Volume 267, number 1, 160–166 FEBS 08578 July 1990

The human islet amyloid polypeptide (IAPP) gene

Organization, chromosomal localization and functional identification of a promoter region

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Received 25 April 1990

We report the isolation and characterization of the human gene encoding islet amyloid polypeptide (IAPP). Previously characterized cDNA sequences correspond to three exons of which the first is noncoding. A functional promoter region was identified in the 5′ flanking DNA, however, this was farther upstream than expected. Northern blot analysis of human insulinoma RNA revealed three IAPP mRNAs of sizes 1.2, 1.8 and 2.1 kb, in agreement with three polyadenylation signals present in the 3′ end of the gene. In situ hybridization to metaphase chromosomes resulted in two distinct peaks on chromosome 12, at 12p12-p13 and 12q13-q14. Southern blot analysis of genomic DNA suggested a single IAPP locus but also indicated the presence of additional homologous sequences in human genomic DNA.

1. INTRODUCTION

Islet amyloid polypeptide (IAPP) (also referred to as 'amylin' or 'diabetes-associated peptide' (DAP) is the major constituent of islet amyloid developing in conjunction with type 2 diabetes mellitus in humans and cats [1–4] (for a recent review, see [5]). IAPP is 37 amino acid residues long and displays a 43–46% sequence identity with members of the calcitonin-gene-related peptide family (CGRP-1 and -2) [1–7]. cDNA cloning of human IAPP has indicated that the 37 amino acid peptide is a carboxy-terminally amidated proteolytic fragment of an 89 amino acid precursor [8–10]. IAPP mRNA expression and immunoreactivity is restricted to normal β-cells in the islets of Langerhans and to insulinomas [3,10–12]. The presence of a putative signal peptide in the IAPP precursor, the co-localization of IAPP and insulin in islet β-cell secretory granules [13,14] and recent measurements of IAPP released from cultured islets [15] suggest that IAPP is secreted together with insulin in response to increased glucose levels. The normal physiological function of IAPP is not known. However, its effects as an inhibitor of insulin-stimulated glycogen synthesis in skeletal muscle cells in vitro [16] and as an inducer of glucose intolerance in cats in vivo [17] indicate that it might take part in the regulation of blood glucose levels.

The occurrence of islet amyloid in type 2 diabetic subjects suggests the development of locally increased IAPP concentrations in the islets of these individuals. Whether this reflects changes in transcription, protein synthesis, rate of secretion of paracellular clearance of IAPP is not known. In order to study the mechanisms for cell-specific expression of the IAPP gene and its transcriptional regulation in relation to that of the insulin gene, we have isolated and structually characterized the human IAPP gene. We have also identified a putative promoter region by means of function and present data concerning the sublocalization of the hIAPP locus on human chromosome 12.

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Abbreviations: hIAPP, human islet amyloid polypeptide; cDNA, complementary deoxyribonucleic acid; mRNA, messenger ribonucleic acid; bp, base pairs, kbp, kilobase pairs; CAT, chloramphenicol acetyltransferase

2. MATERIALS AND METHODS

2.1. *General methods*

Standard methods in molecular biology [18] were utilized where not otherwise indicated. DNA sequencing was performed using dideoxynucleotide methodology [19] following subcloning in M13mp18/19 vectors. RNA was prepared using the LiCl/urea method [20].

2.2. *Isolation and characterization of human IAPP genomic clones*

Human genomic libraries, made in λEMBL-3 and λEMBL-4 vectors using partially *Sau*3A-digested and fully *Eco*RI-digested human macrophage DNA, respectively, were screened at high stringency using a human IAPP cDNA sequence corresponding to a 300 bp *Bam*HI fragment of a cDNA clone previously described [9]. λDNA was isolated from positive clones and inserts were characterized by diges-

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00145793/90/$3.50

tion with various restriction enzymes, Southern blotting and DNA sequencing.

