The Human Colipase Gene: Isolation, Chromosomal Location, and Tissue-Specific Expression†

Harold F. Sims and Mark E. Lowe*

Department of Pediatrics, Washington University School of Medicine, St. Louis, Missouri 63101

Received January 2, 1992; Revised Manuscript Received March 26, 1992

ABSTRACT: The digestion of dietary triglycerides occurs in the duodenum through the action of triglyceride lipase, a pancreatic exocrine protein. The activity of pancreatic lipase is inhibited by the bile salts normally found in the gut lumen. Another pancreatic exocrine protein, colipase, restores the lipolytic activity of triglyceride lipase. The synthesis and secretion of both triglyceride lipase and colipase is increased by dietary fats and secretin. An increase in mRNA accompanies the increased activity, suggesting that the genes for triglyceride lipase and colipase contain nucleotide elements responsive to dietary fats or secretin or both. To study the regulation of colipase expression, we have first isolated the gene for human colipase from a cosmid library with a cDNA probe. The gene was localized to chromosome 6 and is organized into three exons contained in a single 3.3-kb *Bam*HI fragment. The 5′-flanking region of the gene contains a TATA box, a GC box, and a 28-bp region with homology to the rat pancreatic-specific enhancer. This region directs the tissue-specific expression of the chloramphenicol acetyltransferase gene in a transfected rat pancreatic acinar cell line, AR42-J. The same construct is inactive in HEPG2, C2C12, and COS-1 cells. These results demonstrate that the isolated gene for human colipase contains tissue-specific promoter activity in the 5′-flanking DNA. The 28-bp region specifically binds to a factor in nuclear extracts.

Dietary triglycerides are hydrolyzed into more polar fatty acids and monoglycerides in the duodenal lumen by pancreatic triglyceride lipase. The activity of triglyceride lipase is inhibited by the presence of bile salts but is restored by a small molecular weight protein, colipase. Optimal activity occurs at a 1:1 molar ratio of colipase and lipase (Borgstrom & Erlanson-Albertsson, 1984). Both colipase and triglyceride lipase are synthesized and secreted by the pancreas. The coordinated secretion of these proteins is crucial to the efficient digestion of fats.

Several observations suggest that net triglyceride hydrolysis may be controlled by colipase levels. Colipase, but not lipase, is secreted as a proform that poorly restores lipase activity against mixed lipid emulsions (Brockman et al., 1973; Erlanson-Albertsson & Larsson, 1981). After cleavage of an NH_3-terminal pentapeptide, colipase is fully active (Wieloch et al., 1981). Clearly, the rate of cleavage would affect the hydrolysis rate of triglycerides. Additionally, colipase is present at least than 1 mol/mol of lipase in pancreatic secretions and may be the rate-limiting component of triglyceride hydrolysis in the duodenum (Gaskin et al., 1982).

Although lipase and colipase are functionally linked, little is understood about the control of their expression. Colipase and triglyceride lipase are only expressed in pancreatic acinar cells (Lowe et al., 1989, 1990). Thus, tissue-specific elements must regulate their expression. The activity of both proteins increases in pancreatic secretions in response to increased dietary fats and to secretin (Erlanson-Albertsson et al., 1987; Wicker et al., 1988; Rausch et al., 1986; Scheele et al., 1988). The magnitude of the increase varies depending on the amount and type of fat. The rise in triglyceride lipase activity in response to high-fat diets is accompanied by an increase in lipase mRNA level as much as 6.5-fold (Wicker & Puigserver, 1989). Colipase mRNA levels also increase in response to high-fat diets (Wicker & Puigserver, 1990). These results suggest that transcription of both colipase and lipase may be regulated by elements that are controlled by dietary lipids. The isolation and characterization of the gene for colipase is a first step in understanding the control of this gene.

EXPERIMENTAL PROCEDURES

Screening of Cosmid Library. A human genomic library in pTCF was kindly provided by Dr. R. Wetsel (Washington University School of Medicine). Approximately 700 000 colonies were screened with a random primer ^{32}P-labeled probe generated from human colipase cDNA (Lowe et al. 1990). Twenty positive clones were selected and two were purified to homogeneity by repeated subcloning. Cosmid DNA was purified from broth cultures with a Qiagen column.

DNA Blot Analysis of Cosmid Clones. Cosmid DNA was digested with a series of restriction enzymes and the products were separated by agarose gel electrophoresis. The DNA was transferred to nylon membranes by capillary transfer using 0.5 M NaOH and 1.5 M NaCl. The membranes were neutralized with 2× SSC, cross-linked with UV light, and hybridized as described previously with probes from the whole colipase cDNA generated by random primer labeling (Lowe et al., 1990). The blots were washed in 0.1× SSC at 50 °C.

DNA Sequence Analysis. Both strands were sequenced in pGEM3z by the dideoxynucleotide chain termination method using Sequenase. Universal primers and synthetic oligonucleotides complementary to internal sequences were utilized as primers.

