Biochemical Pharmacology, Vol. 43, No. 12, pp. 2527–2533, 1992.
Printed in Great Britain.

0006-2952/92 $5.00 + 0.00

γ-GLUTAMYLTRANSFERASE: NUCLEOTIDE SEQUENCE OF THE HUMAN PANCREATIC cDNA

EVIDENCE FOR A UBIQUITOUS γ-GLUTAMYLTRANSFERASE POLYPEPTIDE IN HUMAN TISSUES

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(*Received* 29 *November* 1991; *accepted* 16 *March* 1992)

Abstract—γ-Glutamyltransferase (GGT, EC 2.3.2.2) is an enzyme involved in glutathione metabolism and drug and xenobiotic detoxification. Using human hepatoma Hep G2 GGT cDNA as probe, we isolated a cDNA from a human pancreatic cDNA library. Analysis of the nucleotide sequences revealed a 2244-bp insert that includes an open reading frame of 1710 bp, encoding a protein identical to the Hep G2 and human placenta GGTs. Similarly, the 5′ untranslated region, though shorter, is highly homologous to that of Hep G2 cDNA. These data suggest strongly that the same gene encodes human GGT in the placenta, Hep G2 and the pancreas. We further studied the distribution of the corresponding mRNA, called type *I* mRNA, in different human tissues. Using a highly sensitive method associating reverse transcription with specific amplification by polymerase chain reaction, cDNA was synthesized from total RNA isolated from the tissues and GGT specific fragments were amplified. We observed the presence of a specific cDNA fragment corresponding to the type *I* mRNA in the human tissues and cells tested, providing the evidence for a ubiquitous expression of this GGT mRNA in human tissues.

γ-Glutamyltransferase (GGT,† EC 2.3.2.2) is a plasma membrane-anchored glycosylated enzyme catalysing the hydrolysis of glutathione and its derivatives, thus supposedly involved in the synthesis of mercapturic acids, the metabolic pathway of leukotrienes and in the regulation of the glutathione levels and the associated detoxification processes [1]. Widely distributed in various tissues and body fluids, the human mature and active enzyme is composed of two non-identical subunits (M_r 50,000–62,000 and 22,000–30,000) [2]. Both subunits are encoded by a common mRNA, translated into a single polypeptide precursor, further cleaved and glycosylated depending on the tissue origin [3]. GGT activity levels in plasma are used extensively in clinical chemistry as a marker of preneoplastic processes, hepatobiliary disorders and alcohol abuse, and in clinical pharmacology as an indirect marker of drug induction [4]. GGT activity exhibits a variability that has not yet been explained by any molecular data.

GGT is encoded by a single gene in the rat, whereas the presence of a multigenic family has been reported in humans [5]. The cDNA sequences related to mRNAs encoding the human placenta [3], fetal liver [6] and Hep G2 [7] GGTs reported to date have not given any clue regarding which gene(s) is (are) expressed.

Human pancreatic GGT exists as two isoforms [8], differing by their sialic acid content [9]. The enzyme is essentially located in the membrane of zymogen granules in the apical cytoplasm of acinar cells [10], and the intercalated and the intra- and interlobular ducts, but not in the islets of Langerhans [11]. Adult normal pancreas contains high GGT activity levels which are exceeded only by those in the kidney [12]. Similarly to the kidney, but in contrast to the liver where the enzyme is induced, GGT activity decreases in the pancreas after chronic ethanol administration [13] and during its neoplastic transformation [14].

In order to elucidate the molecular regulation mechanisms involved in the variability of GGT activity in the human pancreas as well as those implicated in the tissue specificity of the GGT gene expression, we isolated a cDNA coding for the human pancreatic enzyme and studied the distribution of the pancreas-type mRNA in several human tissues.

MATERIALS AND METHODS

Materials. The λgt11 human pancreatic cDNA library and the *Escherichia coli* strain Y1090 (Δ*lac*U169, *pro*A$^+$, Δ*lon*, *ara*D139, *str*A, *sup*F, [*trp*C22::Tn10] (pMC9)) were purchased from Clontech. 5′-[α-^{32}P]dCTP (3000 Ci/mmol) and nitrocellulose filters were from Amersham (U.K.). The 2.4-kb full length cDNA probe to the human hepatoma Hep G2 GGT [7] was kindly provided by Prof. H. C. Pitot (McArdle Institute for Cancer

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† Abbreviations: GGT, γ-glutamyltransferase; PCR, polymerase chain reaction; UTR, untranslated region(s).