2.3. *Promoter constructs, cell transfections and CAT assays*

Various length hIAPP 5′ flanking DNA segments were isolated and cloned in front of the bacterial chloramphenicol acetyltransferase (CAT) gene. The constructs were made by substituting the rat insulin I promoter/enhancer region in the pOK1 vector (provided by Dr Thomas Edlund, Umeå University; the pOK1 vector [21] contains 410 bp of rat insulin I gene 5′ flanking DNA, the entire CAT coding sequence [22] and simian virus 40 splice and polyadenylation signals in the pUC-18 plasmid) with hIAPP sequences. The insulin 5′ sequence was excised by *Hin*dIII. *Hin*dIII fragments of 435 and 1388 bp, respectively, corresponding to hIAPP genomic sequences extending 5′ from a *Hin*dIII site identified in the 5′ untranslated region of the previously characterized hIAPP cDNA sequence, were obtained by partial *Hin*dIII digestion of a plasmid containing a 3 kbp *Bam*HI/*Eco*RI fragment from λhIAPP-EMBL4b (Fig. 1) in pUC-13. The resulting CAT constructs were $CsCl_2$ gradient purified and transfected into the hamster insulinoma cell line HIT-T15 M.2.2.2 [23,24] (obtained from Dr Thomas Edlund, Umeå University), using the $CaCl_2$ method. Cell transfection solutions were purchased from Pharmacia (Uppsala, Sweden) and used according to manufacturers' recommendations. Forty-eight hours following transfection, cultures were harvested, cell lysates prepared and CAT-assays [22] performed using [^{14}C]chloramphenicol (Amersham, UK).

2.4. *Primer-extension and RNase-protection assays*

Primer extension [18] was performed using the oligonucleotide 5′AAGCTTCAAGAAAAGAGTCCAA (complementary to positions −88 to −67 in Fig. 1). Briefly, the primer was annealed overnight to 1 μg of heat-denatured poly(A$^+$) RNA in a solution containing RNase inhibitor and actinomycin D. Extension was performed in the presence of [α-^{32}P]dCTP for 30 min at 37°C using AMV reverse transcriptase (Pharmacia). For RNase A protection assays [18], various DNA fragments covering the putative promoter region were subcloned in Bluescript vectors and ^{32}P-labeled cRNA synthesized following linearization of the vector with an appropriate restriction enzyme. The cRNA probe was gel-purified and hybridized to 1 μg of poly(A$^+$) RNA at 45°C for at least 12 h. RNase A digestion was at 30°C for 30 min using an enzyme concentration of 75 μg/ml. Both primer extension and Rnase A protection reactions were analyzed on 8% polyacrylamide/urea sequencing gels and product sizes were estimated by comparison with M13 sequences run in parallel.

2.5. *In situ hybridization*

A 300 bp fragment derived from the 5′ region of a hIAPP cDNA clone [10] was labeled by nick-translation with [^{3}H]dTTP and [^{3}H]dATP to high specific activity (4×10^7 cpm/μg DNA). Prometaphase chromosomes, prepard from methotrexate-synchronized normal peripheral lymphocytes from two donors, were hybridized in situ as described previously [25,26]. The hybridizations were performed in a moist chamber at 37°C for 16 h at a probe concentration of 20 or 100 ng/ml in the hybridization mixture. Washes after hybridization were at 40 or 43°C. Autoradiographs were exposed for 6–16 days and subsequently developed and G-banded [27]. Metaphases were analyzed directly under the microscope for the presence of silver grains overlaying or touching the chromatides.

3. RESULTS

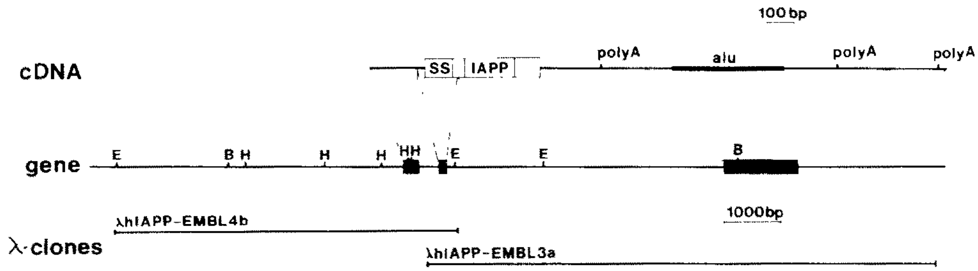
3.1. *Characterization of human IAPP genomic clones*

The outline of two overlapping hIAPP genomic clones, a consensus restriction map of the hIAPP locus and the DNA sequences of exons, exon/intron junctions and a 5′ flanking region are shown in Fig. 1. Previously known mRNA (cDNA) sequence localized to three regions (exons) being 112, 95 and 390–1244 bp long, respectively (the variation of the length of the 3rd region reflects the use of alternative polyadenylation signals [8–10]). The intron between the first of these was only 326 bp whereas the second intron was approximately 5 kb. Comparison with the cDNA sequence [8–10] shows that the first exon is non-coding, the second exon contains the translation initiation site and encodes the signl sequence and part of the N-terminal propeptide of the hIAPP precursor and the third exon encodes the remaining part of the N-terminal propeptide, mature IAPP as well as the C-terminal propeptide(s). Three consecutive polyadenylation signals are found in the 3rd exon, all of which appear to be utilized, as judged from the characterization of polyadenylated cDNAs [8–10]. An Alu repeat sequence is situated between the 1st and 2nd of these, and has been shown to be included in cDNAs polyadenylated at the 2nd or 3rd of the polyadenylation signals [8–10].