Chromosome Analysis. DNA blots of hamster–human somatic cell hybrid DNA digested with *Pst*I were obtained from Bios Corp. The blot was probed with random primer labeled colipase cDNA according to the instructions. Two bands hybridized to the probe in the positive hybrids and in human genomic DNA digested with *Pst*I. No binding was detected to hamster DNA.

Construction of pCAT Vectors and Transfection. The initial constructs were made by digesting the *Bam*HI fragment

† This research was supported by National Institutes of Health Grant HDK334887.

* To whom correspondence should be addressed.

0006-2960/92/0431-7120$03.00/0

in pGEM3z with *Nco*I and creating a blunt end with T4 DNA polymerase (Sambrook et al., 1989). Then, the DNA was digested in the multiple cloning region with *Hin*dIII and the 5′-flanking region from +32 to −635 was then isolated from an agarose gel with DEAE paper. Both pCAT Basic and Enhancer plasmids (Promega) were digested with *Xba*I and blunt-ended with T4 DNA polymerase followed by digestion with *Hin*dIII. The blunt-end/*Hin*dIII fragment was ligated into each plasmid. The ligation reaction was used to transform TB1 cells and colonies with plasmids containing insert were isolated. DNA was prepared from broth cultures with Qiagen columns. The insert was sequenced with primers complementary to the plasmid sequence flanking the insert site.

Other constructs were made by the polymerase chain reaction (Ho et al., 1989; Higuchi, 1988). The 3′-primer was complementary to bp +2 to −25 and contained an *Xba*I site at the 3′-end. The 5′-primer was complementary to 27 bp at the 5′-end of a given construct as defined in the figures and contained an *Sph*I site at the 5′-end. The 3.3-kb *Bam*HI fragment was used as template. Only five cycles of the polymerase chain reaction with 500 ng of template were done to minimize errors. The product was isolated from agarose gels by the DEAE paper method. The isolated insert was digested with *Xba*I and *Sph*I and cloned into *Xba*I/*Sph*I-digested pCAT Basic plasmid. The pCAT Enhancer constructs were made by subcloning the *Xba*I/*Hin*dIII-digested insert from the appropriate pCAT Basic plasmid into *Xba*I/*Hin*dIII digested pCAT Enhancer plasmid.

A concatemer of the region between −121 and −150 was made by ligating together synthetic oligonucleotides made to that region. The oligonucleotides contained complementary *Xma*I overhangs. The mixture of concatemers was ligated into *Xma*I-digested pGEM3z, and colonies were isolated. The largest insert contained five copies of the enhancer sequence in head-to-tail orientation by sequence analysis. The 5-mer was ligated 5′-ward of the SV-40 promoter and 3′-ward of the CAT gene in pCAT vectors containing the SV-40 promoter.

The various cell lines were transfected with the selected plasmids by calcium phosphate coprecipitation (Graham & Van der Eb, 1973). Twenty micrograms of test plasmid and 5 μg of a plasmid containing the β-galactosidase gene driven by an RSV or MSV promoter were transfected into cells on a 60-mm tissue culture dish. The cells were harvested 60 h after transfection. β-galactosidase activity was measured colorimetrically (Norton & Coffin, 1985). Chloramphenicol acetyltransferase (CAT) was measured with ^{14}C-chloramphenicol and *n*-butyryl-coenzyme A as substrates. The amount of product was determined by xylene extraction and scintillation counting (Seed & Sheen, 1988). The assay was linear from 1000 to 150 000 dpm (40–45% conversion). Background was defined as the CAT activity present in cells transfected with the pCAT vector lacking either a promoter or enhancer. The background (500–1000 dpm) was subtracted from the experimental values. The CAT activities were adjusted for β-galactosidase activity to correct for transfection efficiency in each cell type.

Preparation of Nuclear Extracts. Extracts were prepared from the isolated nuclei of HEPG2 and human acinar cells (a gift from Drs. J. Rosenblum and D. Sharp, Washington University School of Medicine) by a published method (Ausubel et al., 1991). The nuclei were extracted as described with high-salt buffer containing 0.8 M KCl and 1.0 mM benzamidine. The final extract was stable for months at −70 °C and for several weeks at −20 °C. Storage at 4 °C resulted in the loss of activity over several days.

