Komaromy & Schotz, 1987; Stahnke et al., 1987; Datta et al., 1988; Martin et al., 1988; Semenkovich et al., 1989). The human gene for hepatic

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lipase has been assigned to chromosome 15 in the region 15q15 → q22 (Datta et al., 1988). There is considerable sequence homology among hepatic lipase, lipoprotein lipase, and pancreatic lipase, suggesting that the three enzymes may belong to a multigene family (Ben-Zeev et al., 1987; Komaromy & Schotz, 1987; Kirchgessner et al., 1987; Datta et al., 1988; Semenkovich et al., 1989). In this paper, we present the structure of the human hepatic lipase gene as deduced from multiple cosmid clones. The gene shows a marked similarity to the recently published human lipoprotein lipase gene (Deeb & Peng, 1989).

Experimental Procedures

Construction of Human Cosmid Library. Human chromosomal DNA was prepared from peripheral blood lymphocytes (Kan & Dozy, 1978) and partially digested with *Sau*3A restriction endonuclease. Digested DNA was layered onto 1.25–5 M NaCl gradients in 20 mM Tris (pH 7.5) and 1 mM EDTA and centrifuged for 3.5 h at 36000 rpm in a Beckman SW40 rotor. DNA fragments in the individual 1-mL fractions were analyzed by agarose gel electrophoresis. Fragments of 30–45 kb were pooled. The cosmid vector pCV001 DNA (Lau & Kan, 1983, 1984) or pWE15 (Wahl et al., 1987) was completely digested with *Bam*HI and treated with calf intestinal phosphatase (Boehringer Mannheim) (Maniatis et al., 1982). Completeness of the dephosphorylation was monitored by the failure of self-religation. Mixtures of 0.8 μg of the 30–45-kb human DNA and 2.5 μg of dephosphorylated linearized cosmid DNA were ligated in 20 μL with T4 DNA ligase for 16 h at 12 °C. Ligated DNA was packaged by using λ DNA in vitro packaging kit (Amersham) following the procedure described by the supplier. Two milliliters of packaged cosmids diluted in SM was used to transduce 2 mL of bacterial host DK1, which had been grown overnight in L broth with 0.4% maltose and then resuspended in 10 mM $MgSO_4$ (half the original growth volume). After 30 min of incubation at 37 °C, 16 mL of L broth was added to each transduction mixture. The incubation was continued for an additional 45 min. Fifty microliters of transduced bacteria was plated onto an L plate containing 50 μg/mL ampicillin to quantitate the number of transformants. The remaining cells were plated directly onto a Biodyne filter (ICN) in a 150-mm L plate containing ampicillin as described (Thomas et al., 1976). Replica filters were prepared and used for screening.

Screening of the human genomic cosmid library was performed by filter hybridization as described by Benton and Davis (1977). The human hepatic lipase cDNAs λHL2 and λHL3 (Datta et al., 1988) were ^{32}P-labeled by nick translation to a specific activity of $\sim 2 \times 10^8$ cpm/μg and used as a probe. Ten recombinant cosmid clones containing hepatic lipase sequences were identified from 1.5×10^6 colonies. DNA was prepared from them for further analysis by standard procedures (Maniatis et al., 1982).

Subcloning of Cosmid Genomic DNA into pGEM-Blue. Various restriction enzyme digested DNA fragments from recombinant cosmid DNA were hybridized to the human hepatic lipase cDNA probes. Positive fragments were isolated by agarose gel electrophoresis and subcloned into pGEM-Blue. pGEM-Blue was digested with the appropriate restriction enzymes, treated with calf intestinal phosphatase, ligated with the genomic DNA fragments, and used to transform *Escherichia coli* K12 strain DH5α (Maniatis et al., 1982). Bacterial colonies containing recombinant plasmids bearing the appropriate restriction fragments were identified by colony hybridization with the nick-translated human hepatic lipase cDNA probes λHL2 and λHL3 and by Southern blot analysis of the individual clones. Large-scale preparations of plasmid DNAs were prepared as described (Maniatis et al., 1982).

