JCI The Journal of Clinical Investigation

Genes encoding pancreatic polypeptide and neuropeptide Y are on human chromosomes 17 and 7.

T Takeuchi, D L Gumucio, T Yamada, M H Meisler, C D Minth, J E Dixon, R E Eddy, T B Shows

J Clin Invest. 1986;77(3):1038-1041. https://doi.org/10.1172/JCI112357.

Research Article

Pancreatic polypeptide and neuropeptide Y share 50% amino acid homology (18 out of 36 residues), suggesting that they may have common ancestral origins. cDNA clones complementary to human mRNAs encoding pancreatic polypeptide and neuropeptide Y were used to detect specific human genomic DNA sequences in human-mouse somatic cell hybrid lines. The pancreatic polypeptide gene (PPY) segregated with human chromosome 17, while the neuropeptide Y gene (NPY) segregated with human chromosome 7. Examination of cell hybrids with chromosomal rearrangements assigned PPY to the p11.1-qter region and NPY to the pter-q22 region of their respective chromosomes.

Genes Encoding Pancreatic Polypeptide and Neuropeptide Y are on Human Chromosomes 17 and 7

Toshiyuki Takeuchi, Deborah L. Gumucio, Tadataka Yamada, Miriam H. Meisler, Carolyn D. Minth, Jack E. Dixon, Roger E. Eddy, and Thomas B. Shows

Departments of Internal Medicine and Human Genetics, University of Michigan Medical School, Ann Arbor, Michigan 48109; Department of Biochemistry, Purdue University, West Lafayette, Indiana 47907; and Department of Human Genetics, Roswell Park Memorial Institute, Buffalo, New York 14263

Abstract

Pancreatic polypeptide and neuropeptide Y share 50% amino acid homology (18 out of 36 residues), suggesting that they may have common ancestral origins. cDNA clones complementary to human mRNAs encoding pancreatic polypeptide and neuropeptide Y were used to detect specific human genomic DNA sequences in human-mouse somatic cell hybrid lines. The pancreatic polypeptide gene (*PPY*) segregated with human chromosome 17, while the neuropeptide Y gene (*NPY*) segregated with human chromosome 7. Examination of cell hybrids with chromosomal rearrangements assigned *PPY* to the p11.1-qter region and *NPY* to the pter-q22 region of their respective chromosomes.

Introduction

Pancreatic polypeptide and neuropeptide Y are two members of a family of peptide hormones that exhibit considerable structural homology. Pancreatic polypeptide was isolated initially as a by-product of insulin purification from the pancreas (1, 2), but as with many other gastrointestinal peptides, pancreatic polypeptide-like immunoreactivity was also localized to mammalian central nervous system tissues by histochemical techniques (3). Tatemoto and Mutt (4) isolated pancreatic polypeptide-like peptides from porcine gut and brain and named them peptides YY, since they had tyrosine residues at both amino- and carboxyl-terminal ends. Amino acid sequence analysis of peptide YY from the brain revealed it to be slightly different from gut peptide YY, thus it was renamed neuropeptide Y (5).

Recently we and others isolated cDNA clones encoding pancreatic polypeptide from human pancreatic endocrine tumors (6–8) and neuropeptide Y from a pheochromocytoma (9). The nucleotide sequences indicated that the human pancreatic polypeptide and neuropeptide Y precursors consist of 95 and 97 amino acids, respectively. The amino acids of both hormones were arranged into three regions as shown in Fig. 1 *A:* putative leader sequences of 29 amino acids for pancreatic polypeptide and 28 amino acids for neuropeptide Y, biologically active peptide regions of 36 amino acids for both peptides, followed by a common Gly-Lys-Arg linking complex and carboxyl-terminal extensions of 27 and 30 amino acids, respectively. The structural similarities between the two peptides were most conspicuous in the biologically active peptide core region, with 50% amino acid homology and 68% homology in the nucleotide sequence as shown in Fig. 1 *B*. Because of this marked structural similarity, we hypothesized that pancreatic polypeptide and neuropeptide Y may originate from a common ancestral gene. To explore the possibility of a chromosomal linkage between the genes encoding the two peptides we used human-mouse somatic cell hybrids to ascertain their chromosomal assignments.

