coop
CF-A4SN
70

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

稲 垣 暢 也

70
CF-A4SN
coop

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）

稲 垣 暢 也

主論文

Gastric Inhibitory Polypeptide: Structure and ChromosomalLocalization of the Human Gene

Nobuya Inagaki, Yutaka Seino, Jun Takeda, Hideki Yano, Yuichiro Yamada, Graeme I. Bell, Roger L. Eddy, Yoshimitsu Fukushima, Mary G. Byers, Thomas B. Shows, and Hiroo Imura

Second Division, Department of Medicine
(N.I., H.Y., Y.Y., H.I.)
and Division of Metabolism and Clinical Nutrition
(Y.S., J.T.)
Kyoto University School of Medicine
Kyoto 606, Japan

Howard Hughes Medical Institute and
Departments of Biochemistry and Molecular Biology, and of
Medicine (G.I.B.)
The University of Chicago
Chicago, Illinois 60637

Department of Human Genetics
(R.L.E., Y.F., M.G.B., T.B.S.)
Roswell Park Memorial Institute
New York State Department of Health
Buffalo, New York 14263

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 (λhGIP-1, -3, and -7) were selected for detailed analysis. The gene spans

0888-8809/89/1014-1021$02.00/0
Molecular Endocrinology
Copyright © 1989 by The Endocrine Society

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 located 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, $GTGG^{AAA}_{TTT}$ G (10), occurs in the region around residue −138. Regions closely resembling the consensus sequences of AP-1 (TGA$^{C}_{G}$TCA) (11, 12) and AP-2 ($^{T}_{C}$C$^{C}_{G}$CC$^{A}_{C}$N$^{GCG}_{CGC}$) (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

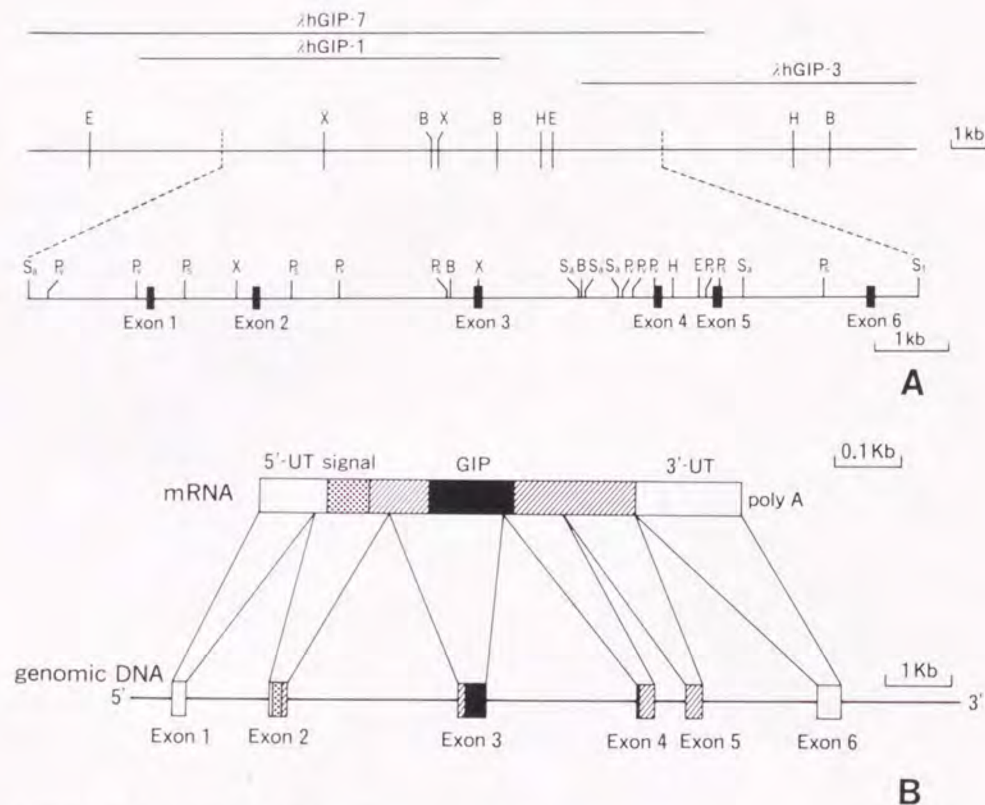


Fig. 1. Organization of the Human GIP Gene

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, *Hin*dIII; 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.

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.