Sequencing of Hepatic Lipase DNA Fragments. Hepatic lipase DNA fragments subcloned in pGEM-Blue were sequenced by the dideoxynucleotide chain termination technique (Sanger et al., 1977). Synthetic oligonucleotide primers (20-mers) were synthesized on an Applied Biosystems 380A DNA synthesizer. Sequenase (U.S. Biochemical Co.) was used for the analysis which was performed on the double-stranded cloned DNA fragments.

5′ Mapping of Hepatic Lipase mRNA by Primer Extension. Total RNA was isolated from cultured HepG2 cells by the guanidinium isothiocyanate method (Chirgwin et al., 1979). Poly(A) RNA was partially purified from the total RNA by a single passage over an oligo(dT)–cellulose column (Aviv & Leder, 1972). Primer extension was performed by a modification of the procedure of Wei et al. (1985). A nucleotide primer with the sequence 5′-d(TCCAGGCTTTCTTGGTAA)-3′ complementary to the 5′ noncoding region of the mRNA was end-labeled with [γ-^{32}P]ATP by polynucleotide kinase. Fifty nanograms of the labeled primer and 3 μg of oligo(dT)-purified human liver poly(A) RNA were heated to 68 °C for 5 min in 50 μL of 78% formamide, 0.4 M NaCl, 9 mM Pipes, pH 7.4, and 1 mM EDTA and then incubated at 38 °C for 10 h. The RNA–DNA hybrid was precipitated with ethanol and dissolved in 12 μL of RT buffer (50 mM Tris-HCl, pH 8.3, 50 mM KCl, 5 mM $MgCl_2$, 40 mM dithiothreitol). Extension of the primer was performed as described by Wei et al. (1985). A genomic subclone covering this region of the lipase gene was sequenced in a parallel reaction with the same primer. The reaction was terminated by the addition of 10 μL of 95% formamide, 10 mM EDTA, 0.1% bromophenol blue, and 0.1% xylene cyanol. Samples were heated at 100 °C for 3 min before they were loaded on an 8% polyacrylamide sequencing gel (Maniatis et al., 1982).

Results

Isolation and Characterization of the Human Hepatic Triglyceride Lipase Gene. Two human genomic cosmid libraries were constructed in the vectors pCV001 (Lau & Kan, 1983, 1984) and pWE15 (Wahl et al., 1987). After multiple screenings of a total of 1.5×10^6 clones, we isolated 10 cosmid clones that together contain all the 9 exons and most of the 8 introns that separate them. Within intron 1, there is a gap that is not covered by the genomic clones. The exon–intron organization of the human hepatic triglyceride lipase gene is shown in Figure 1. The gene spans over 60 kb. Exons with the cloned genomic DNA were confirmed by plasmid subclones identified by restriction mapping and Southern blot hybridization using human hepatic lipase cDNA clones as probes. All the exon sequences and the nucleotide sequences at the exon–intron boundaries (Table I) were established by direct DNA sequence comparison of cDNA and genomic subclones. All the introns begin with dinucleotides GT and end with dinucleotides AG and are consistent with the consensus sequence found around exon–intron junctions (Breathnach & Chambon, 1981).

Sequence of the Human Hepatic Triglyceride Lipase Exons. All the clones containing exon sequences were subcloned into pGEM-Blue and were subjected to direct double-stranded DNA sequencing by using the dideoxynucleotide chain termination technique (Sanger et al., 1977). The exon sequences (Figure 2) completely matched our previously published cDNA sequence (Datta et al., 1988) except for two bases: codon 193 is AAT encoding Asn instead of AGT encoding Ser in the cDNA sequence, and codon 202 is ACG instead of ACC in