Methods

In order to distinguish between human and mouse genes, genomic DNA was isolated from human leukocytes or mouse liver and digested with the restriction endonuclease Pst I. Restriction fragments were separated by electrophoresis on 0.7% agarose gels and transferred to nitrocellulose filters by the method of Southern (10). Hybridization was carried out with ^{32}P-labeled pancreatic polypeptide or neuropeptide Y cDNA probes, (Fig. 1 *B*) as described in the legend to Fig. 2.

For chromosomal segregation analysis, DNA was prepared from a panel of human/mouse hybrid cell lines containing a normal complement of mouse chromosomes plus variable numbers and combinations of human chromosomes. The human chromosome content of each hybrid line was determined either by electrophoretic analysis of marker enzymes whose genes had already been assigned or by karyotype determination (Table I). Pst I fragments from each hybrid cell were hybridized with the pancreatic polypeptide and neuropeptide Y cDNAs.

Results

When human DNA was hybridized with the pancreatic polypeptide cDNA, two Pst I fragments of 4.5 and 1.5 kilobases (kb) were detected but no hybridization to mouse DNA was observed with this probe. The neuropeptide Y cDNA hybridized with three human genomic DNA fragments of 6.7, 4.9, and 0.5 kb and also hybridized with mouse DNA fragments of 9.2 and 2.6 kb. These experiments demonstrated that the hybridizing fragments of human and mouse DNA could be clearly distinguished with both probes, permitting chromosomal segregation analysis in human-mouse somatic cell hybrids.

Human DNA fragments could be detected in certain hybrid cell lines with each of the probes, as demonstrated in Fig. 2. The fragments hybridizing with the pancreatic polypeptide cDNA

Address reprint requests to Dr. Takeuchi, 3912 Taubman Center, University of Michigan Medical Center, Ann Arbor, MI 48109-0362. Please send correspondence regarding this work to Dr. Yamada at the same address.

Received for publication 2 December 1985.

