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(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 eDNA 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.

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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. 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 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 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. 2, 25) and a binding site for Sp1 (Fig. 2) (9). A sequence homologous with the enhancer core sequence, AGGTCA (10), occurs in the region around residue -138. Regions closely resembling the consensus sequences of AP-1 (TGA/CTCA) (11, 12) and AP-2 (C(C/G)CC(G/A)) (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 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, jejunum, 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 chromosomes. The human GIP DNA probe hybridized to three human BamH1 fragments of 15, 9.9, and 1.5 kb.

**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 depicted 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, BamH1; E, EcoRI; H, HindIII; K, PstI; P, PvuII; S, SacI; St, StuI; and X, XhoI. B, Schematic representation of the human GIP mRNA (top lines) and gene (bottom lines). 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.

**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. Asterisks denote the 5'-end of our previously published cDNA sequence (7). One base substitution relative to the cDNA sequence is noted 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.
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 28S and 18S ribosomal RNA are indicated.

The exon-intron organization of the human GIP gene is very similar to that 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 (19-10 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 superfam-

ily genes is localized on a different chromosome: glucagon, chromosome 2q16-q27 (21, 22); VIP, chromosome 6q16-q22 (23, 24); and GRF, chromosome 29q (20, 23). Interestingly, the genes for two other gastrointestinal peptides, gastrin (25) and pancreatic polypeptide (27, 28), have also been localized to chromosomal regions.

**DISCUSSION**

The evidence for the organization of the human GIP gene indicates that the gene has not been moved from its ancestral location on chromosome 17q21.3-q22. This suggests that the GIP gene may have been derived from a single ancestral gene that was present in all members of the glucagon superfamily. The observation that the GIP gene is localized on chromosome 17q21.3-q22 supports this hypothesis, and suggests that the GIP gene may have been derived from a single ancestral gene that was present in all members of the glucagon superfamily. Further studies are needed to determine the structure and function of the GIP gene and its role in the regulation of gastric acid secretion.
The regions of the mRNA or precursor encoded by each exon (3, 19) are indicated. The 5'- and 3'- untranslated regions are shown by the dotted line (31). The sequences are on the mRNA (32). A schematic comparison of the exon-intron organization of the glucagon, GIP, and GRF is shown (33). All intron/exon boundaries are indicated by the cross-hatched boxes. Solid lines between the boxes indicate intron regions. The approximate size of each exon is shown at the right.

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