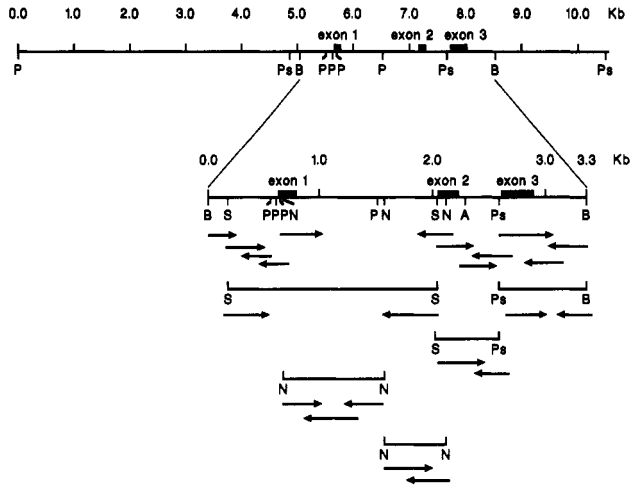

FIGURE 1: Sequencing strategy for the human colipase gene. The portion of the cosmid clone containing the exons and flanking fragments is shown in the upper line. The *Bam*HI fragment encompassing the exons and introns is shown below. The subclones of the *Bam*HI fragment that were sequenced are shown by line drawings below the *Bam*HI fragment. The arrows indicate regions sequenced by universal or synthetic oligonucleotide primers. A, *Acc*I; B, *Bam*HI; N, *Nco*I; P, *Pvu*I; Ps, *Pst*I; S, *Sac*I.

Gel Shift Assay of DNA Binding. The DNA-binding assay was as described with the low ionic strength PAGE system (Ausubel et al., 1991). The probe was prepared by phosphorylating complementary oligonucleotides with ^{32}P and annealing them together. Synthetic oligonucleotides corresponding to the sequence GCCACCAGGTACTCACCCAGTTTCCCTT were labeled. Binding reactions were done in 5 μL with the following components: 2 μL of 2× binding buffer (50 mM HEPES, pH 7.9, 8 mM Tris-HCl, pH 7.9, 25% glycerol, 200 mM KCl, 2 mM EDTA, 2 mM $CaCl_2$, 10 mM $MgCl_2$, 2 mM DTT, 200 ng/μL poly(dI-dC), 200 ng/μL sonicated herring sperm DNA, and 300 ng/μL BSA), 2 μL of labeled probe in H_2O (10 000–20 000 cpm, 5–10 fmol), and 1 μL of nuclear extract or purified binding protein. If competitors were added, they were mixed with the labeled probe and added together in 3 μL. The mixture was incubated for 15–30 min at 30 °C and the bound probe was separated from the unbound probe by PAGE.

RESULTS AND DISCUSSION

Cloning and Organization of the Human Colipase Gene. Two positive clones purified by screening a human cosmid library with the human colipase cDNA were examined by restriction digestion and DNA blot analysis with the cDNA probe. Although the restriction digests of the two clones differed in their ethidium bromide banding pattern, the DNA blot analysis of the two clones was identical (data not shown). In particular, both clones contained a single *Bam*HI fragment of 3.3 kb that hybridized to the colipase probe. The *Bam*HI fragment was subcloned into pGEM and sequenced. To facilitate sequencing, restriction fragments from the original subclone were cloned into pGEM (Figure 1). The entire *Bam*HI fragment was sequenced completely on both strands with this approach. The sequencing strategy and partial restriction map is given in Figure 1.

Portions of the sequence are presented in Figure 2. The gene is organized into three exons. The nucleotide sequence of the exons is identical to that determined for the human colipase cDNA (Lowe et al., 1990). The first exon contains the 5′-untranslated region of the mRNA and the sequence encoding the signal peptide, the propiece, and the first 6 amino