J. Clin. Invest.

0021-9738/86/03/1038/04 $1.00
Volume 77, March 1986, 1038–1041

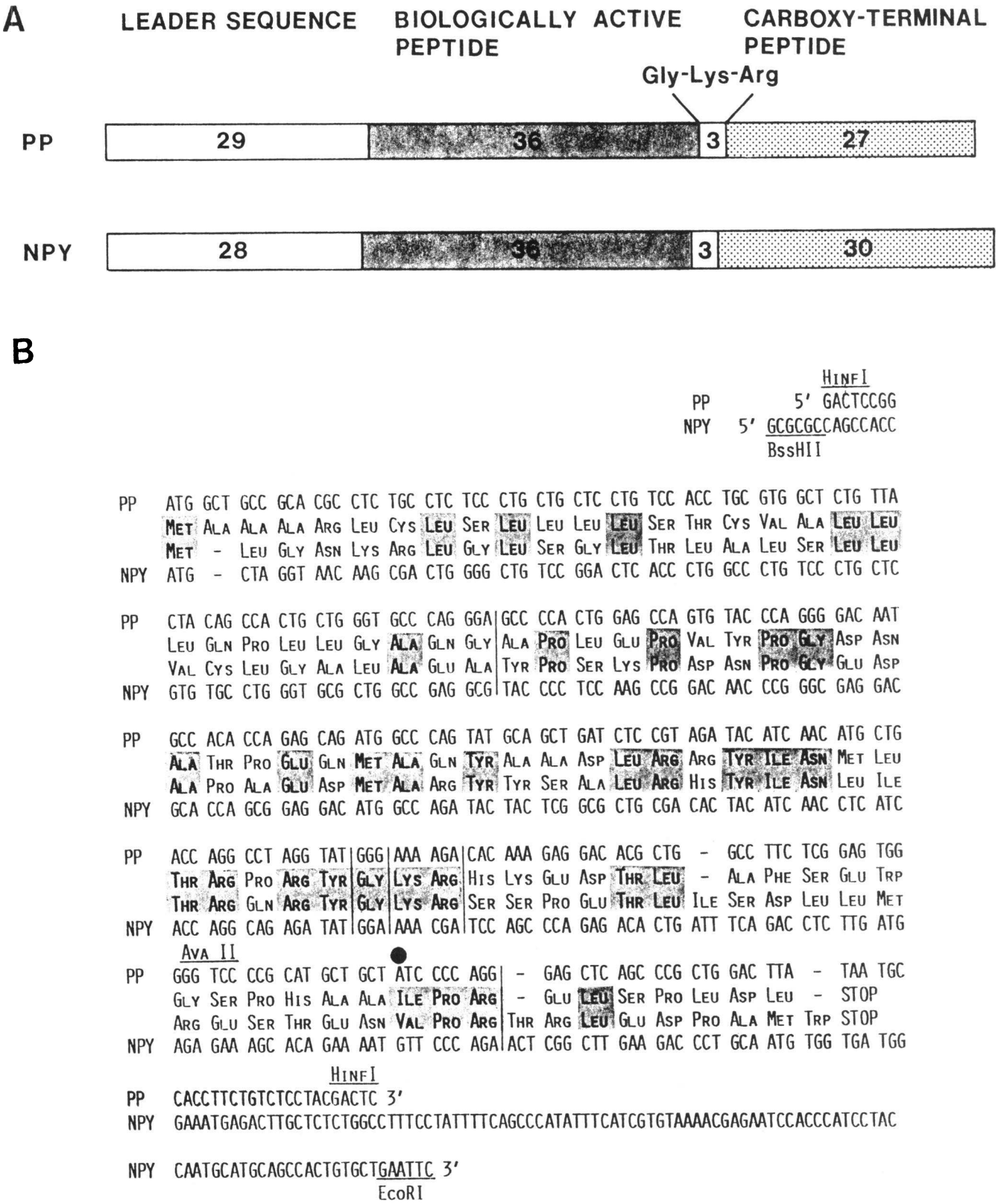

Figure 1. (*A*) Peptide structures of precursors for pancreatic polypeptide (95 amino acids) and neuropeptide Y (97 amino acids). Following leader sequences of 29 amino acids for pancreatic polypeptide and 28 amino acids for neuropeptide Y, both peptides have biologically active cores of 36 amino acids. A linking complex similar to that observed with many alpha-amidated peptides consisting of Gly-Lys-Arg connects the peptide core to a second peptide of 27 amino acids for pancreatic polypeptide and 30 amino acids for neuropeptide Y. (*B*) Comparison of nucleotide sequences for pancreatic polypeptide and neuropeptide Y cDNAs and the encoded amino acid structures. Amino acids common to both peptides are depicted by the shaded blocks and the separate peptide domains are marked by vertical lines. The black dot denotes a site at which the PP cDNA clone utilized for these studies (8) varied from clones reported by others (6, 7). An A-G nucleotide substitution at this position results in an Ile-Val substitution in the deduced amino acid sequence. cDNA probes used to detect pancreatic polypeptide and neuropeptide Y genes were excised using restriction endonucleases Hinf I-Ava II and BssH II-EcoRI, respectively.

Table I. Distribution of Genes Encoding Pancreatic Polypeptide (PPY) and Neuropeptide Y (NPY) with Human Chromosomes in Human-mouse Somatic Cell Hybrids