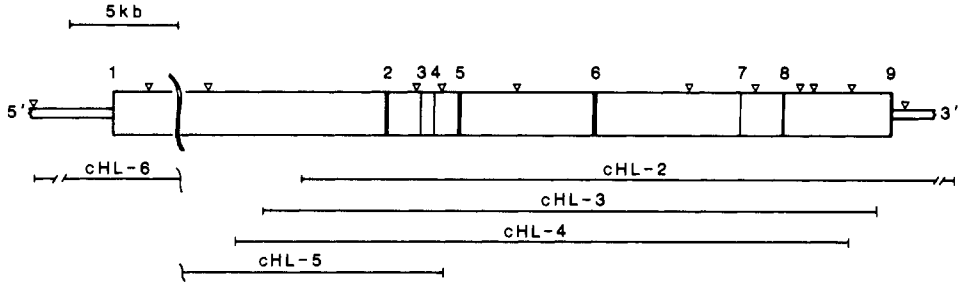

FIGURE 1: Structure of the human hepatic triglyceride lipase gene. The gene is shown in the 5′ to 3′ orientation and is drawn to scale. Exons are denoted by filled-in areas and introns by open areas. The regions encompassed by the genomic cosmid clones are indicated below the map. In the 5′ region covering exon 1, although only one clone, cHL-6, is shown, four other clones covering this region have also been isolated. The triangles denote the *Eco*RI sites. The diagonal lines between exons 1 and 2 represent a gap of unknown size.

Table I: Exon–Intron Organization of the Human Hepatic Triglyceride Lipase Gene

Exon Number	Exon Size (bp)	Sequence at exon-intron junction 5′ Splice Donor	3′ Splice Acceptor	Intron Length (kb)	Preceding or Interrupted Amino Acid
1	165	AAA CCA G gtaaga.....	.....aatcttatattgcag AG CCA TTT	>40	Glu (8)
2	185	GGG TGG TCG gtagga.....	.....ctgtcccctcctcag GTG GAC GGC	1.4	Ser (69)
3	183	TGG CTG GAG gtaccg.....	.....gctgctgtcttccag GAA TCT GTT	0.55	Glu (130)
4	127	ATC ACA G gtaacc.....	.....ctgctttcccattag GG CTG GAT	1.1	Gly (170)
5	234	TTC AAT G gtgaga.....	.....tgccctgtgttccag CC ATC ACC	5.9	Ala (248)
6	243	TTC AAA G gtgagt.....	.....tgtctctctctctag TT TAT CAT	6.7	Val (329)
7	118	ATC ACT CT gtgagt.....	.....ctttgtgtattcaag G GGC AAA	2.3	Leu (368)
8	219	CAG CAA AG gtgact.....	.....ctgttttctattcag A ATG ACA	4.9	Arg (441)
9	158				

the cDNA, both encoding Thr.

Primer Extension of the Human Hepatic Triglyceride Lipase cDNA. The CAP site of the human hepatic lipase gene was mapped by primer extension. A major extension product of 58 nucleotides (including the 18-base primer) was seen (Figure 3). Therefore, the major species of human hepatic lipase mRNA has an initiation site 77 bases upstream of the translation initiation codon AUG, and transcription of the human hepatic lipase gene starts at an adenine residue, a purine nucleotide, in agreement with transcription initiation sites in other eukaryotic genes. We also observed other minor mRNA species containing 76 and 78 bases of the 5′ untranslated region that were present in much lower concentrations (Figure 3).

The 5′ and 3′ Flanking DNA of the Human Hepatic Triglyceride Lipase Gene. We determined the sequence of 845 bp of the 5′ flanking DNA of the human hepatic triglyceride lipase gene (Figure 2). A TATA-boxlike structure with the sequence TATTAA is present 25 bp upstream of the transcription initiation site. Further upstream at −349 bp from the CAP site is located a sequence CAATTT that could potentially be a CAAT-box structure. We sequenced 254 bp of the 3′ flanking DNA for the human hepatic lipase gene (Figure 3). There is a duplicate copy of the polyadenylation signal AATAAA 61 bp downstream to the polyadenylation site identified in our cDNA sequence (Datta et al., 1988). It is not known if this second signal is used in vivo because the previously published cDNA sequences (Stahnke et al., 1987; Datta et al., 1988; Martin et al., 1988) all ended in a site proximal to this sequence.

