
Gastric Inhibitory Polypeptide:

Structure and Chromosomal Localization of
the Human Gene

(ヒト・GIP遺伝子の構造、遺伝子マッピング)

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Gastric inhibitory polypeptide (GIP) is a 42-amino-acid hormone which may have a role in the regulation of insulin secretion. The characterization of cDNA clones encoding this hormone indicates that it is derived by proteolytic processing of a 153-amino-acid precursor. The human gene coding for the human GIP precursor spans approximately 10 kilobase pairs and consists of six exons. Similar to genes encoding other members of the glucagon superfamily, each exon appears to encode a distinct region of the GIP precursor or its mRNA. The promoter region of the human GIP gene contains potential binding sites for a number of transcriptional factors including Sp1, AP-1, and AP-2. The human GIP gene has been assigned to chromosome 17q21.3→q22. (Molecular Endocrinology 3: 1014-1021, 1989)

INTRODUCTION

Gastric inhibitory polypeptide (GIP) is a 42-amino-acid peptide hormone (1) which was first isolated from por-

cine small intestine (2). It is a member of the glucagon superfamily (3) which also includes glucagon, secretin, vasoactive intestinal peptide (VIP), and GRF. Although GIP was originally considered to be enterogastrone because of its inhibitory action on gastric acid secretion (2, 4), subsequent studies suggested that it may be involved in the regulation of insulin secretion (5, 6).

In order to study the biosynthesis and regulation of expression of GIP, we isolated cDNA clones encoding GIP and determined the primary structure of its precursor (7). In this report, we describe the isolation and characterization of the human GIP gene and its promoter region. We also have determined the chromosomal localization of the human GIP gene and examined the tissue distribution of human GIP mRNA.

RESULTS

Cloning and Sequencing of the GIP Gene

Eight of approximately one million phage from two different human genomic libraries hybridized with the human GIP cDNA, and based on preliminary restriction analysis, three of these clones (λ hGIP-1, -3, and -7) were selected for detailed analysis. The gene spans

approximately 10 kilobase pairs (kb) and has six exons separated by five introns (Fig. 1 and 2). Exon 1 includes the 5'-untranslated region of the mRNA. The signal peptide and a small portion of the amino-terminal peptide are encoded by exon 2. Exon 3 encodes most of the mature GIP peptide. Exons 4 and 5 encode the carboxyl-terminal peptide. Exon 6 contains the 3'-untranslated region of the mRNA. The nucleotide sequences of the coding and 5'-untranslated regions are identical to the sequence of cDNA (7), and there is one nucleotide difference in the sequence of the 3'-untranslated region of the cDNA and gene (Fig. 2).

The Promoter Region of the GIP Gene

To define sequences which may be important for expression of the GIP gene, we also sequenced 5'-flanking region of the GIP gene. The transcriptional initiation site was determined by primer extension analysis using human duodenal RNA (Fig. 3). Two major extension products were observed: one corresponding to the 5'-end of the cDNA sequence and another lo-

cated 3 bases upstream. One minor band was also evident between these two sites. A number of possible regulatory elements were identified in the 5'-flanking region, including a TATA motif (8, 9) and a binding site for Sp1 (Fig. 2) (9). A sequence homologous with the enhancer core sequence, GTGG^{AAA}G (10), occurs in the region around residue -138. Regions closely resembling the consensus sequences of AP-1 (TGA_G^C-TCA) (11, 12) and AP-2 (C_GCC_CN_G^{CG}) (13) target elements are present at residues -344 and -368, respectively. These factors are involved in the regulation of gene expression by protein kinases A and C (11-13). In addition, there are sequences similar to a cAMP response element (consensus TGACGTCA) (14, 15) at residues -376, -349, and -306. The role of these putative regulatory elements on regulation of GIP expression remains to be determined.