			Human chromosomes																						
Hybrids	NPY*	PPY*	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	X	TL
WIL-2	−	+	−	−	−	−	−	−	−	+	−	−	−	+	−	−	+	−	+	−	−	−	+	−	+	
WIL-6	+	+	−	+	−	+	+	+	+	+	−	+	+	−	−	+	−	−	+	−	+	+	+	−	+	
WIL-8	+	+	+		+	+	+	+	+	+	+	−	+	+	+	+	+	+	+	+	+	+	+	+	−	+
WIL-15	+	+	−	+	+	+	−	+	+	−	−	+	+	+	+	+	−	−	+	+	−	+	+	−	+	
REW-5	+	+	+	+	+	+	+	+	+	+	−	+	+	+	+	+	+	−	+	+	+	−	+	+	+	
REW-8D	−	+	−	−	−	+	−	−	−	+	−	−	−	−	−	+	−	−	+	−	−	+	+	+	+	
REW-11	+	−	−	−	−	+	−	−	+	−	−	−	+	+	+	−	−	+	−	−	−	+	+	−	+	
REW-15	+	+	+	+	+	+	+	+	+	+	−	+	+	+	+	+	+	−	+	+	+	+	+	+	+	
ICL-15	−	+	−	−	−	−	−	−	−	−	−	−	−	+	−	−	−	−	+	−	−	+	+	−	−	
ICL-15CSBF	−	−	−	−	−	−	−	−	−	−	−	−	−	+	−	−	−	−	−	−	−	+	+	−	−	
JSR-17S	+		+	+	+	−	+	−	−	+	+	+	+	+	+	+	+	+	+	+	−	+	+	+	−	7/9
JSR-29	+	+	+	+	+	+	+	+	−	+	−	+	+	+	+	+	+	+	+	+	+	+	+	+	+	7/9
ATR-13	+	+	+	+	+	+	+	+	+	+	−	+	−	+	+	+	+	+	+	+	+	−	−	−	−	5/X
XTR-3BSAgH	−	−	−	−	−	−	−	−	−	−	+	−	−	+	−	−	−	−	−	−	−	+	−	−	−	3/X, 10q
XTR-22	−	−	−	+	−	+	+	+	−	+	−	+	+	−	−	−	+	−	−	+	+	+	+	+	−	X/3
DUA-1CSAzF	+		−	−	−	−	−	−	+	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	
DUA-3BSAgA	+	+	−	+	−	−	−	−	+	+	−	−	−	−	+	+	−	−	+	−	−	−	−	−	−	
DUA-5BSAgA	−	+	−	−	+	−	+	−	−	−	−	−	+	−	−	+	−	−	+	+	−	−	+	−	−	
DUM-13	+	+	+	+	+	−	−	+	+	−	−	+	+	+	−	+	−	+	+	+	+	+	+	+	−	15/X, X/15
REX-11BSAgB	−	−	−	−	+	−	−	−	−	−	−	+	−	−	−	+	+	−	−	+	−	−	−	+	−	
TSL-2	−	+	−	+	−	−	+	+	−	−	−	+	−	+	−	−	−	−	−	+	−	+	+	−	+	17/3
NSL-5	−	+	+	−	−	−	−	−	−	−	−	+	−	+	−	+	−	+	−	+	−	+	−	−	−	17/9, 12q+
NSL-16	+	+	−	−	+	+	+	−	+	−	−	+	−	+	−	+	+	+	+	+	−	+	−	−	−	17/9
ITW	−	+	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	+	−	−	−	−	−	−	
ICL-4‡	+	+	−	+	+	+	+	+	+	−	−	+	+	+	+	+	−	+	+	−	+	+	+	+	+	
ICL-6‡	−	+	+	−	−	+	−	−	−	−	−	+	−	+	+	−	−	−	+	+	−	+	−	−	−	
TSL-8‡	−	+	−	−	−	+	−	−	−	−	−	−	−	−	−	−	−	−	+	−	−	−	−	−	−	
XER-15‡	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	−	+	−	+	
DUM-23‡	+	−	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	−	−	+	−	+	+	+	+	
JSR-1‡	−	−	−	−	−	−	−	−	−	+	−	−	−	−	+	−	−	+	−	+	−	−	−	−	+	
VTL-12‡	+	+	−	−	−	−	−	+	+	+	−	+	+	+	+	−	+	−	+	−	+	+	−	+	+	
VTL-13‡	−	+	−	−	−	−	−	−	−	−	−	−	−	+	+	−	+	−	+	−	−	−	+	−	−	
VTL-18‡	−	+	−	−	−	−	−	−	−	−	−	−	−	−	−	+	−	−	+	−	−	−	−	−	−	
% Discordancy NPY			30	18	24	27	27	21	0	30	42	24	18	33	21	24	30	27	36	42	21	42	39	36	27	
% Discordancy PP			52	45	48	45	48	45	42	52	77	39	52	35	48	32	52	61	0	45	52	45	42	65	45	