3.2. *Primer-extension, Northern blot and RNase protection analyses:*

The largest hIAPP cDNA clones characterized to date have their 5′ end close to two *Hin*dIII sites located in the noncoding exon (Fig. 1) [8–10]. To map the transcriptional start site, primer-extension analysis was performed using a synthetic antisense oligonucleotide primer complementary to positions −88 to −67 in Fig. 1. Extended products, up to 40 nucleotides long, were seen using IAPP-positive human insulinoma mRNA as template, but not with IAPP-negative mRNA (Fig. 2a). The 'ladder' of products indicates some degradation in the 5′ end of the IAPP mRNA or incomplete extention, possibly due to mRNA secondary structures. The latter seemed most probable in the light of the hIAPP mRNA sizes revealed by Northern blotting (Fig. 2b). The size differences of the three transcripts (1.2, 1.8 and 2.1 kb), identified specifically in human insulinoma RNA, correlate with the positions of the three polyadenylation signals (Fig. 1). However, the coding sequences between the polyadenylation signals and the 5′ ends of previously characterized cDNA clones [8–10] and our primer-extension products (Fig. 2a) is only about 0.6, 1.2 and 1.5 kb, respectively. A comparison with the sizes of the mRNAs indicates the existence of some 600 bp of 5′ untranslated sequence not present in the cDNA clones.

To explore the possibility of additional 5′ untranslated sequence, RNase A protection assays were performed using human insulinoma poly(A$^+$) RNA and ^{32}P-labeled antisense RNA corresponding to different regions of the 5′ flanking sequence. Fig. 3 illustrates the probes used and shows the fragment protected. The 435 bp *Hin*dIII fragment extending from the 5′ end of the cDNAs protected a slightly smaller RNA fragment (Fig. 3), whereas the 125 bp *Kpn*I/*Hin*dIII fragment immediately upstream of the 435 bp region, did not give any detectable protection (data not shown). Fragments



(b)