Sequence of the 3'-Flanking Region of the GIP Gene

The nucleotide sequence of the 3'-flanking region of the GIP gene contains a GT-rich region which may be

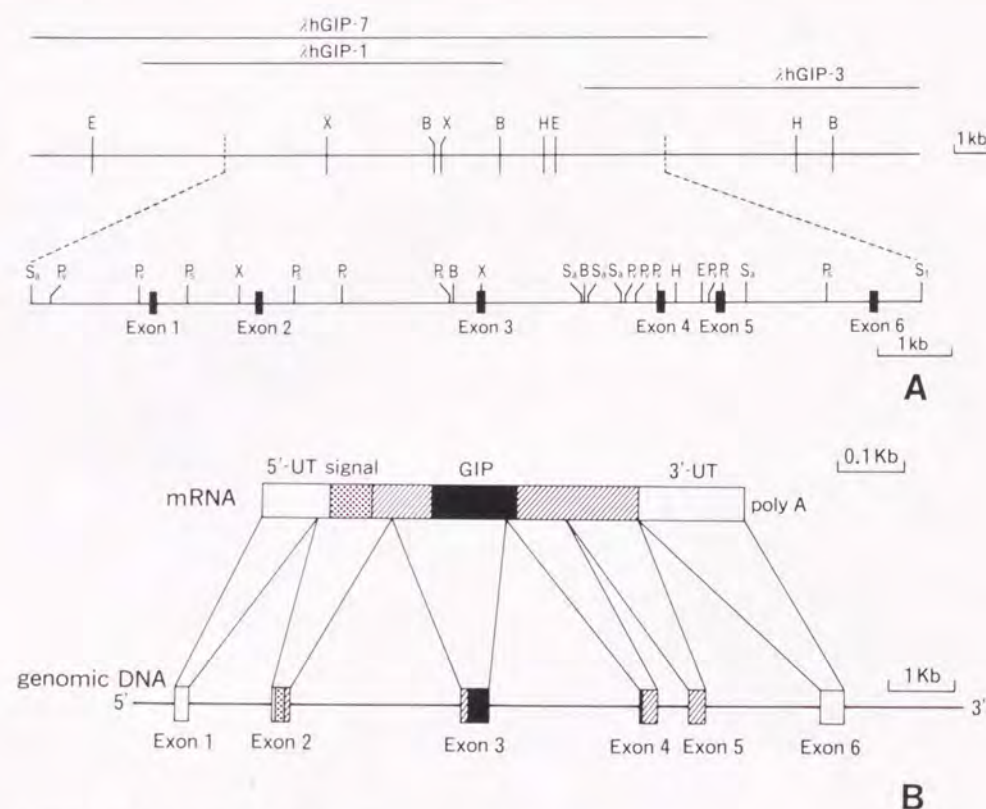


Fig. 1. Organization of the Human GIP Gene

A, Restriction map and organization of the human GIP gene. The top lines represent the overlapping DNA inserts contained within the genomic clones. The region containing the GIP gene and its flanking region is expanded to show a detailed restriction map at the bottom. The six exons and the five introns of the GIP gene are represented by boxes and lines, respectively. Restriction sites: B, *Bam*HI; E, *Eco*RI; H, *Hind*III; Ps, *Pst*I; Pv, *Pvu*II; Sa, *Sac*I; St, *Stu*I; and X, *Xho*I. B, Schematic representation of the human GIP mRNA (top line) (7) and gene (bottom line). The 5'-untranslated region (5'-UT) and 3'-untranslated region (3'-UT) are indicated by open boxes. The putative signal peptide is indicated by a dotted box. Shaded areas represent the amino and carboxyl-terminal peptides. The dark box represents GIP.

