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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)
Gastric Inhibitory Polypeptide: Structure and Chromosomal Localization of the Human Gene

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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 porcine 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 (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 carboxy-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 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 located 3 bases upstream. One minor band was also evident among these two bands. A number of possible regulatory elements were identified in the 5'-flanking region, including a TATA box (Fig. 1) and a binding site for Sp1 (Fig. 2) (9). A sequence homologous with the enhancer core sequence, OTGTGTT (10), occurs in the region around residue -138. Regions closely resembling the consensus sequences of AP-1 (TGA/TCA) (11-13) and AP-2 (C(CCG)G13) (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 TGAACGTCAGA) (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 involved in transcription termination/polyadenylation (Fig. 2) (16).

Tissue Distribution of GIP mRNA

The tissue distribution of GIP mRNA was examined by Northern 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, cecum, and cardia of the stomach, or esophagus. These results are consistent 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 chromosome spreads. The human GIP cDNA probe hybridized to three human BamHI 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 present 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 (17pter→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→q22.

**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 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 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 2p16→q37 (21, 22); VIP, chromosome 6q16→q22 (23, 24); and GRF, chromosome 20q (20, 23). Interestingly, the genes for the two other gastrointestinal peptides, gastrin (25) and pancreatic polypeptide (27, 28), have also been localized to chro-

![Fig. 4. RNA Blot Analysis](image)

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.
mosome 17; however, it is unknown if they are adja-
cent 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 Cheron 4A library prepared from fetal liver DNA (20, 51) and a Cheron 4A library (93), were screened by plaque hybridization (30, 31) using a "p-h labeled human GIP cDNA probe (7). Prehy-
bridization was carried out at 50 °C overnight in a solution of 50% formamide, 5 X SSC (150 mM sodium chloride/ 15 mM sodium phosphate), 2% Denhardt's solution (27), 0.2% BSA/0.2% polyvinylpyrrolidone/0.2% Ficoll, 20 mM sodium phosphate buffer (pH 7.4), 100 ng/ml heat-denatured salmon sperm DNA, and 0.2% NaDODSO4. Hybridization was done at 42 °C. Hybridization and washing were carried out as described above. The probe used was a 552-base pair AvaiI-PstI fragment of the cDNA (7).

Gene Mapping

The chromosomal localization of the human GIP gene was determined by hybridization of the p-h labeled GIP cDNA to Southern blots of BamHI-digested DNA from 35 human 

To determine if any of the DNA fragments transferred to nitrocellulose contained a currently recognized gene, the blot was hybridized with a charac-
terized cDNA probe coding for chicken TSH-beta (57) and a cDNA probe coding for a 3' flanking region of the mouse p16 tumor suppressor gene (58). Both hybridizations were scored negative. DNA fragments transferred to nitrocellulose that hybridized to the probe for the human growth hormone releasing factor gene but not to the TSH-beta probe were hybridized to a p-h labeled human c-myc cDNA probe. This hybridization showed no specificity for chromosome 7.爆

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RNA Blot Analysis

Total RNAs were isolated from various human tissues by the guanidinium isothiocyanate/cesium chloride procedure (34). Twenty micrograms of total RNA were denatured with formaldehyde, 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 552-base pair AvaiI-PstI fragment of the cDNA (7).

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