Research, Madison, WI, U.S.A.). Plasmid pBS-(M13+) has been purchased from Stratagene Inc., and oligonucleotides from Appligene (Illkirch, France).

Restriction enzymes, DNA ligase, polynucleotide kinase, T7 DNA polymerase, "RNA guard" and the oligolabelling and sequencing kits were from Pharmacia. Moloney-murine leukemia virus reverse transcriptase was purchased from Bethesda Research Laboratories and the *Thermus aquaticus Taq* DNA polymerase from Cetus. All other reagents and chemicals were of the purest grade available.

Human samples. All samples were prepared from tissues which had been frozen in liquid nitrogen as quickly as possible after sampling. Adult tissues were obtained through the courtesy of Prof. J. P. Boissel (Centre Hospitalier Universitaire Régional, Vandoeuvre-lès-Nancy, France) during the course of surgical operations. Fetal tissues were kindly offered by Dr B. Leheup (Maternité Régionale, Nancy, France) from a 22-week-old fetus without any pathological features. Lymphocytes were prepared from 10-mL samples of whole blood from volunteers, according to Hanash *et al.* [15]. Human hepatoma Hep G2 cell line was obtained from ECCAC (U.K.) and cultured in Petri dishes as described previously [16].

Screening of library and analysis of positive clones. Hep G2 GGT cDNA was radiolabelled by random priming using the Klenow fragment of DNA polymerase. It was used as probe to screen the human pancreatic cDNA library. Positive clones were selected out of 1×10^6 plated clones after four consecutive screenings under the following conditions: hybridization at 65° in [6 × SSC, 5 × Denhardt's solution, 0.1% (w/v) SDS]; washing at 65° in (0.1 × SSC, 0.1% SDS). The pancreatic cDNA fragment cloned by us was analysed by restriction mapping and sequenced using the dideoxy method with T7 DNA polymerase and specific oligonucleotides as primers.

RNA preparation. Total cellular RNA was isolated from lymphocytes, Hep G2 cells and human tissues by the method of guanidium thiocyanate [17] and phenol/chloroform extraction.

cDNA synthesis. The conversion of RNA to cDNA was carried out in a final volume of 50 μL containing 1 μg of total RNA from human tissues or Hep G2 cells, 50 mM Tris–HCl, pH 7.6, 70 mM KCl, 10 mM $MgCl_2$, 4 mM dithiothreitol, 10 μg oligod$(T)_{12\text{-}18}$ primer, 1 mM each dNTP, 40 U of "RNA guard" and 200 U of reverse transcriptase. Before adding "RNA guard" and reverse transcriptase, the reaction mixture was heated at 65° for 6 min and quenched on ice. The reaction was carried out at 37° for 60 min and stopped by cooling on ice.

Amplification method. cDNA was amplified with 2 U of *Taq* DNA polymerase in a thermal cycler (Perkin Elmer-Cetus). Samples were prepared with 10 μL of the cDNA solution in a total volume of 100 μL containing the following PCR mixture: 1 × PCR buffer [10 mM Tris–HCl, pH 8.0, 50 mM KCl, 1.5 mM $MgCl_2$, 0.001% (w/v) gelatine], 250 mM each dNTP and 1 μM of each 5′ and 3′ primers. After a first denaturation at 96° for 6 min, the *Taq* polymerase was added and the mixture was overlaid with mineral oil (Sigma) and then incubated for amplification. Steps were denaturation for 1 min at 94°, primer annealing for 2 min at 55°, and primer extension for 2 min at 72°. A first PCR was conducted with primers # 1 (CGTGCTGGCCCTCATCCTCA) and 2 (pTGACCGGGGAGCGGACC) during 30 cycles. A second PCR was performed during 35 cycles in a fresh mixture containing 1 μL of the first PCR product and primers # 3 (pCAAGTTTGTGGATGTGACTGAG) and 4 (pTCAGAGATCTGGGCCCG), identical to those used by Pawlak *et al.* [18] enclosing the region where a 22-bp insertion can occur.

Aliquots of intermediary or final products were loaded on a non-denaturing 8% (w/v) polyacrylamide gel, electrophoresed for 2 hr at 150 V, and visualized under UV illumination with intercalated ethidium bromide.