Table II: Differences in Nucleotide Sequence in the Coding Region of the Human Hepatic Lipase DNA

study	residue no.[a] 133	193	202	234
Stahnke et al. (1987)	GT<u>T</u>[b] (Val)	AAT (Asn)	ACG (Thr)	T<u>T</u>C (Phe)
Datta et al. (1988)	GTG (Val)	A<u>G</u>T (Ser)	AC<u>C</u> (Thr)	TCC (Ser)
Martin et al. (1988)	GTG (Val)	AAT (Asn)	ACG (Thr)	TCC (Ser)
present study	GTG (Val)	AAT (Asn)	ACG (Thr)	TCC (Ser)

[a] Refers to the mature peptide. [b] The bases unique to only one of the four sequences are underlined.

Discussion

To date, four sequences of the human hepatic triglyceride lipase mRNA as deduced from their cDNA (Stahnke et al., 1987; Datta et al., 1988; Martin et al., 1988) and genomic (present study) sequences have been published. By direct peptide sequencing, Martin et al. (1988) showed that the NH_2 terminus is a Leu residue. The deduced amino acid sequence predicts a 477 amino acid mature polypeptide preceded by a 22-residue signal peptide. The mRNA sequence deduced from the genomic structure in the present study (Figure 2) is identical with that deduced from the cDNA published by Martin et al. (1988). Comparison of these two sequences with those of Stahnke et al. (1987) and Datta et al. (1988) reveals discrepancies in the sequence of the following four codons (Table II): codon 133 is GTT in Stahnke et al. and GTG in the others, both encoding valine; codon 193 is AGT encoding