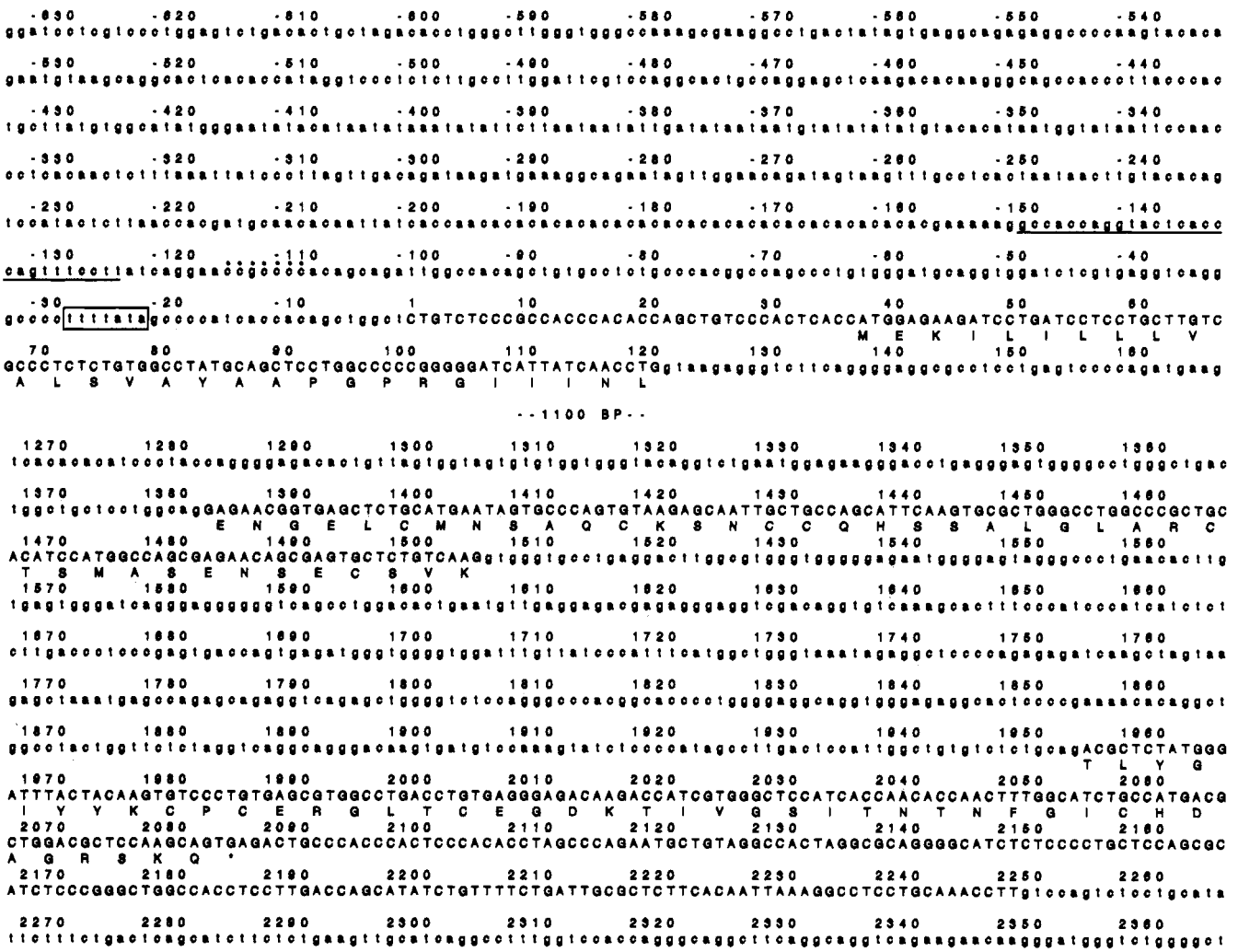

FIGURE 2: Nucleotide sequence of the human colipase gene. The exons, selected portions of the introns, and flanking regions are given. The nucleotides in the exons are in capital letters. The predicted amino acid sequence is given in the single-letter code below the exons. The TATA box is boxed. The putative tissue-specific enhancer is underlined. The nucleotides of the SP1 binding site have dots above them.

acids of the mature protein. This pattern differs from the gene organization of other pancreatic exocrine proteins in which the signal peptide is encoded by a separate exon. Thus, the colipase gene is an exception to the earlier prediction that a separate exon would encode the signal peptide for pancreatic secretory proteins (Scheele & Jacoby, 1983). The remaining two exons encode the rest of the protein and the 3′-untranslated region.

Chromosomal Location of the Human Colipase Gene. To determine which chromosome contains the colipase gene, a DNA blot of human–hamster somatic DNA was probed with the entire colipase cDNA. We had previously determined that a *Pst*I digest of human and hamster genomic DNA gave two bands in the human DNA and no detectable bands in the hamster DNA under our hybridization conditions (unpublished data). DNA from each of the cell lines that contained human chromosome 6 hybridized to the colipase probe (Table I). In contrast, the probe did not hybridize to the DNA from the cell lines lacking chromosome 6. The concordance/discordance ratios indicate that the human colipase gene is located on chromosome 6.

Potential Regulatory Regions of the Human Colipase Gene. The first 635 bp of the 5′-flanking region are shown in Figure 2. Within this region, there is a TATA box 28 bp from the transcription start site which was previously determined by primer extension analysis (Lowe et al., 1990). A CAAT consensus sequence is located 207 bp from the transcription start site, and a single SP1 consensus sequence is present at bp −115. A poly(dC-dA) region of 36 bp is present from bp −159 to −194. Poly(dC-dA) or poly(dG-dT) regions are commonly found in intergenic segments or in introns but are rarely located near the transcription start site (Rich et al., 1984).

Tissue-Specific Promoter Activity. The presence of a functional promoter and tissue-specific elements in the 5′-flanking region of the isolated colipase gene was tested by transient expression assays. Nucleotides +37 to −635 were linked to the CAT gene in a transfection vector. The SV-40 enhancer element was included in some constructs to determine if the addition of an enhancer would permit expression, particularly in nonpancreatic tissues. The colipase vector and a control plasmid consisting of the SV-40 promoter and enhancer driving the CAT gene were introduced into several cell types and CAT activity was determined (Figure 3). Expression of the colipase construct in the rat acinar cell line, AR42-J, was 2.5 times the levels obtained with the position control (Figure 3A). The levels of CAT activity were 10–25 times background. The expression of CAT activity was not changed by the presence of the SV-40 enhancer (Figure 3B). In contrast, little or no activity was detected in three heterologous cell types unless the SV-40 enhancer was present. Then, low levels of expression relative to the positive control were found in the HEPG2 and C2C12 cells, but no detectable activity was present in the COS-1 cells. These results