RESULTS

Cloning and nucleotide sequence of the human pancreas GGT cDNA

Using human hepatoma Hep G2 GGT cDNA as a probe, we screened a human pancreatic cDNA library. Under highly stringent conditions, we isolated two clones showing a strong hybridization signal. Of these two clones, only one was large enough to contain a full length fragment capable of encoding the human pancreatic GGT and has been further characterized. A 2.3-kb *Eco*RI fragment was obtained from this clone and was subcloned into the *Eco*RI site of pBS(M13+) vector. After confirming that this fragment hybridized to the Hep G2 cDNA, used as probe in a Southern blot, and that it contained restriction sites identical to those of Hep G2 cDNA, the nucleotide sequence was determined and analysed.

Analysis revealed a 2244 bp-long insert (Fig. 1) that includes a 5′-untranslated region of 358 bp, an open reading frame of 1710 bp and a 3′-untranslated region of 156 bp with a 20 bp-long poly(A) tail. The 5′-untranslated region (5′UTR) is 129 bp shorter than that of Hep G2 (Fig. 2a). Comparison of the nucleotide sequence in the common region showed an identity of 99.4% between the pancreas and the hepatoma sequence. Only two discrepancies were identified at positions 102 and 336 of our clone (Fig. 1), where a G and a C exist instead of an A and a T in the homologous Hep G2 position, respectively. Despite these point mutations which could result from interindividual genetic variability, splicing in this region of the pre-mRNA seems to be identical in hepatoma and pancreas, which could suggest that both are regulated through the same mechanisms. However, human pancreas cDNA differs from the Hep G2 sequence as it contains additional base pairs at the 3′UTR and a 20-bp poly(A) tail (Fig. 2B). The differences observed with the fetal liver and placenta GGT cDNAs consist of several deletions, suggesting other tissue-specific sequences located in the 3′UTR.

The open reading frame of the human pancreatic cDNA is entirely homologous to those in human placenta and Hep G2 cDNA [3, 7], all encoding a unique and identical type of GGT polypeptide.

Besides this type of mRNA, Pawlak *et al.* [19] reported the existence, in human kidney first, of a second type of GGT mRNA exhibiting a deletion of 30 nucleotides, corresponding to the positions 1568–1597 in our clone (Fig. 1). Type *I* corresponds to the pancreatic cDNA we cloned, but also to the cDNA of the placenta and Hep G2. We showed previously that it is translated into an active protein, by expressing the latter in *E. coli* [20] and in Chinese hamster lung fibroblasts V79 cells [21]. Thus, it can be reasonable to assume that all or part of the GGT polypeptide found in human organs or cells is encoded by this type of mRNA.

Distribution of type I *GGT mRNA in various human tissues*

In order to verify the presence of the type *I* mRNA in human tissues, we developed a strategy (illustrated in Fig. 3) to amplify selectively this type of mRNA.

Total RNA was prepared from different human tissues and cells and was used to synthesize cDNA using reverse transcriptase. Both types of GGT cDNA were probably synthesized. Therefore, for the first PCR step, we designed a primer complementary to the region where nucleotides are deleted in the type *II* cDNA. PCR with this primer would selectively amplify a fragment specific for the type *I* cDNA. For this PCR1, primers were complementary to regions 1210–1229 (# 1) and 1579–1595 (# 2) of pancreas GGT cDNA (Fig. 1). To ascertain this we amplified the correct fragment, designed two more primers, complementary to regions 1357–1378 (# 3) and 1421–1437 (# 4), which would amplify 81- and 103-bp internal sequences, depending on the possible presence of the 22-bp insertion, in a region which would be surrounded by primers # 3 and 4 [18].

cDNA fragments corresponding to type *I* GGT mRNA from several human tissues and cells were synthesized. Figure 4 shows the presence, in each human sample we tested, of the two products expected. The signal corresponding to the 81-bp fragment is far more intense than that corresponding to the 103-bp fragment, but quantification would be difficult from such an experiment. Nonetheless, our results demonstrate unambiguously the presence of the type *I* GGT mRNA in human tissues. These results provide information complementary to that obtained by Pawlak *et al.* [18], showing also that the method we used is sensitive enough to detect, in a shorter time and without radiolabelled compounds, the presence of a specific mRNA species, present even in very low concentrations in total RNA sample, as in human lymphocytes.