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T C C G C C C G T G T C G T C C T C C C A A G C G C T G G G A T T A C A G A C G T G A T T C A T C G C G C C T G G C C T
  Sp1      CRE      AP-2      CRE      AP-1
A C A A G T A T C C C T T C T A A C T G A G G G C A A C T G T A A A G G A A G T A G G G C A T A G A A A T A G T A G
  CRE
T G G G C C C T G T G C C C A G A A G G G A G G A G G A A G A A A A A G G G G T A C T T A G G G T A C C T A C
  CRE
C C A C A G G C A G G C C C C A G A C A G C A G C T G G A G A T A G C C A A A T G T T A A T C A C C A A T T A G C A C A
  CCAAT
G T T C A G G T G G A A A G G G C A A C T C T A T T A T G T G T A A A A T G G A C T T A A T G T G G C A G G A G C T G T
  enhancer core
G G G C T T A A T T T C T T G A G A T A A G A T G C T T T T A G G C T A A T C A G C A G G T C T A T G C C T A A T A T A
  TATA
A A G G A G C T G G G G C A T G A T T T C T T C A G C A G G C T C A G A A G G T C C A G A A A T C A G G G A A G G A G
  TATA
A C C C C T A T C T G T C C T T C T T C T G G A A G A G C T G G A A G G A A G T C T G G T A A G A G A A G
  (intron 1: 1.3kb)
T C T G T C T T A G C T C A G G A A A T A A C C T T G G A A G A T G
  Met
G T G G C C A C G A A G A C C T T T G C T C T G C T G C T G T C C C T G T T C C T G G C A G T G G G A C T A G G A
  Val Ala Thr Lys Thr Phe Ala Leu Leu Leu Ser Leu Phe Leu Ala Val Gly Leu Gly
G A G A A G A A A G G G T C A C T T C A G G T A G G A T G G
  (intron 2: 2.7kb)
G L u L y s L y s G L u G L y H i s P h e S e
T T G T T T C C A G C G C T C T C C C C T C C C T G C C T G T T G G A T C T C A T G C T A A G G T G A G C
  r Ala Leu Pro Ser Leu Pro Val Gly Ser His Ala Lys Val Ser
A G C C C T C A A C C T C G A G G C C C A G G T A C G C G G A A G G G A C T T T C A T C A G T A C A G T A T T
  Ser Pro Gln Pro Arg Gly Pro Arg Tyr Ala Gly Thr Phe Ile Ser Asp Tyr Ser Ile
G C C A T G G A C A A G A T T C A C C A C A A G A C T T T G T G A A C T G G C T G C T G G C C A A A A G G G A A G
  Ala Met Asp Lys Ile His Gln Gln Asp Phe Val Asn Trp Leu Leu Ala Gln Lys Gly Lys
A A G A A T G A G T G A G T C G C T
  (intron 3: 2.5kb)
L y s A s n A s
C C C A G C T G G A A A C A C A C A T C A C C C A G A G G G A G G C T C G G G C G C T G G A G C T G G C C A G T C A A
  p Trp Lys His Asn Ile Thr Gln Arg Glu Ala Arg Ala Leu Glu Leu Ala Ser Gln
G C T A A T A G G A A G G A G G A G G C A G T G G A G C C A C A G A G G T G A G T C A G G
  Ala Asn Arg Lys Glu Glu Ala Val Gly Pro Gln Ser
  (intron 4: 0.7kb)
T C C C T T G C A G C T C C C C A G C C A A G A A C C C A G C
  r Ser Pro Ala Lys Asn Pro Ser
G A T G A A G A T T T G C T G C G G G A C T T G C T G A T T C A A G A G C T G T T G G C C T G C T T G C T G G A T C A G
  Asp Glu Asp Leu Leu Arg Asp Leu Leu Ile Gln Glu Leu Leu Ala Cys Leu Leu Asp Gln
A C A A C C T C T G C A G G C T C A G G T A G G G G A G T
  (intron 5: 1.9kb)
T h r A s n L e u C y s A r g L e u A r
C T C T C C C C A G G T C T C G G T G A C T C T G A C C A C A C C C A G C T C A G G A C T G G A T T
  G Ser Arg
C T G C C C T T C A C T T A G C A C C T G C C T C A G C C C C A C T C C A G A A T A G C C A A G A G A A C C C A A A C C
  A T A A A G T T T A T G C T A A G T C G A G C C C A T T G T G A A A A T T T A T T A A A T G A C T A C T G A G C A C
  Poly (A)
T A A C A G G G C A T G T T T G T C T T T G T G T A T C T C T G T G T G T G G G C A C C A C C C A G T G A A A A G G A A
  A T A T G G T C T G G G C A T G G T G C A T A T G C C T G T

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Fig. 2. Partial Nucleotide Sequence of the Human GIP Gene