Table I: Segregation of Colipase with Human Chromosomes in *Pst*I-Digested Human–Hamster Somatic Cell Hybrid DNA[a]

	Human Chromosome Number																							
Hybrid cell number	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	X	Y
Positives																								
860	-	-	+	-	+	+	-	-	-	+	-	-	-	-	-	-	-	-	+	-	+	-	-	-
909	-	-	-	-	+	+	-	+	-	-	-	-	-	+	-	-	-	-	-	-	-	-	+	-
756	-	-	-	-	+	+	+	-	-	-	-	+	+	+	-	-	-	-	+	+	+	-	-	+
904	-	-	-	-	+	+	-	-	-	-	-	+	-	-	-	+	-	-	-	-	+	-	-	+
Negatives																								
867	+	-	-	-	+	-	-	-	-	-	-	-	+	+	-	-	-	+	+	-	-	-	-	-
854	-	+	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
423	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
803	-	-	-	+	+	-	-	+	-	-	-	-	-	-	-	-	-	+	-	-	+	-	-	-
1006	-	-	-	+	+	-	+	+	-	-	-	-	+	-	+	-	-	-	+	-	+	-	-	-
811	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	+	+	-	-	-	-	-	-
967	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-
734	-	-	-	-	+	-	-	-	+	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-
968	-	-	-	-	+	-	-	-	+	-	-	-	+	-	-	-	-	-	-	-	-	-	+	-
683	-	-	-	-	+	-	-	-	-	-	+	+	-	+	-	-	-	-	+	-	+	+	-	-
507	-	-	+	-	+	-	-	-	-	-	-	+	-	+	-	-	-	-	-	+	-	+	-	+
750	-	-	-	-	+	-	-	-	-	-	-	-	+	+	+	-	-	-	+	-	-	-	-	-
1099	+	-	-	-	+	-	-	-	-	-	-	-	+	-	-	-	-	-	+	-	+	+	-	-
324	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-
940	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-
983	-	-	-	-	+	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-
937	+	-	-	-	+	-	-	-	-	-	-	-	-	+	+	-	+	-	-	-	+	-	-	-
1079	-	-	+	-	+	-	-	-	-	+	-	-	-	-	+	+	-	-	-	-	-	-	-	-
862	-	-	-	-	+	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
1049	-	-	-	-	+	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-
212	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+
Chromosome #	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	X	Y
# Concordant	18	20	19	19	7	25	21	19	18	21	18	21	17	18	17	20	19	16	18	20	19	18	21	21
# Discordant	7	5	6	6	18	0	4	6	7	4	7	4	8	7	8	5	6	9	7	5	6	7	4	4
% Discordancy	28	20	24	24	72	0	16	32	28	16	28	16	32	28	32	20	24	36	28	20	24	28	16	16

[a] DNA blots of human–hamster somatic cell hybrid DNA were hybridized with random primer labeled probes from the whole cDNA as described in Experimental Procedures. Concordant hybrids have a positive signal when a specific chromosome is present and a negative signal when a specific chromosome is absent. Discordant hybrids have a positive signal when a specific chromosome is absent or a negative signal when a specific chromosome is present. The percent discordancy indicates the degree of discordant segregation of a probe and a chromosome. Chromosome assignment is made when 0% discordancy is present.

demonstrate the presence of both a functional promoter and tissue-specific elements in the first 635 bp of the colipase gene 5′-flanking region.

The location of the tissue-specific element was identified by transfecting additional constructs with progressive 5′-deletions of the flanking region into AR42-J and HEPG2 cells (Figure 4). The SV-40 enhancer was included in some constructs to help locate the basal promoter element. None of the constructs were active in the HEPG2 cells unless the SV-40 enhancer was present (Figure 4). All of the constructs had low but detectable activity with the SV-40 enhancer. The highest activity was seen with the −121 vector, suggesting that the minimal promoter is located in the first 121 bp of the 5′-flanking region.

In the AR42-J cells, low levels of expression were obtained with the +2 to −87 and −121 constructs. If an additional 29 bp was added, 90-fold more activity relative to the −87 vector was present in the AR42-J cells. Adding additional 5′-ward sequence decreased the activity 2–3-fold. The presence of the SV-40 enhancer did not affect the levels of expression in the pancreatic cells.

Decreases in CAT activity upon extending the 5-flanking sequence past −150 bp suggests the presence of a negative regulatory element in the region past the enhancer. The element is active in both AR42-J and HEPG2 cells and may be a general transcriptional element. Transcription factors that bind to this element may interact with the neighboring tissue-specific factors bound to the pancreas-specific element. The nucleotide sequence of the negative element has not been identified, but it resides in the 65 bp between −150 and −215. Interestingly, a 36-bp dC-dA repeat is located between bp −150 and −215. This region has a high potential to form left-handed Z-DNA and, as such, is a region that may be recognized by trans-acting elements (Rich et al., 1984). The importance of regions with the potential for forming Z-DNA is unclear, but several potential Z-DNA regions are part of the SV-40 enhancer element for the viral early promoter (Rich et al., 1984). Z-DNA regions may be important elements controlling transcription.