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-2395                                                                                     GGATCCATTTTCAAT
-2380  GTTATGTTTGGCACCCTTAACATCTTAGAGAAACAAAAAAGGTTATGAAAATAGAATAAATAACAATGTAGGTAATAAGCAAGTATTTTT
                                              HindIII
-2290  GTCTAAATGAACACTAGACCATGAGTCATAAAGCTAAGCTTGGTCCAGATTCAGCATTAACTAGATGTTTGATGTTGAACTTTATTTAAT
-2200  GTCACTGGCCCTGGTATGCTTTCTACATCACTGTATTTAACGCAGAACAGGTGAGACCCAAACTGGGGCTTAGCCCCTCAAGGGTTCTTG
-2110  GCTTTGTTCATGAAAGAGTTCAGGGGCAAGTTGGAGGTAGAAGAAAACAGCTTTATTGAAGAGGCGGTGTTACAGCTCTGGCATTTATAA
-2020  CTCTGTGACTGCTCCTGCAGGGCAGGCTAACCCATATGCAGAGAGTAGCAGCTTACGGCGGTTTTGCAGTCATATTTATACCCACTTTTA
-1930  ACTACATGCAGATTAAGGGATGGTTTATGCGGAAATTTCAAGGGAAGTGGTAGTAACTTTTGGGTCATCTGGTCATTGCCCTGGAAAGAG
-1840  GCAGTAACTCCTGGGTGTTGTTATAGCAATGGTAAACTGACATGGCACACTAGGGGGCTGGTCTTATGGAAGCTGCTTTCCATTGGCCCT
-1750  GTTTTATCTAGTTCTCAATTTGGTCTGGTGTTCAAGCCCTGCCTCTGGAGTCTAGTCCTGCCTCCTACCTCATATTCACCTTGTTAAGTA
-1660  CATTATCTTCTTACGGAAATGCATTATCATTCCATTTCTTTTGATTTTTTTTCTTTTGGAATATCAGTAAATTTCACAGAAATATTAAAA
-1570  TTGTAGAATCATTGAATAATTAGAACAAGGATTCTAAACAAGTTTCTTTCCTCCTGAAATCTCTACCAAACACCTTCAAACAATGTTACA
       HindIII
-1480  AAGCTTTTAAAGAAGTATATGAAATCAATGAACTAATGTTTCTAGTTAGAGAACTTCAATTTTTTTAAGATTACACAGGCATGCACATTT
-1390  TCACTGACAATTGAAATTTATTTGCTTTACATGACACTAAGTTGTTCTATTAATAACTAAGATACCAAATATAATTTAGAAATACAAAAA
                Alu repeat sequence
-1300  AAGAGGATGGGTGCGGTCGTGACGCCTGTAATCCCAGCACTTTGGGAGGCAGAAGTGGGCGGATCACCTGAGGTTGGGAGTTCGAGACCA
-1210  GCCTGACCAACATAGAGAATCCCCGTCTCCACTAAAAATACAAAATTAGCCATACATGGTGATGCATGCCTGTAATCCCAGCTACTCTGT
-1120  AGGCTGAGGCAGGAGAATCACTTGAACCCGGGAGGCACAGGTTACAGTGAGCCGAGATCGCACCCTTGTACTCCAGCCTGGGCAACAAAG
-1030  AGTGAAACTTCATCTCAAAAAAAAAAGAAAAAAAAAGAAGAGAGAAAGAAATACAAGAAAAAGAAAAAGCAATTTGGAGAAAATTTTAAAA
-940   AGCAAATCATTTTAAAAGACAAACTACAAAGTACTGTGTGAGCTAAGCGATTCATAATGTTCTCATGGAGCTAATGGGAAACAAATCTGT
-850   TCTTTCAAAATAATTGTAAAATTTCTGTGTAAGAATGAATGGAAAAGGTCATCCAAAAACTACTTGACTACATATACCTATGGATCTCTA
-760   TCTAAGTGCTGAAAAATGAATTTTTTTATTCATTTGGTTATGTAGCATTTGTTCATTCTAATTTTGTTTTGTTCCCTGTCATATCTCTGG
-670   TACCTAGAATAATCCCTACCACAGAGTAGGTCTTCCATTACTCTTATGCTTTTAAATCTCCCCTCACCTCATTGTAAATGACTTTTGATT
                                                                     HindIII
-580   TCTCTTTTATGCCCTTTTTATACACCTTTCCCTTATATCTCCATTTATTCCTGAAGCTTCATGGGATTCAGCCATTGAGGTCACTTGGGT
-490   TTAGATATACCAAAAGTCTGTGATTTCTCTGTTTGCATATATGCACATTTGTTGTTATCCTTACCCTTTTCTATCAGTTCCTTACCATAA
-400   CATACACTTAATTCTTGGAAATTCACTCATGTCTTACAAAGATGGCAAATTCACACTTCTGCTGTGTATGACACACCATTAACTGCACAA
-310   GGACACTGTGTATTTGCTACGTTAATATTTACTGATGAGTTAATGTAATAATGACCCATCCGCTTCTGCTGCCTGTGAGGTACTTTCTAT
-220   CTATAGGGATGGAAATTAATGACAGAGGCTCTCTGAGCTGCCTGATGTCAGAGCTGAGAAAGGTGTGAGGGGTATATAAGAGCTGGATTA
         ++++++++++++                            HindIII              HindIII
-130   CTAGTTAGCAAATGAGGGGTAAATATTCCAGTGGATACAAGCTTGGACTCTTTTCTTGAAGCTTTCTTTCTATCAGAAGCATTTGCTGAT
-40    ATTGCTGACATTCAAACATTAAAAGgtaaagaatttcctatttctgggaaagttttatttatttagagaaatgcacacttggtgttaaat
       tcatggtttatttcaaagaaaggctaaagggagaatgtattacaatataaatgttcagattgcttagagaaggaaattgggaaagtaaaa
       atctcgaaattacttgaaaagtggacaatattaagggactgatcataaatttgtccttgtaaattacgttttaaaaagatgtttcttta
-15    aaaactaagctctaatttaaaattacatcaattagaactgtaagaaatctcttgatttcagtgctggattattctttgcagAAAATTTGA

              1               5                  10                 15                 20
              Met Gly Ile Leu Lys Leu Gln Val Phe Leu Ile Val Leu Ser Val Ala Leu Asn His Leu Lys
-6     GAAGCA ATG GGC ATC CTG AAG CTG CAA GTA TTT CTC ATT GTG CTC TCT GTT GCA TTG AAC CAT CTG AAA

                   25
       Ala Thr Pro Ile Glu Se
37     GCT ACA CCC ATT GAA AG gttggtaacttaaaat   ....   5 kb intron

                                    30                 35                 40
                              r His Gln Val Glu Lys Arg Lys Cys Asn Thr Ala Thr Cys Ala Thr Gln Arg
81       tgttccatgttaccag T CAT CAG GTG GAA AAG CGG AAA TGC AAC ACT GCC ACA TGT GCA ACG CAG CGC