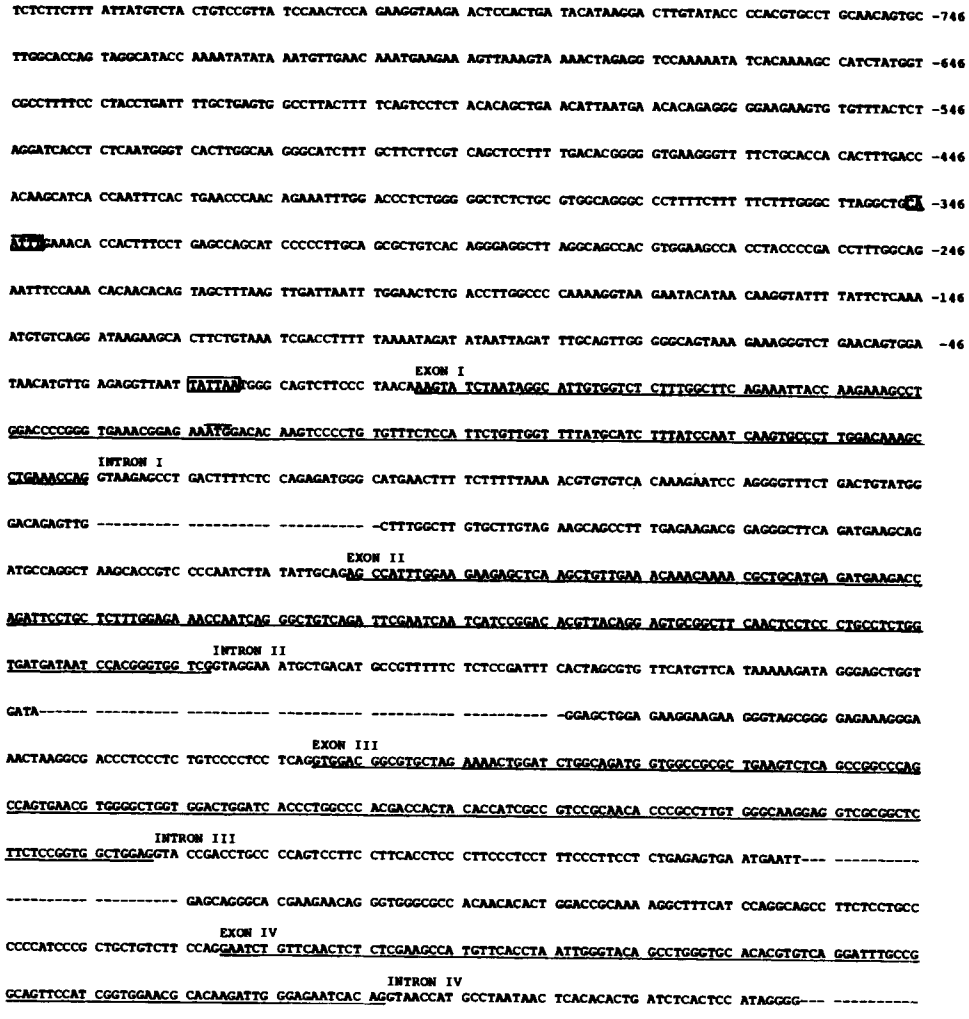

FIGURE 2: Sequence of the 5′ and 3′ flanking DNA, exons, and exon–intron junctions of the human hepatic triglyceride lipase gene. All the exons are underlined and marked. Individual introns are also labeled. The 5′ flanking DNA is numbered by taking the first base preceding the transcription initiation site as −1. The putative TATA box and CAAT box are boxed. The translation initiation codon is marked by a line above it. The translation termination codon and polyadenylation signals are marked by double underlines.

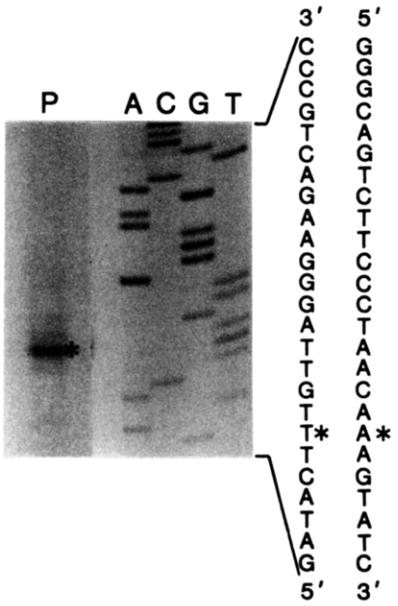

FIGURE 3: Primer extension of human hepatic triglyceride lipase mRNA. Primer extension was performed by using the oligonucleotide primer 5′-d(TCCAGGCTTTCTTGGTAA)-3′ that is complementary to the 5′ untranslated region of human hepatic lipase mRNA according to the procedure described under Experimental Procedures. The ^{32}P-labeled reverse-transcribed product was fractionated on an 8% polyacrylamide sequencing gel next to the sequence reaction products of a genomic covering the same region and primed by the same oligonucleotide. The autoradiograph is shown on the left, and the corresponding sequence and complementary sequences are shown on the right. The major product is marked with an asterisk. Minor products that are longer or shorter by a single base are also detected at much lower concentrations.

Ser in Datta et al. and AAT encoding Asn in the others; codon 202 is ACC in Datta et al. and ACG in the others, both encoding Thr; finally, codon 234 is TTC encoding Phe in Stahnke et al. and TCC encoding Ser in the others. It is not clear if these differences represent true sequence heterogeneity, cloning artifacts, or sequencing errors. We resequenced the cDNA clones that we reported previously (Datta et al., 1988) and confirmed that the sequence was accurate. We note that if the Ser-234/Phe-234 substitution represented bona fide protein polymorphism, the allelic difference may be functionally significant because it involves the replacement of a polar residue by a nonpolar one.