The nucleotide sequences of all exons, exon-intron boundaries, and 5'- and 3'-flanking regions are shown. Numbering is from the proposed cap site and the introns are not numbered. Arrows indicate the beginning of exons and the three possible poly(A) addition sites. The larger letters represent exons of the gene. The first and last 10 nucleotides of each intron are shown and the approximate length of each intron is also indicated. Amino acids indicated in italic print are those of the mature 42-amino-acid GIP peptide. Asterisk denotes the 5'-end of our previously published cDNA sequence (7). One base substitution relative to the cDNA sequence is dotted under the sequence (at nucleotide residue 589). The TATA box, CCAAT box (8), and Sp1 binding sequence are boxed. Sequence homologous with the consensus sequences of the enhancer core element, AP-1 and AP-2 target elements, and cAMP response element (CRE) are underlined. Putative poly(A) addition signals are double underlined. The GT cluster found in the 3'-flanking region is shown by a wavy line.

involved in transcription termination/polyadenylation (Fig. 2) (16).

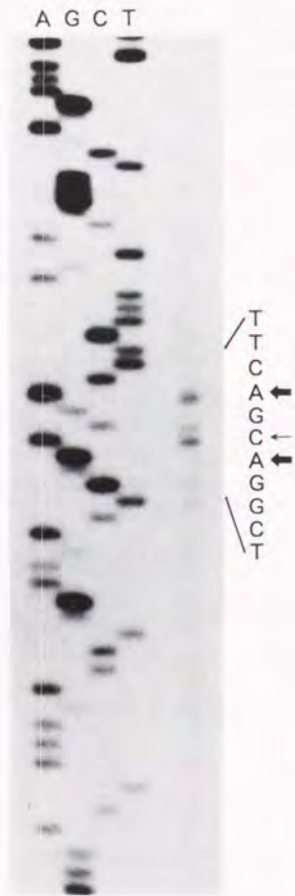
Tissue Distribution of GIP mRNA

The tissue distribution of GIP mRNA was examined by RNA blotting (Fig. 4). The 800-base GIP transcript was detected only in RNA prepared from the duodenum. No hybridizing signals were detected in the gallbladder, pancreas, liver, descending colon, antrum, and cardia of the stomach, or esophagus. These results are con-

sistent with immunocytochemical localization of GIP (17).

Chromosomal Localization of the GIP Gene

The chromosomal assignment of the human GIP gene was determined from analysis of its segregation in a panel of reduced mouse-human somatic cell hybrids as well as by *in situ* hybridization to prometaphase chromosomes. The human GIP cDNA probe hybridized to three human *Bam*HI fragments of 15, 9.9, and 1.5 kb,



readily distinguishable from the single mouse DNA fragment of 1.7 kb (Fig. 5). Hybridization to DNA from a panel of 35 somatic cell hybrids indicated that the human DNA fragments were presented only in those hybrids which retained chromosome 17 (Table 1). The probe also hybridized to two cell hybrids, TSL-2 and NSL-5 (Fig. 5), derived from human cells with translocations of chromosome 17 (17qter→17p13 :: 3p21→3pter and 17qter→17p11.2 :: 9q12→9qter, respectively), localizing the gene to the p11.2→qter region of chromosome 17. *In situ* hybridization to prometaphase chromosomes (Fig. 6) confirms the localization to the long arm of chromosome 17, and indicates that the GIP gene is in the region 17q21.3→22.

DISCUSSION

The exon-intron organization of the human GIP gene is very similar to those of other members of the glucagon superfamily (Fig. 7) (3). Each of the various

Fig. 3. Determination of the 5'-End of the Human GIP mRNA by Primer Extension
Two bold arrows indicate the major bands: the lower band corresponds to the 5'-end of our previously published cDNA sequence. One minor band is indicated by a thin arrow. A sequencing ladder is shown in adjacent lanes.