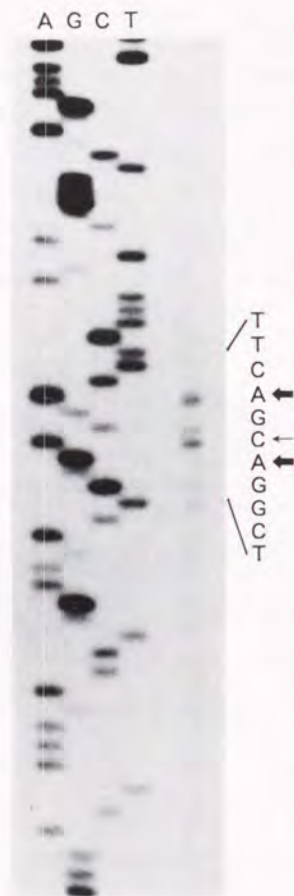
involved in transcription termination/polyadenylation (Fig. 2) (16).

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 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 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.

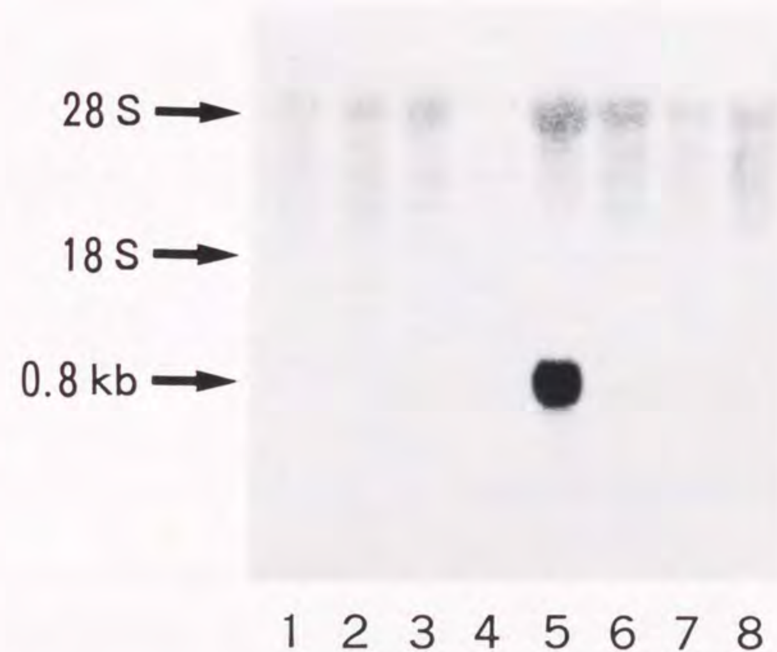


Fig. 4. RNA Blot Analysis

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 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-

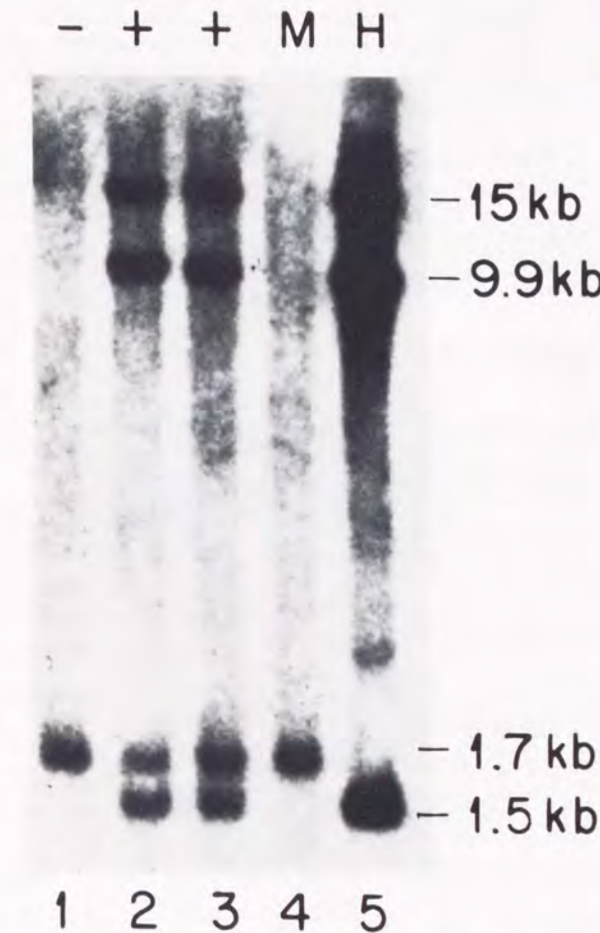


Fig. 5. Hybridization of the Human GIP cDNA to *Bam*HI-Digested Human-Mouse Cell Hybrid DNAs

Lane 1, ICL-15CSBF; lane 2, TSL-2; lane 3, NSL-5; lane 4, LM/TK- (mouse control); and lane 5, FB51 (human control).