       45                  50                 55                 60                 65
       Leu Ala Asn Phe Leu Val His Ser Ser Asn Asn Phe Gly Ala Ile Leu Ser Ser Thr Asn Val Gly
133    CTG GCA AAT TTT TTA GTT CAT TCC TGC AAC AAC TTT GGT GCC ATT CTC TCA TCT ACC AAC GTG GGA

                   70                 75                 80                 85
       Ser Asn Thr Tyr Gly Lys Arg Asn Ala Val Glu Val Leu Lys Arg Glu Pro Leu Asn Tyr Leu Pro
199    TCC AAT ACA TAT GGC AAG AGG AAT GCA GTA GAG GTT TTA AAG AGA GAG CCA CTG AAT TAC TTG CCC
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                89
                Leu  *
265     CTT TAG AGGACAATTGTAACTCTATAGTTATTGTTTTATGTTCTAGTGATTTSTGTTAATTTAACAGTGCCCTTTTCATCTCCAGTGT

353     GAATATATGGTCTGTGTGTCTGATGTTTGTTGCTAGGACATATACCTTCTCAAAAGATTGTTTTATATGTAGTACTAACTAAGGTCCCAT
        polyA
443     AATAAAAAGATAGTATCTTTTAAAATGAAATGTTTTTGCTATAGATTTGTATTTTAAAACATAAGAACGTCATTTTTGGGACCTATATAT

533     CTCAGTGGCACAGGTTTAAGAACGAAGGAGAAAAAGGTAGTTTGAACCTTGGTAAATTGTAAACAGCTAATAATGAAGTTATTCTTGACA
                                  Alu repeat sequence
623     TGAGAAAATCAGTAATTGGACCAGGCGCGGTGGCTCTTGCCTGTATCCCAGCACTTTGGGAGGCCGAGGCAGGCAGATCACAAGGTCAGG

713     AGTTCGAGACCAGCCTGACCAACAATGGTGAAACCCTGTCTCTACTGGAAATACGGAAATTAGCCGGGGGTGGTGACATGTGCCTGTAAT

803     CCCAGCTACTCAGGAGGCTAAGGCAGGAGAATCGCTTGAACCCAGGAGGCGGAGGTTGCAGTGAGCCGAGATTGCACCACTGCACTCCAG

893     CCTGGGTGGCAGAGTGAGACTCGTCTCAAAAAAAGAAAGAAAATTAGTAATTGTAAGTACCCCTGATAAGCAAATTAGTAATTGTCAATA
                                                                                    polyA
983     CCCCTGTTAAGCAATTCCTTTTTGCTGTATATTTCTGAAATGACAGAATGCTGTTTTAAAAACAAAGAAATAAAATCCTGCTCCTGACTC

1073    GGTCAAAATATTTTTTAAAGTGTCTATTGTTTGTTGTGCTTGCTGGTACTAAGAGGCTATTTTAAAAGTATAAAACTGCTTTGTATCGAT

1163    AGGGTTTCATTGTGTGTTAGCAGCAGTGAGCTTCTATTAAATGTATATGTCATTTATTTTGTTTAAGTGGCTTTCAAGCAAACCTCAGTC
                                                                                polyA
1253    TATTCTTATGCAGGGTATTGCGAAACAACTTGTGTTCTATTAATCGTGTCTTCAATTAAAAAGACCACAGACTT
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Fig. 1. (a) Outline of the hIAPP cDNA as previously characterized [8–10] and its relationship to the gene as determined through the characterization of two λ-clones. Within the protein-coding sequence in the cDNA (boxed), the signal sequence (SS) and IAPP-encoding region are indicated. In the gene, the positions of sequence corresponding to the cDNA (exons) are indicated (filled boxes). Restriction sites for *Eco*RI (E), *Bam*HI (B) and *Hin*dIII (H) are marked. (b) Sequence of the hIAPP gene and 5′ flanking DNA. Small letters are used for known intron sequences. Restriction sites used for promoter-CAT constructs are indicated as well as two Alu repeat sequences, one in the 5′ flanking DNA and one in the 3′ untranslated region. The sequence complementary to the oligonucleotide used for primer extension analysis is overlined and the position of primer extension products marked by arrows Polyadenylation signals in the 3′ part of the gene are underlined.

corresponding to regions further upstream protected a series of small fragments, ranging from 20 to 70 bp (data not shown), in agreement with the presence of an Alu repeat sequence in this region (Fig. 1). Our data are thus compatible with the existence of a large portion of 5′ untranslated sequence which is missing in the hIAPP cDNA clones isolated so far [8–10]. The reason for this, as well as for the presumably incomplete primer-extension shown in Fig. 2a, is not known. However, poor reverse transcription of long 5′ untranslated sequences is not uncommon and may be due to the presence of stable secondary structures in these regions.