DISCUSSION

In man, a multigene family of at least four genes or pseudogenes related to GGT has been reported [5]. From the cDNA sequences known to date, encoding the human GGTs from the placenta [3], fetal liver [6] and hepatoma cell line Hep G2 [7], it is not clear how many of these genes are actually expressed. Pawlak *et al.* [19] demonstrated the existence of two types of mRNA, suggesting that at least two genes of the GGT multigene family would be transcribed. Type *I* mRNA corresponds to the sequences determined from the cDNAs encoding the human placenta, Hep G2 and pancreas GGTs. Type *II* mRNA, in contrast, differs from type *I* in containing a 30-nucleotide deletion in the light subunit-encoding sequence and several other point mutations [18]. It was first observed in human kidney, where it seems to be the major species. However, no evidence has been reported for the ability of this type *II* mRNA to be translated into an active form of GGT. Furthermore, in human liver, type *I* mRNA was shown to be differentially spliced, generating a species containing a 22-nucleotide insertion, putatively translated into a truncated polypeptide (M_r 39,000) presumably lacking GGT activity [18].

In this report, we describe the cloning and the nucleotide sequence of the cDNA encoding human pancreatic GGT. We observed that the open reading frame coding for the protein is identical to that reported for the human placenta and hepatoma Hep G2 GGTs [3, 7]. However, discrepancies identified in the 3'UTR may indicate that this region could contain specific signals for eventual post-transcriptional regulation processes. We do not believe that these discrepancies reflect a polymorphic difference dependent on the individual from which each cDNA library was prepared. As a matter of fact, polymorphisms are characterized by point mutations which are dispersed rather than being together in a short DNA segment, as in our case.

GGT activity often evolves in opposite ways in the liver and the pancreas. Hepatic levels decrease with growth [22] but are increased in hepatoma [23], whereas reverse fluctuations are observed in the pancreas [14, 24]. Thus, considering the presumed involvement of 5'UTR in the tissue specificity of gene expression, we were surprised to find no divergence in the organization of the 5'UTR between the pancreatic and the Hep G2 cDNAs, even though the former was shorter. Earlier data showed differences in the organization of the 5'UTR of various GGT cDNAs, resulting from a difference in splicing probably supporting tissue-specific GGT expression [18]. According to the proposed organization of human GGT mRNAs, the Hep G2 5'UTR is composed of seven exons, whereas fetal liver and placenta 5'UTRs consist of four and six exons, respectively. In the pancreas cDNA, 5'UTR results in only five exons, identical to the corresponding exons of Hep G2 and obviously sharing regulatory signals involved in the tissue-specific post-transcriptional regulation of the expression of the mRNA. However, a common regulation seems rather unlikely to control such different variabilities in GGT level as in the human pancreas and liver. Both organs derive from the same primitive tissue, namely the duodenal endoderm, which could partially explain the common use of a highly conserved coding mRNA further regulated by tissue-specific mechanisms occuring after cell differentiation. Whether pancreas-specific GGT expression is supported by a shorter 5'UTR and a distinct 3'UTR or whether the sequence we cloned is not the full length cDNA has to be determined and is under investigation. Nonetheless, these data suggest

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1                                                                CGGGGCAAGTGAGGTGCTGCCGTCATCCAGGCTGGACA