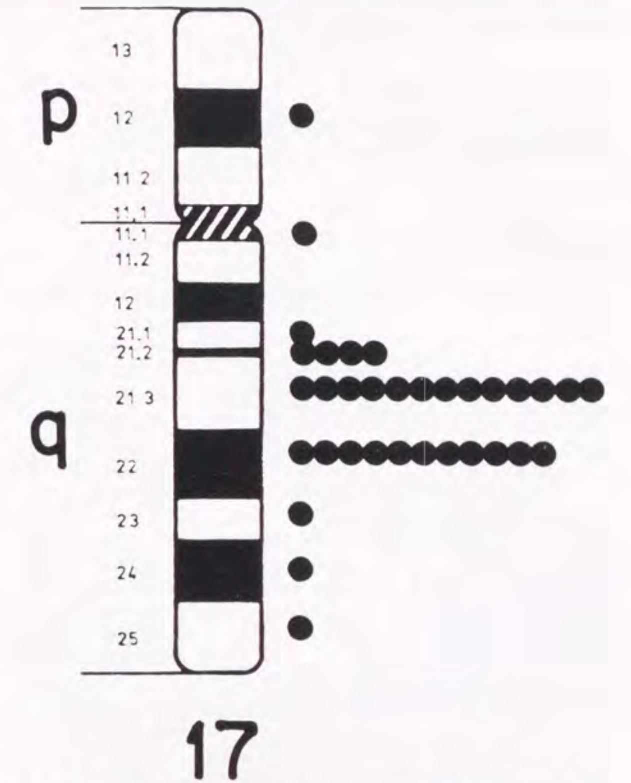


Fig. 6. Ideogram of Human Chromosome 17 Showing Silver Grain Distribution after Hybridization with the GIP cDNA

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.

Table 1. Segregation of the GIP Gene with Human Chromosomes in *Bam*HI Digested Human-Mouse Cell Hybrid DNA

	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 makrer and a chromosome. A 0% discordancy is the basis for chromosome assignment.

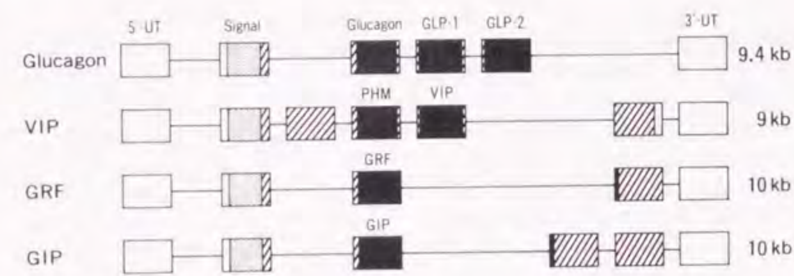


Fig. 7. Schematic Comparison of the Exon-Intron Organization of the Glucagon, VIP, GRF, and GIP Genes

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 ^{32}P-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% $NaDodSO_4$. Hybridization was done at 42 C overnight in a solution similar to the above except with 10% dextran sulfate and approximately 1×10^6 cpm/ml heat-denatured probe. The filters were washed twice with 0.1× SSC/0.1% $NaDodSO_4$ 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'-CAGACTTCCTTTCCAGC-3', complementary to nucleotides 64 to 80 (Fig. 2), was synthesized, and end-labeled with [γ-^{32}P]ATP (33). Fifty micrograms of human duodenal total RNA was dissolved in 11.25 μl H_2O and heated at 65 C for 15 min; 2 μl ^{32}P-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 $MgCl_2$, 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 *Ava*II-*Pst*I fragment of the cDNA (7).

Gene Mapping

The chromosomal localization of the human GIP gene was determined by hybridization of the ^{32}P-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 ^{3}H-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).

Acknowledgments

We are very grateful to Dr. S. Ishii (Riken), Dr. S. Nakanishi and Dr. H. Fukumoto (Kyoto University) for their valuable advice. We would also like to thank T. Mitani (Sanwa Kagaku Kenkyusho) for preparation of the synthetic oligonucleotide used for primer extension analysis, S. Ogaya for excellent technical assistance, and H. Tachikawa for preparing the manuscript.

Received February 10, 1989. Revision received March 17, 1989. Accepted March 20, 1989.