3.3. *Functional identification of a promoter region*

Two DNA fragments, extending 435 and 1388 bp, respectively, from the most 5′ of the two *Hin*dIII sites

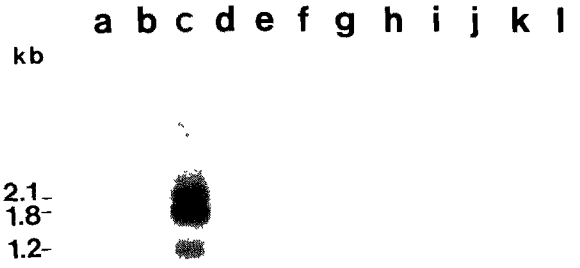

Fig. 2. (a) Primer extension analysis using human insulinoma (Ins) or melanoma (mel) mRNA (from the human melanoma cell line WM 266-4 [37]) in comparison with an M13mp18 sequence. Two independent reactions are shown. nt, nucleotides. (b) Northern blot analysis of poly(A^+) RNA (10 μg/lane) from human fibroblasts (lane a), human WM 266-4 melanoma cells [37] (lane b), a primary human insulinoma (lane c), human U-343 MGa Cl 2:6 glioblastoma cells [38] (lane d), human A431 epidermal carcinoma cells [39] (lane e), rat liver (lane f), rat spleen (lane g), rat brain (lane h), rat kidney (lane i), rat intestine (lane j), rat testis (lane h) and porcine thyroid (lane l). The probe was a ^{32}P-labeled hIAPP cDNA probe. kb = kilobases.

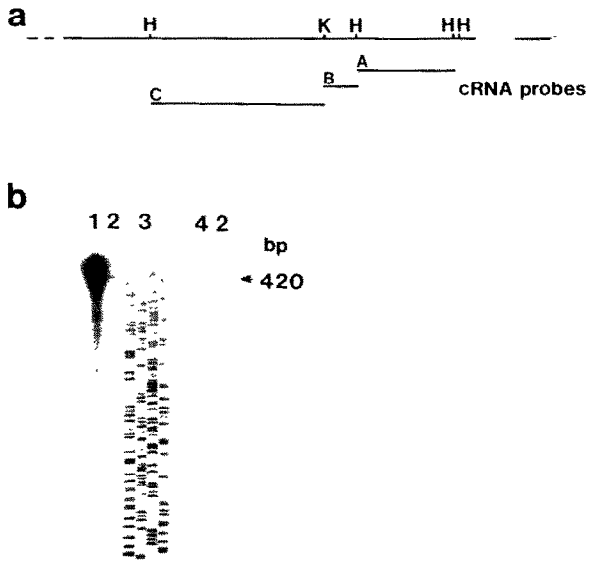

Fig. 3. RNase protection analysis of human insulinoma RNA. (a) Illustration of the 5′ flanking region of the gene and the probes used. (b) Autoradiogram showing a 420 bp protected fragment in human insulinoma RNA using probe A (lane 2). This product was absent in the control reaction utilizing tRNA instead of insulinoma poly(A^+) RNA (lane 4). The protected fragment was slightly smaller than the probe used (lane 1). Fragment sizes were estimated by comparison with the M13mp18 sequence run in parallel (lane 3). H = *Hin*dIII site, K = *Kpn*I site; bp, base pairs.