The 45-fold increase in CAT expression of the −150 construct over the −121 construct in the AR42-J cells suggests that an enhancer element resides between −121 and −150 bp. Additional evidence supporting an enhancer function for this region was obtained by transfecting SV-40 promoter pCAT constructs containing multiple copies of the putative enhancer sequence (Figure 5). The presence of the enhancer concatemer either 5′-ward of the promoter or 3′-ward of the CAT gene allowed expression in the AR42-J cells. The promoter pCAT without any enhancer gave no detectable CAT activity. Neither enhancer construct was expressed in transfected HEPG2 cells (data not shown). The ability of the region between −121 and −150 to be moved and to confer tissue specificity to a heterologous promoter is consistent with an enhancer function for the sequence.

The sequence in this region also shows homology to the rat pancreatic-specific enhancer (Table II) (Boulet et al., 1986). This element is present in the 5′-flanking region of genes for many pancreatic exocrine proteins and is required for pancreas-specific expression of reporter genes in transfected cells and transgenic mice (Boulet et al., 1986; Hammer et al., 1987).

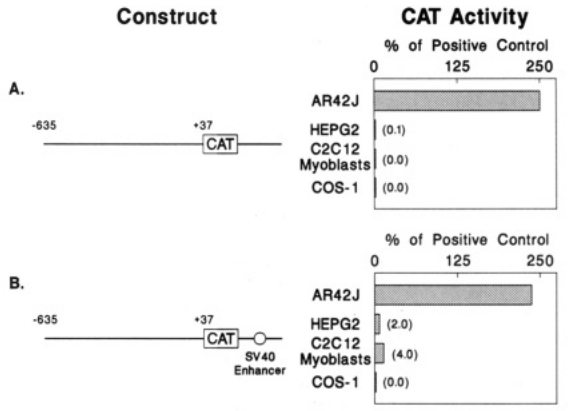

FIGURE 3: Cell-specific activity of human colipase gene 5′-flanking sequences. A portion of the 5′-end of the colipase gene from bp +37 to −635 was fused to the 5′-end of the CAT gene in a transfection vector. The constructs were transfected into various cell types. AR42-J, rat pancreatic acinar cell line; COS-1, monkey kidney epithelium cell; C2C12, mouse myoblast cell line; HEPG2, human hepatoma cell line. Transfections were done as described in Experimental Procedures. CAT activity is expressed as a percent of the CAT activity in each cell type transfected with a vector containing the CAT gene driven by the SV-40 promoter and SV-40 enhancer, positive control. The results are the average of 6–10 separate experiments for each cell type. Panel A shows the results of transfections with the construct missing the heterologous enhancer. Panel B shows the results of transfections with the construct containing the SV-40 enhancer. CAT activity expressed as dpm per hour per 60-mm dish in each cell type transfected with the positive control was 10 000 for Ar42-J, 35 000 for C2C12, and 400 000 for HEPG2 and COS-1 cells. The HEPG2 and COS-1 cells had 10-fold and the C2C12 cells 4-fold more protein per dish than the AR42-J cells.

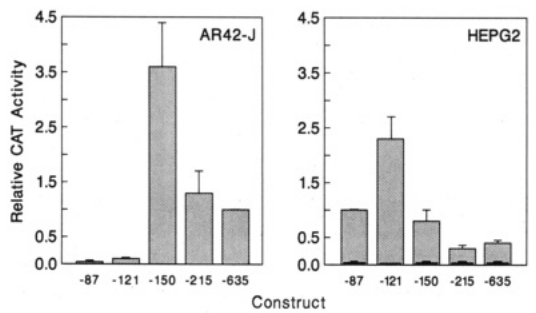

FIGURE 4: Effect of 5′-deletions on the activity of the colipase 5′-flanking sequences. Vectors with portions of the colipase gene 5′-flanking region starting at nucleotide +2 and ending at various distances 5′-ward were transfected into AR42-J and HEPG2 cells. Vectors with and without the SV-40 enhancer were tested. Six to 10 separate experiments were done with construct. The bars represent the average of these data. Plus one standard deviation is given for each construct. Only the data for the constructs containing the SV-40 enhancer are shown for the AR42-J cells. The constructs without the SV-40 enhancer gave identical results. The CAT activity was expressed relative to the −635 construct for the AR42-J cells and to the −87 construct for the HEPG2 cells. The activity in HEPG2 with the constructs minus the SV-40 enhancer are shown in the bars inserted in the hatched bars. The hatched bars are the results for the constructs with the SV-40 enhancer present.