Address requests for reprints to: Dr. Nobuya Inagaki, Kyoto University School of Medicine, Second Division, Department of Medicine, 54 Shogoin Kawahara-cho, Sakyo-ku, Kyoto 606, Japan.

This research was supported in part by Grant-in-Aid for Scientific Research 63440042 from the Ministry of Education, Science, and Culture, Japan (to H.I.), by Uehara Memorial

Foundation, and grant-in-aid from the Mochida Memorial Foundation for Medical and Pharmaceutical Research (to Y.S.), and by NIH Grants GM-20454 and HD-05196 (to T.B.S.).

REFERENCES

1. Moody AJ, Thim L, Valverde I 1984 The isolation and sequencing of human gastric inhibitory peptide (GIP). FEBS Lett 172:142–148
2. Brown JC, Mutt V, Pederson RA 1970 Further purification of a polypeptide demonstrating enterogastrone activity. J Physiol 209:57–64
3. Bell GI 1986 The glucagon superfamily: precursor structure and gene organization. Peptides 7:27–36
4. Pederson RA, Brown JC 1972 Inhibition of histamine-, pentagastrin-, and insulin-stimulated canine gastric secretion by pure 'gastric inhibitory polypeptide.' Gastroenterology 62:393–400
5. Dupré J, Ross SA, Watson D, Brown JC 1973 Stimulation of insulin secretion by gastric inhibitory polypeptide in man. J Clin Endocrinol Metab 37:826–828
6. Anderson DK, Elahi D, Brown JC, Tobin JD, Andres R 1978 Oral glucose augmentation of insulin secretion. Interactions of gastric inhibitory polypeptide with ambient glucose and insulin levels. J Clin Invest 62:152–161
7. Takeda J, Seino Y, Tanaka K, Fukumoto H, Kayano T, Takahashi H, Mitani T, Kurono M, Suzuki T, Tobe T, Imura H 1987 Sequence of an intestinal cDNA encoding human gastric inhibitory polypeptide precursor. Proc Natl Acad Sci USA 84:7005–7008
8. Breathnach R, Chambon P 1981 Organization and expression of eucaryotic split genes coding for proteins. Annu Rev Biochem 50:349–383
9. Dynan WS, Tjian R 1985 Control of eukaryotic messenger RNA synthesis by sequence-specific DNA-binding proteins. Nature 316:774–778
10. Weiher H, König M, Gruss P 1983 Multiple point mutations affecting the simian virus 40 enhancer. Science 219:626–631
11. Angel P, Imagawa M, Chiu R, Stein B, Imbra RJ, Rahmsdorf HF, Jonat C, Herrlich P, Karin M 1987 Phorbol ester-inducible genes contain a common *cis* element recognized by a TPA-modulated *trans*-acting factor. Cell 49:729–739
12. Lee W, Mitchell P, Tjian R 1987 Purified transcription factor AP-1 interacts with TPA-inducible enhancer elements. Cell 49:741–752
13. Imagawa M, Chiu R, Karin M 1987 Transcription factor AP-2 mediates induction by two different signal-transduction pathways: protein kinase C and cAMP. Cell 51:251–260
14. Montiminy MR, Sevarino KA, Wagner JA, Mandel G, Goodman RH 1986 Identification of a cyclic-AMP-responsive element within the rat somatostatin gene. Proc Natl Acad Sci USA 83:6682–6686
15. Montiminy MR, Bilezikjian LM 1987 Binding of a nuclear protein to the cyclic-AMP response element of the somatostatin gene. Nature 328:175–178
16. McLauchlan J, Gaffney D, Whitton JL, Clements JB 1985 The consensus sequence YGTGTTYY located downstream from the AATAAA signal is required for efficient formation of mRNA 3' termini. Nucleic Acids Res 13:1347–1368
17. Polak JM, Bloom SR, Kuzio M, Brown JC, Pearse AGE 1973 Cellular localization of gastric inhibitory polypeptide in the duodenum and jejunum. Gut 14:284–288
18. White JW, Saunders GF 1986 Structure of the human glucagon gene. Nucleic Acids Res 14:4719–4730
19. Tsukada T, Horovitch SJ, Montiminy MR, Mandel G, Goodman RH 1985 Structure of the human vasoactive intestinal polypeptide gene. DNA 4:293–300