* *NPY* is localized to human 7pter-7q22 by the 7/9 translocation; *PPY* is localized to human 17p11-17qter by the 17/3 and 17/9 translocations. ‡ These human-mouse hybrids were characterized by chromosome-specific enzyme markers only; the remaining hybrids were characterized by both chromosome-specific enzyme markers and chromosome analysis.

segregated concordantly with markers for human chromosome 17 (Table I). This association was confirmed by comparison of genomic DNA from hybrid line ICL-15 and from the subclone ICL-15CSBF, which had been isolated from ICL-15 by selection for growth in the presence of BUDR. The only difference between the two lines is the presence of chromosome 17 in ICL-15 and its absence in the subline. The pancreatic polypeptide probe hybridized with DNA from ICL-15 but not from the subline, confirming the assignment of the gene to human chromosome 17. This gene is designated *PPY*.

Regional localization of *PPY* on chromosome 17 was determined by analysis of hybrid cell lines containing translocation chromosomes. Line TSL-2 carries a 17/3 translocation chromosome which includes the region 17p13-qter (11). Hybrid lines NSL-5 and NSL-16 contain a 17/9 translocation chromosome which includes the region 17p11.1-qter (12). Since all three of these lines were positive for hybridization with the pancreatic polypeptide cDNA (Table I), the *PPY* gene appears to be localized to the region 17p11.1-qter.

In contrast to pancreatic polypeptide the human genomic fragments hybridizing with the neuropeptide Y probe segregated concordantly with human chromosome 7 (Table I). DNA from the hybrid line JSR-29, carrying a 7/9 translocation chromosome containing 7pter-q22 (13) also hybridized with the neuropeptide Y cDNA. Thus, the gene encoding neuropeptide Y, designated *NPY*, appears to be localized to the region 7pter-q22.

Discussion

Two other gene families that have similar localizations as *PPY* and *NPY* are the *erb* oncogenes and the alpha chains of collagen. The localization of genes encoding the alpha-1-chains of types I and IV collagen and the oncogene *erb-A* overlaps with that of *PPY* on chromosome 17, while the localization of genes encoding the alpha-1-chain of type III collagen and the alpha-2-chain of type I collagen, as well as the oncogene *erb-B*, overlaps with that of *NPY* on chromosome 7 (14). These data are consistent with clustering of the three gene families on the two human chromosomes, a phenomenon of particular interest in light of recent studies indicating that *erb-A* and *erb-B* genes are syntenic in the mouse (15).

Gene families are thought to have originated by tandem gene duplication. The duplicated gene copies may remain linked on the original chromosome, or they may eventually be segregated