The human hepatic triglyceride lipase gene contains 9 exons separated by 8 introns. It spans over 60 kb with the exons accounting for only 1.6 kb of its total length. All except one (intron 3) of the introns are longer than 1 kb. They are located in the coding portion of the gene, all of them interrupting exons in the mature peptide region, there being no intron in the 5′ or 3′ untranslated regions or the signal peptide region of the gene.

In the 5′ flanking region of the hepatic lipase gene, we identified a TATA-box and CAAT-boxlike structure at −25 and −349 bp, respectively. In addition, there are four 6-bp GC-rich motifs at −398, −387, −188, and −77 bp from the transcription initiation site. These sequences show only partial homology to the Sp1 consensus binding sequence 5′-$^{GG}_{TA}$C-CCC$^{GGGC}_{TAAT}$-3′ (Briggs et al., 1986), and it is unclear whether any of them represent bona fide GC boxes.

The two "endothelial" lipases, lipoprotein lipase and hepatic triglyceride lipase, and digestive pancreatic lipase have highly homologous structures and may share a common evolutionary origin (Ben-Zeev et al., 1987; Kirchgessner et al., 1987; Datta et al., 1988). While we were preparing this paper for publication, Deeb and Peng (1989) published the structure of the human lipoprotein lipase gene. Comparison of the hepatic lipase and lipoprotein lipase genes showed an almost identical structural organization. The amino acid sequence alignment and intron locations for these two genes are shown in Figure 4. The introns are all located at identical sites with the following three exceptions: (i) intron 1 is located in a region of the sequence where there are major deletions in lipoprotein lipase and the alignment is ambiguous; (ii) there is a slight shift in the position of intron 4; in hepatic lipase, it is between the first and second base of Gly-170 and in lipoprotein lipase, it immediately precedes Gly-154; and (iii) intron 9 is present only in the lipoprotein lipase gene, interrupting the termination codon TG↓A. The additional exon in lipoprotein lipase containing 1948 bp of the 3′ untranslated sequence is the largest of all the exons in both genes.

In the human hepatic triglyceride lipase gene, we can identify the following exons that correspond to specific

FIGURE 4: Alignment and intron locations of the human hepatic lipase (HL) and lipoprotein lipase (LPL) genes. The introns are indicated by the solid triangles when they interrupted an amino acid codon and by arrows when they were inserted between two codons. The numbering of the introns is shown for HL only except for intron 9, which is present only in the LPL gene; it interrupts the termination codon TG↓A. Symbols: ---, missing residues; *, identical residues. The intron locations for the human LPL gene are taken from Deeb and Peng (1989).

structural and putative functional domains of the enzyme: exon 4, the interfacial lipid-binding region by which the enzyme binds to its substrate (Senda et al., 1987); exon 5, a conserved sequence with high homology among the various lipases (see identical residues represented by asterisks in Figure 4) that might serve important catalytic function (Komaromy & Schotz, 1987; Wion et al., 1987; Datta et al., 1988); exons 6 and 9, domains rich in basic amino acids (Wion et al., 1987) that may be responsible for the enzyme binding to the acidic domain of heparan sulfate on the surface of the capillary endothelium. Deeb and Peng (1989) recently identified the same exons in the human lipoprotein lipase gene which contain analogous domains that perform the same functions as those in hepatic lipase. These observations lend further support to the hypothesis that the two genes shared a common ancestor during evolution, a conclusion also supported by direct sequence comparison of the two lipases (Ben-Zeev et al., 1987; Kirchgessner et al., 1987; Datta et al., 1988).

The elucidation of the genomic structures of human hepatic lipase and lipoprotein lipase together with knowledge on the complete cDNA sequence of the enzymes (Wion et al., 1987; Stahnke et al., 1987; Datta et al., 1988; Martin et al., 1988) provides the necessary background for investigations on the genetic basis for heritable deficiencies in these two enzymes (Breckenridge et al., 1982; Little & Connelly, 1986; Carlson et al., 1986; Brunzell, 1989) and the molecular mechanism of the tissue-specific regulation of these two otherwise very similar enzymes.

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