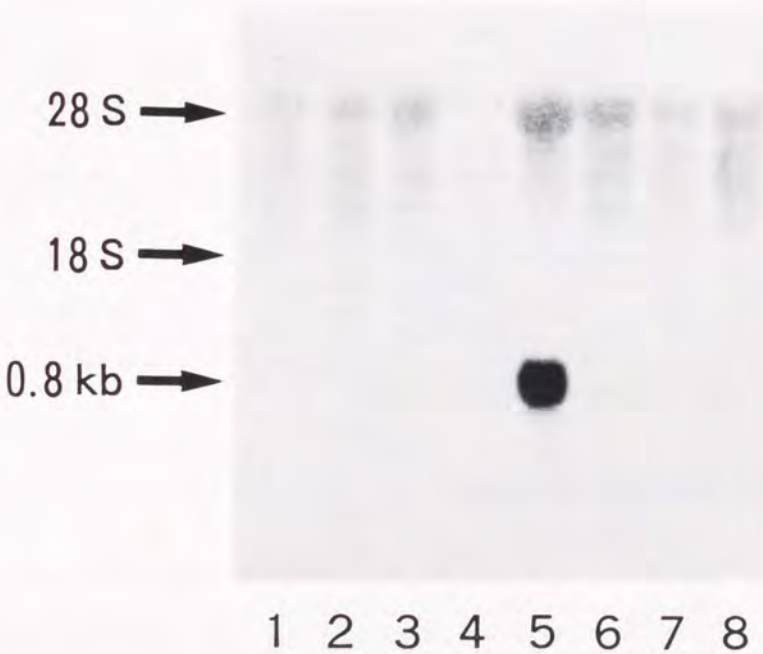


Fig. 4. RNA Blot Analysis
Total RNAs were prepared from the following human tissues: lane 1, gallbladder; 2, pancreas; 3, liver; 4, descending colon; 5, duodenum; 6, antrum; and 7, cardia of the stomach; and 8, esophagus. Positions of 28 S and 18 S ribosomal RNA are indicated.

domains of the precursors of glucagon (18), VIP (19), GRF (20), and GIP is encoded by a unique exon. The size of each of these genes also is similar (≈9–10 kb) (18–20). This comparison suggests that the ancestral gene of the family consisted of four exons which encoded the 5'-untranslated region of the mRNA, the signal peptide, the hormone, and the 3'-untranslated region of the mRNA, and that the glucagon superfamily arose by amplification of this basic motif. Subsequent

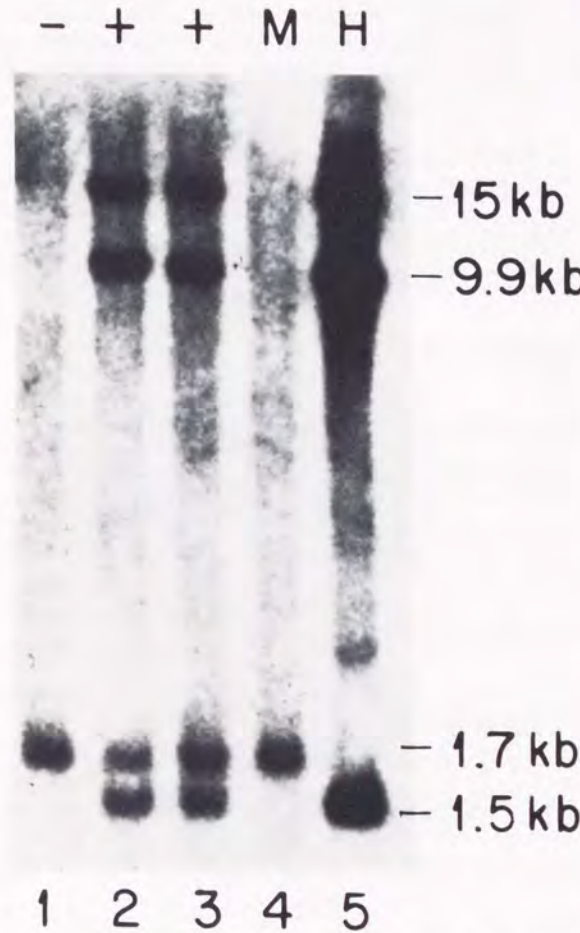


Fig. 5. Hybridization of the Human GIP cDNA to *Bam*HI-Digested Human-Mouse Cell Hybrid DNAs
Lane 1, ICL-15CSBF; lane 2, TSL-2; lane 3, NSL-5; lane 4, LM/TK- (mouse control); and lane 5, FB51 (human control).