DNase I footprint analysis, gel mobility-shift experiments, and methylation interference studies have identified two binding domains in the consensus sequence, CACCTG and TTTCC (Boulet et al., 1986; Hammer et al., 1987; Meister et al., 1989; Kruse et al., 1988; Cockell et al., 1989). Two nearly identical proteins, Pan-1 and Pan-2, that bind the CACCTG motif have been identified by cDNA cloning (Nelson et al., 1990). Pan-1 and Pan-2 are homologous to other DNA-binding proteins that also recognize the CACCTGT sequence. These proteins include E12 and E47, which bind to the immunoglobulin κE2 element, AP-4, MyoD, myogenin, Myf-5, and the *Drosophila* daughterless protein. One difference between the sequence identified in the colipase gene and the other known genes with the CACCTG motif is the substitution of adenine for thymine in the fifth position. In the other sequences, the thymine is always present.

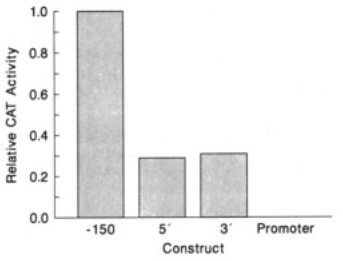

FIGURE 5: Enhancer activity of the sequence from −121 and −150. Vectors containing the SV-40 promoter driving CAT expression and with a concatemer (5-mer) of the sequence between −121 and −150 were transfected into AR42-J cells. The CAT activity was expressed relative to the −150 pCAT construct. 5′ indicates the position of the concatemer upstream from the promoter. 3′ indicates a position downstream from the CAT gene. Promoter is the SV-40 promoter pCAT without any enhancer.

Table II: Comparison of Human Colipase Gene Sequence with Rat Pancreatic-Specific Enhancer Consensus Sequence[a]

rat consensus	gtCACCTGt--------gctTTTCCctg	
human colipase	gcCACCAGgtactcacccagTTTCCtta	
	−150	−125

[a] Sequence from the 5′-flanking region of the human colipase gene was aligned with the rat consensus sequence for the pancreatic-specific enhancer (Boulet et al., 1986; Cockell et al., 1989). The putative binding domains for transcription factors in the rat sequence are in capital letters. Homologous sequences in the human sequence are also capitalized. Dashes were introduced in the rat sequence to optimize alignment. The numbers indicate the position of the sequence in the human colipase gene relative to the start site.

A 28-bp synthetic, double-stranded oligonucleotide corresponding to the region from −122 and −150 was tested as a ligand in a gel shift assay with nuclear extracts. A single major species in human acinar cell nuclear extract bound the labeled oligonucleotide (Figure 6). Unlabeled oligonucleotide competed from the binding in a specific fashion. An unrelated oligonucleotide did not compete for binding when present in 100-fold molar excess. A similar activity was also present in the HEPG2 cells. If the binding protein in the HEPG2 cells is identical to that detected in the pancreatic acinar cells, then the tissue specificity ascribed to that region by the transfection experiments must arise from interactions of the detected binding protein with other regions of the DNA or with other binding proteins that are tissue-specific. mRNA for Pan-2, which binds a similar element in the rat, was found in a variety of tissues, leading to the speculation that other factors are needed to confer tissue specificity (Nelson et al., 1990).

The transfection studies clearly demonstrate the presence of a tissue-specific promoter in the human colipase gene and suggest the presence of negative regulatory elements. The constructs with varying 5′-deletions located the tissue-specific element to the region between −121 and −150. Furthermore, this region is a binding site for a putative trans-acting factor. The location of other regulatory elements will require further experimentation. The isolation and characterization of the human colipase gene reported in this paper is a first step in

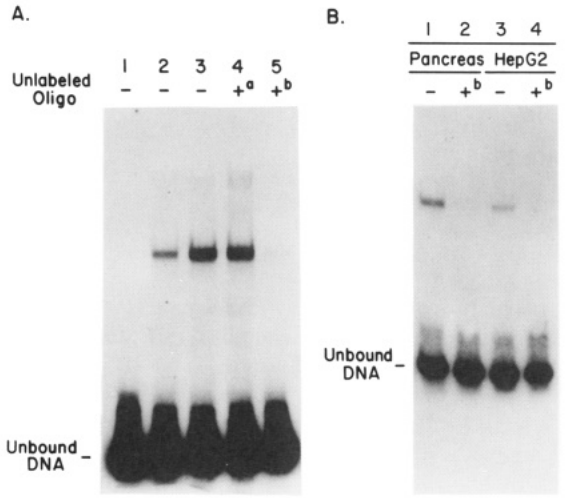

FIGURE 6: Binding of pancreatic nuclear extract to sequence from −121 to −150. Panel A shows the gel shift analysis of the binding of nuclear extract components to a labeled synthetic oligonucleotide corresponding to the region from −121 and −150. Lane 1 is the labeled oligonucleotide without extract. Lane 2 shows the effect of adding 5.0 μg of extract protein. Lane 3 has 10 μg of extract protein added. Lane 4 contains 10 μg of protein and a 100-fold molar excess of an unrelated oligonucleotide. Lane 5 contains 10 μg of protein and a 100-fold molar excess of unlabeled oligonucleotide. Panel B shows the gel shift analysis of pancreatic and HEPG2 nuclear extracts with the −121 to −150 probe. Lane 1 has 5 μg of pancreatic extract protein. Lane 2 contains a 100-fold molar excess of unlabeled oligonucleotide. Lane 3 has 5 μg of HEPG2 extract protein. Lane 4 contains a 100-fold molar excess of unlabeled oligonucleotide. [a] Reaction contains an oligonucleotide corresponding to 30 bp of the 5′ coding region of the human lipase cDNA. [b] Reaction contains the −121 to −150 oligonucleotide.

understanding the molecular events that control the expression of colipase and other pancreatic proteins involved in lipolysis.