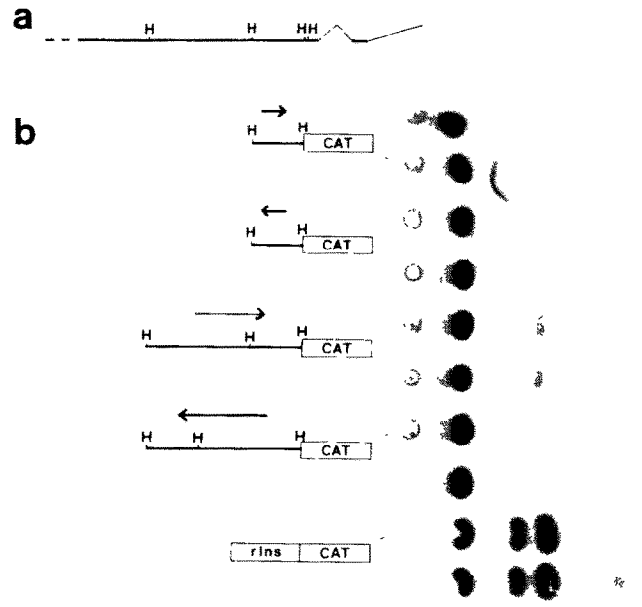

Fig. 4. CAT assays using HIT-T15 M.2.2.2 cells. Constructs containing various length and orientation DNA fragments from the 5′ flanking region of the hIAPP gene (indicated at the top of the figure; compare with Fig. 1a) in front of a CAT gene, were assayed for promoter activity in comparison with a rat insulin I promoter (rIns)-CAT construct. Arrows indicate orientation of the 5′ flanking DNA fragment. H = *Hin*dIII site.

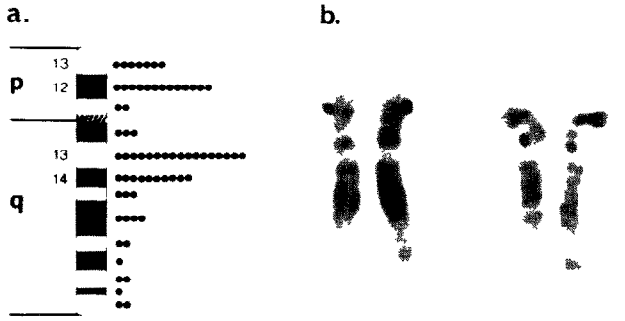

Fig. 5. Distribution of labeled sites on human chromosome 12 (a) after in situ hybridization to a ^{3}H-labeled hIAPP cDNA probe (20 ng probe/ml hybridization solution), and G-banded hybridized chromosomes 12 (b) with grains at 12p13 and 12q13, respectively.

found in the untranslated sequence of the cDNA clones, were cloned in correct and reversed orientation in front of the bacterial cloramphenicol acetyltransferase (CAT) gene and transfected into hamster insulinoma (HIT-T15 M.2.2.2) cells. Significant CAT activity was seen only when the vector containing the correctly oriented 1388 bp fragment was used (Fig. 4). Neither the 1388 bp fragment in reversed orientation nor the 435 bp fragment in correct or reversed orientation resulted in significant CAT activity.

At present, we do not know the location of the hIAPP promoter within the 1388 bp fragment. A construct carrying its 5′ 933 bp in correct orientation also induced CAT activity in HIT cells (data not shown), in-

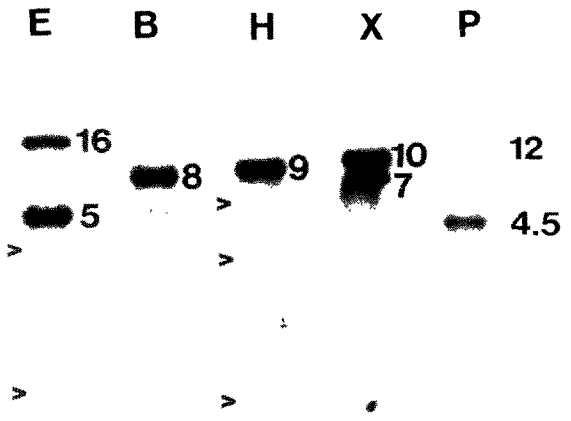

Fig 6. Southern blot analysis of human chromosomal DNA samples digested with *Eco*RI (E), *Bam*HI (B), *Hin*dIII (H), *Xba*I (X) and *Pst*I (P), respectively. The blot was hybridized with a ^{32}P-labeled 300 bp *Eco*RI/*Bam*HI fragment corresponding to the 5′ end of a previously described hIAPP cDNA clone [10]. Numbers refer to approximate sizes (in kilobase pairs) of each fragment. Arrows indicate additional, weakly hybridizing bands

dicating an upstream location in agreement with the estimated mRNA sizes.

The 1388 bp human IAPP 5′ flanking sequence was much less active in HIT cells than a construct containing 410 bp of rat insulin gene 5′ flanking sequence (Fig. 4). This might reflect the respective activities of the two endogenous promoters in HIT cells and agrees with the finding that the level of IAPP mRNA is approximately 100-fold less than that of insulin mRNA in these cells (Betsholtz et al., unpublished result). However, we cannot exclude the possibility that sequences of importance for efficient IAPP expression in HIT cells (i.e. tissue-specific enhancers) are located upstream of the 1388 bp region analyzed in this study.