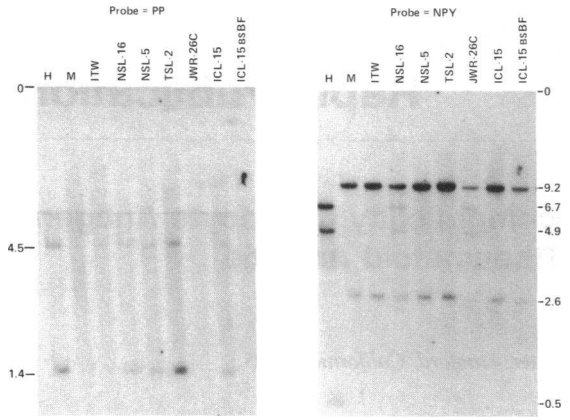

Figure 2. Hybridization of pancreatic polypeptide cDNA and neuropeptide Y cDNA probes to genomic DNA from human/mouse hybrid cell lines. Genomic DNA was digested with the restriction enzyme Pst I and the fragments were separated by electrophoresis on 0.7% agarose gels. After transfer of the DNA to nitrocellulose filters by the method of Southern (10), the baked filters were prehybridized for 2 h at 65° in buffer containing 4 × SSC (1 × SSC = 0.15 M sodium chloride/0.015 M trisodium citrate), 20 mM pyrophosphate solution (10 mM disodium phosphate, 6.6 mM monosodium phosphate, 3.3 mM sodium pyrophosphate), 20 μg/ml sonicated denatured salmon sperm DNA, 0.06% Ficoll, 0.06% polyvinyl pyrrolidone, and 0.1% sodium dodecyl sulfate (SDS). Filters were hybridized overnight at 65° in 10 ml of a solution containing 4 × SSC, 0.1% SDS, 50 mM EDTA, 2 μg/ml sonicated denatured salmon sperm DNA, and ~250 ng (2.5 to 12.5 × 10^7 cpm) of probe which had been nick-translated with [α-^{32}P]dNTPs to a specific activity greater than 10^8 cpm/μg DNA. After hybridization, filters were washed at 65° in solutions containing 0.1% SDS, 20 mM pyrophosphate buffer, and decreasing concentrations of salt as follows: two 30-min washes with 2 × SSC, two 15-min washes with 1 × SSC, and one 10-min wash with 0.1 × SSC. Filters were monitored with a hand held counter throughout the washing procedure. X-ray film (XAR-5; Eastman Kodak Co., Rochester, NY) was exposed to the dry filters for 1–5 d.

to different chromosomes by translocation events. For example, human growth hormone and somatomammotropin, which share a high degree of homology, are encoded by genes which have been localized to a cluster on chromosome 17 (16–18). On the other hand, the gene encoding prolactin, which also appears to have originated from the same ancestral gene as human growth hormone, has become separated to a site on chromosome 6 (19). After duplication, members of a gene family often diverge with respect to structural and regulatory characteristics. In the biologically active core region the structural homology between pancreatic polypeptide and neuropeptide Y is limited to 18 of 36 amino acid residues. In addition, these genes have diverged with respect to tissue specificity of expression, with *PPY* expressed in the pancreatic cells, and *NPY* expressed in the central nervous system. The physiological actions of the two peptides may also be quite different. The third member of this family, peptide YY, appears to be more closely related to neuropeptide Y, since the porcine peptides share 24 out of 36 amino acid residues (20, 21). It will be of great interest to determine whether the gene encoding pancreatic peptide Y is linked to *PPY* or to *NPY*.

Acknowledgments

This work was supported by United States Public Health Service (National Institutes of Health) grants AM33500, GM24872, GM20454, HDD5196, and pilot feasibility funding from the Michigan Gastrointestinal Peptide Research Center (AM34933).

References

1. Chance, R. E., and W. E. Jones. 1974. Polypeptide from bovine, ovine, human and porcine pancreas. *US Patent* No. 3:842,063.

2. Kimmel, J. R., L. J. Hayden, and H. G. Pollock. 1975. Isolation and characterization of a new pancreatic polypeptide hormone. *J. Biol. Chem.* 250:9369–9376.

3. Loren, I., J. Alumets, R. Hakanson, and F. Sundler. 1979. Immunoreactive pancreatic polypeptide (PP) occurs in central and peripheral nervous system: preliminary immunocytochemical observations. *Cell Tissue Res.* 200:179–186.

4. Tatemoto, K., and V. Mutt. 1980. Isolation of two novel candidate hormones using a chemical method for finding naturally occurring polypeptides. *Nature (Lond.).* 285:417–418.

5. Tatemoto, K., M. Carlquist, and V. Mutt. 1982. Neuropeptide Y—a novel brain peptide with structural similarities to peptide YY and pancreatic polypeptide. *Nature (Lond.).* 296:659–660.