ACKNOWLEDGMENT

We thank Dr. Arnold Strauss for his encouragement and critical reading of the manuscript.

REFERENCES

Ausubel, F. M., Brent, R., Kingston, R. E., Moore, D. D., Seidman, J. G., Smith, J. A., & Struhl, K. (1991) *Current Protocols in Molecular Biology*, John Wiley & Sons, New York.

Borgstrom, B., & Erlanson-Albertsson, C. (1984) in *Lipases* (Borgstrom, B., & Brockman, H. L., Eds.) pp 152–183, Elsevier, New York.

Boulet, A. M., Erwin, C. R., & Rutter, W. J. (1986) *Proc. Natl. Acad. Sci. U.S.A. 83*, 3599–3603.

Brockman, H. L., Law, J. H., & Kezdy, F. J. (1973) *J. Biol. Chem. 248*, 4965–4970.

Cockell, M., Stevenson, B. J., Strubin, M., Hagenbuchle, O., & Wellauer, P. K. (1989) *Mol. Cell. Biol. 9*, 2464–2476.

Erlanson-Albertsson, C., & Larsson, A. (1981) *Biochim. Biophys. Acta 665*, 250–255.

Erlanson-Albertsson, C., Larsson, A., & Duan, R. (1987) *Pancreas 2*, 531–535.

Gaskin, K. J., Durie, P. R., Hill, R. E., Lee, L. M., & Forstner, G. G. (1982) *J. Clin. Invest. 69*, 427–434.

Graham, F. L., & Van der Eb, A. J. (1973) *J. Virol. 52*, 455–456.

Hammer, R. E., Swift, G. H., Ornitz, D. M., Quaife, C. J., Palmiter, R. D., Brinster, R. L., & MacDonald, R. J. (1987) *Mol. Cell. Biol. 7*, 2956–2967.

Higuchi, R., Krummel, B., & Saiki, R. K. (1988) *Nucleic Acids Res. 16*, 7351–7367.

Ho, S. N., Hunt, H. D., Horton, R. M. Pullen, J. K., & Pease, L. R. (1989) *Gene 77*, 51–59.

Kruse, F., Komro, C. T., Michnoff, C. H., & MacDonald, R. J. (1988) *Mol. Cell. Biol. 8*, 893–902.

Lowe, M. E., Rosenblum, J. L., & Strauss, A. W. (1989) *J. Biol. Chem. 264*, 20042–20048.

Lowe, M. E., Rosenblum, J. L., McEwen, P., & Strauss, A. W. (1990) *Biochemistry 29*, 823–828.

Meister, A., Weinrich, S. L., Nelson, C., & Rutter, W. J. (1989) *J. Biol. Chem. 264*, 20744–20751.

Nelson, C., Shen, L.-P., Meister, A., Fodor, E., & Rutter, W. J. (1990) *Genes Dev. 4*, 1035–1043.

Norton, P., & Coffin, J. M. (1985) *Mol. Cell. Biol. 5*, 281–290.

Rausch, U., Rudiger, K., Vasiloudes, P., Kern, H., & Scheele, G. (1986) *Pancreas 1*, 522–528.

Rich, A., Nordheim, A., & Wang, A. H.-J. (1984) *Annu. Rev. Biochem. 53*, 191–846.

Sambrook, J., Fritsch, E. F., & Maniatis, T. (1989) *Molecular Cloning A Laboratory Manual*, 2nd edition, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.

Scheele, G. A., & Jacoby, R. (1983) *J. Biol. Chem. 258*, 2005–2009.

Scheele, G. A., Steinhilber, W., Swarovsky, B., & Kern, H. F. (1988) *J. Cell Biol. 107*, 763a.

Seed, B., & Sheen, J.-Y. (1988) *Gene 67*, 271–278.

Wicker, C., & Puigserver, A. (1989) *Eur. J. Biochem. 164*, 301–305.

Wicker, C., & Puigserver, A. (1990) *Biochem. Biophys. Res. Commun. 167*, 130–136.

Wicker, C., Scheele, G. A., & Puigserver, A. (1988) *Biochimie 70*, 1277–1283.

Wieloch, T., Borgstrom, B., Pieroni, G., Pattus, F., & Verger, R. (1981) *FEBS Lett. 128*, 217–220.