39   GTTCAGTGATTTGCCTGAGGCCCCACAGCAGAGTTCAACTGGAGACAGAGAAACCAGCTAGAGGCAGAGGGAGGTAACAC

119  GGAGTCCCCCAGAAAGGTCTGGGCTGCGCGTGCTTCAGGTAACCTCCCTTGACCTTCAGGAGAACGAGAAGGCTGCCTGA

199  TCAGAGAGTCCCTGAAGAAGATTCTGTGGCTACAGGCTTCAGCAGAGTGTGAGGGAGACCCCGGTTATTTCCTCAGCTAT

279  TTCCACCAAATCCTCCTGTCTTTCGTGGCCAACACCCCAGGCAAGGCTTGGGGCCCCCGTCTGCTGCTGGACGCAGAGCC

359  ATG AAG AAG AAG TTA GTG GTG CTG GGC CTG CTG GCC GTG GTC CTG GTG CTG GTC ATT GTC
       M   K   K   K   L   V   V   L   G   L   L   A   V   V   L   V   L   V   I   V
       1                                                                           20
419  GGC CTC TGT CTC TGG CTG CCC TCA GCC TCC AAG GAA CCT GAC AAC CAT GTG TAC ACC AGG
       G   L   C   L   W   L   P   S   A   S   K   E   P   D   N   H   V   Y   T   R
                                           30                                      40
479  GCT GCC GTG GCC GCG GAT GCC AAG CAG TGC TCG AAG ATT GGG AGG GAT GCA CTG CGG GAC
       A   A   V   A   A   D   A   K   Q   C   S   K   I   G   R   D   A   L   R   D
                                           50                                      60
539  GGT GGC TCT GCG GTG GAT GCA GCC ATT GCA GCC CTG TTG TGT GTG GGG CTC ATG AAT GCC
       G   G   S   A   V   D   A   A   I   A   A   L   L   C   V   G   L   M   N   A
                                           70                                      80
599  CAC AGC ATG GGC ATC GGG GGT GGC CTC TTC CTC ACC ATC TAC AAC AGC ACC ACA CGA AAA
       H   S   M   G   I   G   G   G   L   F   L   T   I   Y   N   S   T   T   R   K
                                           90                                     100
659  GCT GAG GTC ATC AAC GCC CGC GAG GTG GCC CCC AGG CTG GCC TTT GCC ACC ATG TTC AAC
       A   E   V   I   N   A   R   E   V   A   P   R   L   A   F   A   T   M   F   N
                                          110                                     120
719  AGC TCG GAG CAG TCC CAG AAG GGG GGG CTG TCG GTG GCG GTG CCT GGG GAG ATC CGA GGC
       S   S   E   Q   S   Q   K   G   G   L   S   V   A   V   P   G   E   I   R   G
                                          130                                     140
779  TAT GAG CTG GCA CAC CAG CGG CAT GGG CGG CTG CCC TGG GCT CGC CTC TTC CAG CCC AGC
       Y   E   L   A   H   Q   R   H   G   R   L   P   W   A   R   L   F   Q   P   S
                                          150                                     160
839  ATC CAG CTG GCC CGC CAG GGC TTC CCC GTG GGC AAG GGC TTG GCG GCA GCC CTG GAA AAC
       I   Q   L   A   R   Q   G   F   P   V   G   K   G   L   A   A   A   L   E   N
                                          170                                     180
899  AAG CGG ACC GTC ATC GAG CAG CAG CCT GTC TTG TGT GAG GTG TTC TGC CGG GAT AGA AAG
       K   R   T   V   I   E   Q   Q   P   V   L   C   E   V   F   C   R   D   R   K
                                          190                                     200
959  GTG CTT CGG GAG GGG GAG AGA CTG ACC CTG CCG CAG CTG GCT GAC ACC TAC GAG ACG CTG
       V   L   R   E   G   E   R   L   T   L   P   Q   L   A   D   T   Y   E   T   L
                                          210                                     220
1019 GCC ATC GAG GGT GCC CAG GCC TTC TAC AAC GGC AGC CTC ACG GCC CAG ATT GTG AAG GAC
       A   I   E   G   A   Q   A   F   Y   N   G   S   L   T   A   Q   I   V   K   D
                                          230                                     240
1079 ATC CAG GCG GCC GGG GGC ATT GTG ACA GCT GAG GAC CTG AAC AAC TAC CGT GCT GAG CTG
       I   Q   A   A   G   G   I   V   T   A   E   D   L   N   N   Y   R   A   E   L
                                          250                                     260
1139 ATC GAG CAC CCG CTG AAC ATC AGC CTG GGA GAC GCG GTG CTG TAC ATG CCC AGT GCG CCG
       I   E   H   P   L   N   I   S   L   G   D   A   V   L   Y   M   P   S   A   P
                               OLIGO # 1   270                                     280