3.4. *Chromosomal sublocalization of the hIAPP gene*

The hIAPP gene has previously been regionally mapped to the 12pter-q14 by using a panel of somatic cell hybrids [28]. For sublocalization of hIAPP, 64 cells with silver grains located on chromosome 12 were analyzed. Two different sites of hybridization were detected, one on the long arm and one on the short arm. Out of the 67 grains recorded, 27 (40%) clustered at 12q13-q14, and 20 (30%) at 12p12-p13 (Fig. 5). The remaining grains were randomly distributed over chromosome 12. The same hybridization pattern was obtained both at standard and high stringency hybridization conditions (data not shown). The only difference that could be noted was that hybridization at a probe concentration of 100 ng/ml resulted in a somewhat greater accumulation of grains at 12q13-q14 as compared to 12p12-p13. This raises the question whether more than one IAPP gene is present on human chromosome 12. Southern blot analysis (Fig. 6) is consistent with a single IAPP locus in the human genome, but the existence of several faintly hybridizing restriction fragments, of sizes not predictable from the structure of the cloned IAPP locus, suggests that additional IAPP-related sequences might be found in the human genome.

4. DISCUSSION

We report here the structural characterization and chromosomal sublocalization of the human IAPP gene. Given the fact that it encodes a precursor protein of only 89 amino acids and a mature protein product which is only 37 residues long, the gene is surprisingly complex. Thus, it consists of at least three exons, of which the first is non-coding. Exon 2 contains the translation initiation codon and encodes the signal peptide as well as part of the N-terminal propeptide. The mature IAPP coding sequence falls entirely within exon 3, as do the C-terminal propeptide(s) and 3′ untranslated sequence.

The identification of a functional IAPP promoter region is a first step in unravelling the mechanisms involved in the regulation of this gene. The coexpression of IAPP and insulin in pancreatic β-cells [3,10–12], the presumed cosecretion of the two hormones in response to increased blood glucose levels [13–15] and their reported opposite effects on the glucose metabolism in skeletal muscle cells [16] and on blood glucose levels [17] highlight questions concerning how their transcriptional regulation. In the insulin gene, transcription is regulated by elements in the 5′ flanking DNA that bind β-cell-specific nuclear proteins [21,24,29,30]. However, no extensive homologies could be seen between the hIAPP and insulin 5′ flanking regions. This may indicate that the two genes have different transcriptional regulation. Such a notion is supported by non-correlating insulin and IAPP mRNA and protein expression patterns in cultured insulinoma cell lines (O. Madsen, personal communication), human insulinoma primary tumors (Westermark et al., unpublished observations) and in experimentally diabetic rats [31].

During the preparation of this manuscript, Nishi et al. reported on the structure and chromosomal localization of the human IAPP gene [32]. In contrast to us, they report a single site for IAPP-hybridizing sequences on chromosome 12. This site coincides with one of our localizations, that on 12p12-p13, which is therefore likely to constitute the site of the hIAPP gene. Recent in situ data from a third group also support the assignment to 12p [33]. Interestingly, our second hybridization site at 12q13-q14 coincides with a suggested alternative localization for the hIAPP gene [33]. We do not know the reason for the two subregional localizations described for hIAPP, but different hybridization conditions used by the various groups could have given hybridization to an IAPP-related sequence in addition to the authentic hIAPP locus, also being present on chromosome 12.

Nishi et al. also reported a transcription initiation site, mapped by primer extension and RNase protection analysis, which corresponds to position −117 in Fig. 1 and thus indicates a promoter region distinct from ours. The basis for this discrepancy is also unknown. A possible explanation might be that alternative promoters exist for the hIAPP gene and that the utilization of these promoters is variable in insulinomas.

Further insight into the regulation of the IAPP gene might have relevance for the understanding of type 2 diabetes mellitus. Evidence is accumulating that this disease is associated with an increased expression of IAPP. Islet amyloid is deposited in diabetic individuals of species with an amyloidogenic structure of IAPP [1–4,10,34,35]. Moreover, increased IAPP immunoreactivity has been shown in conjunction with prediabetic states in the cat [36]. It will be important to know whether this reflects changes in the transcription of the IAPP gene and if so, whether disturbances in this regulation may be caused by hereditary or dietary factors.

Acknowledgements: We thank Drs Thomas Edlund and Olof Karlsson, University of Umeå for HIT-T15 M.2.2.2 cells and the pOK1 vector. This study was supported by the Medical Research Council, Hans von Kantzow's Foundation, Magnus Bergwall's Foundation, the Swedish National Board for Technical Development, the Research Fund of King Gustaf V, the Nordic Insulin Fund and the Swedish Cancer Society.

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