amplification of the exon encoding the hormone domain may have generated the multiple glucagon-like and VIP-like peptides observed in the glucagon and VIP precursors (3).
The human GIP gene was mapped to chromosome 17q21.3→q22. Thus, each of the glucagon superfamily genes is localized on a different chromosome: glucagon, chromosome 2q36→q37 (21, 22); VIP, chromosome 6q16→q22 (23, 24); and GRF, chromosome 20q (20, 25). Interestingly, the genes for two other gastrointestinal peptides, gastrin (26) and pancreatic polypeptide (27, 28), have also been localized to chro-

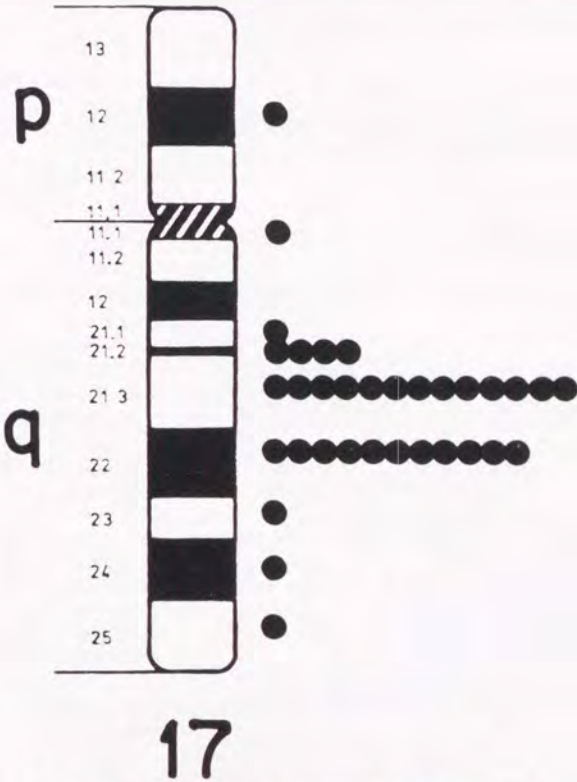


Fig. 6. Ideogram of Human Chromosome 17 Showing Silver Grain Distribution after Hybridization with the GIP cDNA
One hundred metaphase chromosomes were examined and 22% (34/154) of the grains were on chromosome 17. Seventy percent of the grains are localized in 17q21.3→q22. No other human chromosome demonstrated a grain distribution above background.

	Human Chromosomes																							
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	X	
Concordant no. of Hybrids	16	21	17	20	18	21	22	23	14	26	21	21	13	22	20	16	33	21	19	23	25	16	15	
Discordant no. of Hybrids	16	13	17	15	17	14	12	12	19	9	12	14	22	13	14	19	0	14	16	11	10	17	14	
GIP % Discordancy	50	38	50	43	49	40	35	34	58	26	36	40	63	37	41	54	0	40	46	32	29	52	48	

The scores were tabulated by the presence or absence of human DNA fragments in the different somatic cell hybrids. Concordant hybrids have either retained or lost the gene together with a specific chromosome. Discordant hybrids either retained the gene but not a specific chromosome, or the reverse. Percent discordancy indicates the degree of discordant segregation for a marker and a chromosome. A 0% discordancy is the basis for chromosome assignment.

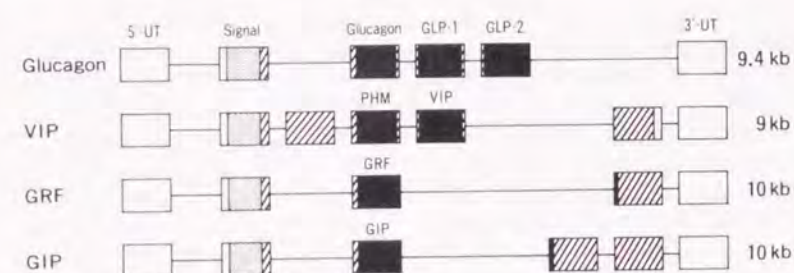


Fig. 7. Schematic Comparison of the Exon-Intron Organization of the Glucagon, VIP, GRF, and GIP Genes

The regions of the mRNA or precursor encoded by each exon (3, 19) are indicated. The 5'- and 3'-untranslated regions are indicated by open boxes. The signal peptide is indicated by a dotted box. Closed boxes represent the biologically active peptides; glucagon, glucagon-like peptide (GLP)-1 and -2, PHM-27, VIP, GRF, and GIP. Amino-terminal, connecting, and carboxyl-terminal peptides are indicated by cross-hatched boxes. Solid lines between the boxes denote intron regions. The approximate size of each gene is shown at right.

mosome 17; however, it is unknown if they are adjacent to one another or to the GIP gene.