1199 CTC AGC GGG CCC GTG CTG GCC CTC ATC CTC AAC ATC CTC AAA GGG TAC AAC TTC TCC CGG
       L   S   G   P   V   L   A   L   I   L   N   I   L   K   G   Y   N   F   S   R
                                          290                                     300
1259 GAG AGC GTG GAG AGC CCC GAG CAG AAG GGC CTG ACG TAC CAC CGC ATC GTA GAG GCT TTC
       E   S   V   E   S   P   E   Q   K   G   L   T   Y   H   R   I   V   E   A   F
                                          310                   OLIGO # 3         320
1319 CGG TTT GCC TAC GCC AAG AGG ACC CTG CTT GGG GAC CCC AAG TTT GTG GAT GTG ACT GAG
       R   F   A   Y   A   K   R   T   L   L   G   D   P   K   F   V   D   V   T   E
                                          330                       OLIGO # 4     340
1379 GTG GTC CGC AAC ATG ACC TCC GAG TTC TTC GCT GCC CAG CTC CGG GCC CAG ATC TCT GAC
       V   V   R   N   M   T   S   E   F   F   A   A   Q   L   R   A   Q   I   S   D
                                          350                                     360
1439 GAC ACC ACT CAC CCG ATC TCC TAC TAC AAG CCC GAG TTC TAC ACG CCG GAT GAC GGG GGC
       D   T   T   H   P   I   S   Y   Y   K   P   E   F   Y   T   P   D   D   G   G
                                          370                                     380
1499 ACT GCT CAC CTG TCT GTC GTC GCA GAG GAC GGC AGT GCT GTG TCC GCC ACC AGC ACC ATC
       T   A   H   L   S   V   V   A   E   D   G   S   A   V   S   A   T   S   T   I
                                   OLIGO # 2                                      400
1559 AAC CTC TAC TTT GGC TCC AAG GTC CGC TCC CCG GTC AGC GGG ATC CTG TTC AAT AAT GAA
       N   L   Y   F   G   S   K   V   R   S   P   V   S   G   I   L   F   N   N   E
                                          410                                     420
1619 ATG GAC GAC TTC AGC TCT CCC AGC ATC ACC AAC GAG TTT GGG GTA CCC CCC TCA CCT GCC
       M   D   D   F   S   S   P   S   I   T   N   E   F   G   V   P   P   S   P   A
                                          430                                     440
1679 AAT TTC ATC CAG CCA GGG AAG CAG CCG CTC TCG TCC ATG TGC CCG ACG ATC ATG GTG GGC
       N   F   I   Q   P   G   K   Q   P   L   S   S   M   C   P   T   I   M   V   G
                                          450                                     460
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1739  CAG GAC GGC CAG GTC CGG ATG GTG GTG GGA GCT GCT GGG GGC ACA CAG ATC ACC ACG GCC
       Q   D   G   Q   V   R   M   V   V   G   A   A   G   G   T   Q   I   T   T   A
                                           470                                     480
1799  ACT GCA CTG GCC ATC ATC TAC AAC CTC TGG TTC GGC TAT GAC GTG AAG CGG GCC GTG GAG
       T   A   L   A   I   I   Y   N   L   W   F   G   Y   D   V   K   R   A   V   E
                                           490                                     500
1859  GAG CCC CGG CTG CAC AAC CAG CTT CTG CCC AAC GTC ACG ACA GTG GAG AGA AAC ATT GAC
       E   P   R   L   H   N   Q   L   L   P   N   V   T   T   V   E   R   N   I   D
                                           510                                     520
1919  CAG GCA GTG ACT GCA GCC CTG GAG ACC CGG CAC CAT CAC ACC CAG ATC GCG TCC ACC TTC
       Q   A   V   T   A   A   L   E   T   R   H   H   H   T   Q   I   A   S   T   F
                                           530                                     540
1979  ATC GCT GTG GTG CAA GCC ATC GTC CGC ACG GCT GGT GGC TGG GCA GCT GCC TCG GAC TCC
       I   A   V   V   Q   A   I   V   R   T   A   G   G   W   A   A   A   S   D   S
                                           550                                     560
2039  AGG AAA GGC GGG GAG CCT GCC GGC TAC TGAGTGCTCCAGGAGGACAAGGCTGACAAGCAATCCAGGGACA
       R   K   G   G   E   P   A   G   Y   *
                                       569
2109  AGATACTCACCAGGACCAGGAAGGGGACTCTGGGGGACCGGCTTCCCCTGTGAGCAGCAGAGCAGCACAATAAATGAGGC