6. Boel, E., T. W. Schwartz, K. E. Norris, and N. P. Fiil. 1984. A cDNA encoding a small common precursor for human pancreatic polypeptide and pancreatic icosapeptide. *EMBO (Eur. Mol. Biol. Organ.) J.* 3:909–912.

7. Leiter, A. B., H. T. Keutmann, and R. H. Goodman. 1984. Structure of a precursor to human pancreatic polypeptide. *J. Biol. Chem.* 259: 14702–14705.

8. Takeuchi, T., and T. Yamada. 1985. Isolation of a cDNA clone encoding pancreatic polypeptide. *Proc. Natl. Acad. Sci. USA.* 82:1536–1539.

9. Minth, C. D., S. R. Bloom, J. M. Polak, and J. E. Dixon. 1984. Cloning, characterization, and DNA sequence of a human cDNA encoding neuropeptide tyrosine. *Proc. Natl. Acad. Sci. USA.* 81:4577–4581.

10. Southern, E. M. 1975. Detection of specific sequences among DNA fragments separated by gel electrophoresis. *J. Mol. Biol.* 98:503–517.

11. Naylor, S. L., R. W. Elliot, J. A. Brown, and T. B. Shows. 1982. Mapping of aminoacylase-1 and Beta-galactosidase-A to homologous regions of human chromosome 3 and mouse chromosome 9 suggests location of additional genes. *Am. J. Hum. Genet.* 34:235–244.

12. Shows, T. B., A. Y. Sakaguchi, S. L. Naylor, D. V. Goeddel, and R. M. Lawn. 1982. Clustering of leukocyte and fibroblast interferon genes on human chromosome 9. *Science (Wash. DC).* 218:373–374.

13. Shows, T. B., J. A. Brown, L. L. Haley, M. G. Byers, R. L. Eddy, B. S. Cooper, and A. P. Goggin. 1978. Assignment of the beta-glucuronidase structural gene to the pter-q22 region of chromosome 7 in man. *Cytogenet. Cell Genet.* 21:99–104.

14. O'Brien, S. J. 1984. Genetic Maps—1984. Cold Spring Harbor Laboratories, Cold Spring Harbor, New York.

15. Zabel, B. U., R. E. K. Fournier, P. A. Lalley, S. L. Naylor, and A. Y. Sakaguchi. 1984. Cellular homologs of the avian erthroblastosis virus ERB-A and ERB-B genes are syntenic in mouse but asyntenic in man. *Proc. Natl. Acad. Sci. USA.* 81:4874–4878.

16. Owerbach, D., W. J. Rutter, J. A. Martial, J. D. Baxter, and T. B. Shows. 1980. Genes for growth hormone, chorionic somatomammotropin, and growth hormone-like gene on chromosome 17 in humans. *Science (Wash. DC).* 209:289–292.

17. George, D. L., J. A. Phillips III, U. Francke, and P. H. Seeburg. 1981. The genes for growth-hormone and chorionic somatomammotropin are on the long arm of human chromosome-17 in region Q21-QTER. *Hum. Genet.* 57:138–141.

18. Chakravarti, A., J. A. Phillips III, K. H. Mellits, K. H. Buetow, and P. H. Seeburg. 1984. Patterns of polymorphism and linkage disequilibrium suggest independent origins of the human growth hormone gene cluster. *Proc. Natl. Acad. Sci. USA.* 81:6085–6089.

19. Owerbach, D., W. J. Rutter, N. E. Cooke, J. A. Martial, and T. B. Shows. 1981. The prolactin gene is located on chromosome 6 in humans. *Science (Wash. DC).* 212:815–816.

20. Tatemoto, K. 1982. Isolation and characterization of peptide YY (PYY), a candidate gut hormone that inhibits pancreatic exocrine secretion. *Proc. Natl. Acad. Sci. USA.* 79:2514–2518.

21. Tatemoto, K. 1982. Neuropeptide Y: Complete amino acid sequence of the brain peptide. *Proc. Natl. Acad. Sci. USA.* 79:5485–5489.