MATERIALS AND METHODS

Isolation and Sequencing of Genomic Clones of Human GIP

Two different human genomic libraries, a Charon 4A library prepared from fetal liver (29) and an EMBL 3 library (Clontech, Palo Alto, CA), were screened by plaque hybridization (30, 31) using a ³²P-labeled human GIP cDNA probe (7). Prehybridization was carried out at 42°C overnight in a solution of 50% formamide, 5× SSC (1× SSC = 0.15 M sodium chloride/15 mM sodium citrate), 2× Denhardt's solution (1× Denhardt's solution = 0.02% BSA/0.02% polyvinylpyrrolidone/0.02% Ficoll), 20 mM sodium phosphate buffer (pH 7.4), 100 μg/ml heat-denatured salmon sperm DNA, and 0.25% Na-DodSO₄. Hybridization was done at 42°C overnight in a solution similar to the above except with 10% dextran sulfate and approximately 1 × 10⁶ cpm/ml heat-denatured probe. The filters were washed twice with 0.1× SSC/0.1% Na-DodSO₄ at room temperature for 30 min and then at 65°C for 30 min before autoradiography. The phage DNAs which gave positive signals were prepared as described (31). Three isolated clones, λhGIP-1 and -3 (from the Charon 4A library) and λhGIP-7 (from the EMBL 3 library) were subjected to sequencing analysis. The nucleotide sequence was determined by the dideoxy chain-termination method (32).

Primer Extension Analysis

The transcriptional start site was determined by primer extension. A 17-mer, 5'-CAGACTTCCTTCCAGC-3', complementary to nucleotides 64 to 80 (Fig. 2), was synthesized, and end-labeled with [γ-³²P]ATP (33). Fifty micrograms of human duodenal total RNA was dissolved in 11.25 μl H₂O and heated at 65°C for 15 min; 2 μl ³²P-labeled oligomer (1 pmol) were added and maintained at the same temperature for 15 min. Next, 4 μl 500 mM Tris-HCl (pH 8.3), 700 mM KCl, and 50 mM MgCl₂, 2 μl 200 mM dithiothreitol, 1.25 μl 10 mM each of the deoxynucleotide triphosphates, and 0.5 μl ribonuclease inhibitor (80 U) were added. The mixture then was allowed to cool slowly to 42°C in 80 min. To initiate primer extension, 1 μl (22 U) RAV-2 reverse transcriptase (Takara, Kyoto, Japan) was added, and the reaction mixture was incubated at 42°C for 1 h. After addition of 2 μl 0.25 M EDTA and 2 μl 2 N NaOH, the mixture was incubated at 37°C for 30 min, extracted with phenol, and precipitated with ethanol. The primer-extended products were denatured at 95°C and

separated on a 5% polyacrylamide-8 M urea gel. A sequencing ladder obtained with this primer on the appropriate template was run in adjacent lanes to ascertain the size of the extended products and the sequence at which the primer extension terminated.

RNA Blot Analysis

Total RNAs were isolated from various human tissues by the guanidine isothiocyanate/cesium chloride procedure (34). Twenty micrograms of total RNA were denatured with glyoxal, electrophoresed on a 1% agarose gel, and then blotted onto a nylon filter (35). Prehybridization, hybridization, and washing were carried out as described above. The probe used was a 532-base pair *Avall*-*Pst*I fragment of the cDNA (7).

Gene Mapping

The chromosomal localization of the human GIP gene was determined by hybridization of the ³²P-labeled GIP cDNA to Southern blots of *Bam*HI-digested DNA from 35 human-mouse somatic cell hybrid cell lines (36, 37). The regional localization was determined by *in situ* hybridization (38, 39) of the ³H-labeled GIP cDNA to normal human prometaphase lymphocyte chromosomes. After autoradiography, the chromosomes were stained for G-banding giving a 550–850 band pattern (40).

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