20. Mayo KE, Cerelli GM, Lebo RV, Bruce BD, Rosenfeld MG, Evans RM 1985 Gene encoding human growth hormone-releasing factor precursor: structure, sequence, and chromosomal assignment. Proc Natl Acad Sci USA 82:63–67
21. Tricoli JV, Bell GI, Shows TB 1984 The human glucagon gene is located on chromosome 2. Diabetes 33:200–202
22. Schroeder WT, Lopez LC, Harper ME, Saunders GF 1984 Localization of human glucagon gene (GCG) to chromosome segment 2q36→37. Cytogenet Cell Genet 38:76–79
23. Nagarajan L, Louie E, Tsujimoto Y, Balduzzi PC, Huebner K, Croce CM 1986 The human c-ros (ROS) is located at chromosome region 6q16→6q22 Proc Natl Acad Sci USA 83:6568–6572
24. Gozes I, Avidor R, Yahav Y, Katznelson D, Croce CM, Huebner K 1987 The gene encoding vasoactive intestinal peptide is located on human chromosome 6p21-6qter. Hum Genet 75:41–44
25. Riddel DC, Mallonee R, Phillips JA, Parks JS, Sexton LA, Hamerton JL 1985 Chromosomal assignment of human sequences encoding arginine vasopressin-neurophysin II and growth hormone releasing factor. Somatic Cell Mol Genet 11:189–195
26. Fukushige S, Murotsu T, Matsubara K 1986 Chromosomal assignment of human genes for gastrin, thyrotropin (TSH-beta subunit) and c-erbB-2 by chromosome sorting combined with velocity sedimentation and Southern hybridization. Biochem Biophys Res Commun 134:477–483
27. Takeuchi T, Gumucio DL, Yamada T, Meisler MH, Minth CD, Dixon JE, Eddy RE, Shows TB 1986 Genes encoding pancreatic polypeptide and neuropeptide Y are on human chromosomes 17 and 7. J Clin Invest 77:1038–1041
28. Miki T, Murphy PD, Pletcher PA, Kidd JR, Ferguson-Smith AC, Ruddle FH, Kidd KK 1987 Hox2 maps to 17q near PPY and NGFR. Ninth International Workshop on Human Gene Mapping. Cytogenet Cell Genet 46:662 (Abstract)
29. Lawn RM, Fritsch EF, Parker RC, Blake G, Maniatis T 1978 The isolation and characterization of a linked δ- and β-globin gene from a cloned library of human DNA. Cell 15:1157–1174
30. Benton WD, Davis RW 1977 Screening of λgt recombinant clones by hybridization to single plaques *in situ*. Science 196:180–182
31. Maniatis T, Fritsch EF, Sambrook J 1982 Molecular Cloning: A Laboratory Manual. Cold Spring Harbor Laboratory, Cold Spring Harbor, NY
32. Sanger F, Nicklen S, Coulson AR 1977 DNA sequencing with chain-terminating inhibitors. Proc Natl Acad Sci USA 74:5463–5467
33. Maxam AM, Gilbert W 1980 Sequencing end-labeled DNA with base-specific chemical cleavages. Methods Enzymol 65:499–560
34. Chirgwin JM, Przybyla AE, MacDonald RJ, Rutter WJ 1979 Isolation of biologically active ribonucleic acid from sources enriched in ribonuclease. Biochemistry 18:5294–5299
35. Thomas PS 1980 Hybridization of denatured RNA and small DNA fragments transferred to nitrocellulose. Proc Natl Acad Sci USA 77:5201–5205
36. Shows TB, Brown JA, Haley LL, Byers MG, Eddy RL, Cooper ES, Goggin AP 1978 Assignment of the β-glucuronidase structural gene to the pter→q22 region of chromosome 7 in man. Cytogenet Cell Genet 21:99–104
37. Shows T, Eddy R, Haley L, Byers M, Henry M, Fujita T, Matsui H, Taniguchi T 1984 Interleukin 2 (IL2) is assigned to human chromosome 4. Somatic Cell Mol Genet 10:315–318

38. Zabel BU, Naylor SL, Sakaguchi AY, Bell GI, Shows TB 1983 High resolution chromosomal localization of human genes for amylase, proopiomelanocortin, somatostatin, and a DNA fragment (D3S1) by *in situ* hybridization. Proc Natl Acad Sci USA 80:6932–6936
39. Nakai H, Byers MG, Shows TB, Taggart RT 1986 Assignment of the pepsinogen gene complex (PGA) to human chromosome region 11q13 by *in situ* hybridization. Cytogenet Cell Genet 43:215–217
40. ISCN 1981 An International System for Human Cytogenetic Nomenclature-High-Resolution Banding. Cytogenet Cell Genet 31:1–32