2189  CACTGTGCCAGGTGCCTCCCTGGCCTGTCTCCCCACAAAAAAAAAAAAAAAAAAAA                    2244
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Fig. 1. Nucleotide sequence and deduced primary structure of human pancreatic GGT. The translated sequence is given in the single-letter code and in italics. Translation regulatory signals and putative polyadenylation signal are displayed in bold. PCR primer annealing regions are underlined.

strongly that the human pancreatic GGT is encoded by the same gene as the human placenta and Hep G2 enzymes.

From these observations, we hypothesized that the type *I* mRNA, encoding human pancreatic GGT, may be a ubiquitous species. Thus, we studied the distribution of the corresponding cDNA in several human tissues. We showed that this cDNA species, corresponding to the type *I* mRNA, was present in all the tissues tested. This is in agreement with the results reported by Pawlak *et al.* [19], describing the simultaneous presence of type *I* and type *II* GGT mRNAs in fetal and adult kidney and the liver. Our data were obtained using a two consecutive PCRs strategy, which first selected type *I*-corresponding cDNA, and then further amplified a highly specific internal region to increase the sensitivity and the accuracy of the analysis of the results. This was chosen since it is not yet known whether the type *II* GGT mRNA contains the 22-nucleotide insertion contained by type *I* or whether type *II* exists in tissues other than the kidney or liver, thus preventing

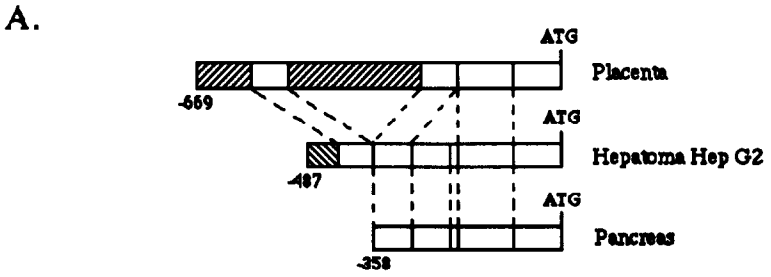

Fig. 2. Comparison of the untranslated regions of different human GGT cDNAs. Sequence of pancreatic cDNA was compared with those reported for cDNAs encoding GGT from human placenta [3], human hepatoma Hep G2 [7], human fetal liver [18]. (A) Analysis of the 5′-untranslated regions. Exons were located according to Pawlak *et al.* [18]. Regions conserved among all sequences are represented with white boxes. Hatched boxes indicate specific regions for each cDNA. (B) Analysis of the 3′-untranslated regions. Gaps (·) are inserted to maximize alignment of the sequences. Polyadenylation signals are underlined.

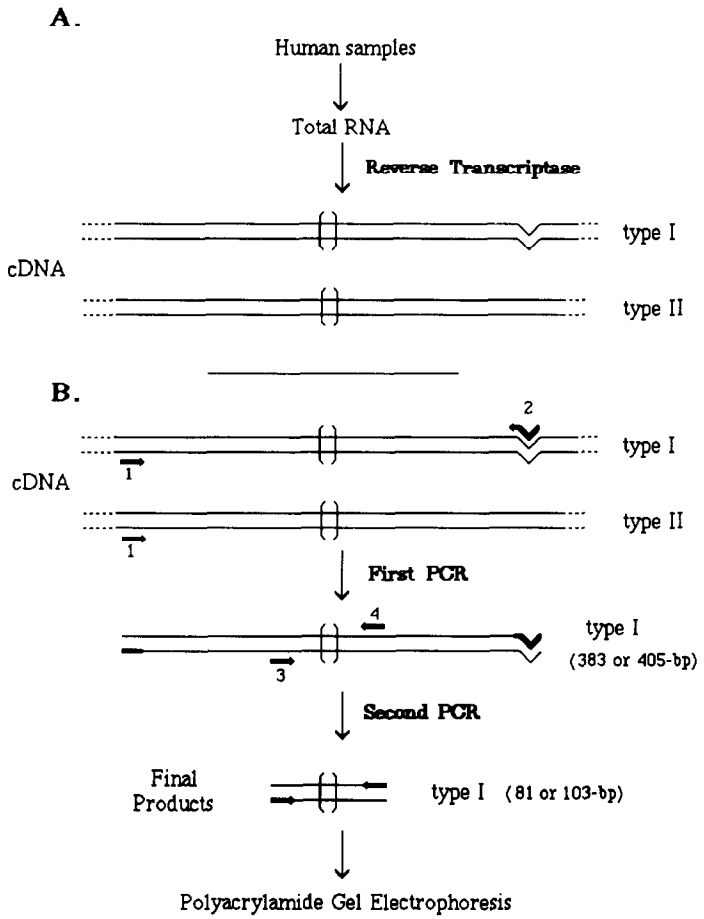

Fig. 3. Strategy of GGT cDNA species in various human samples. (A) cDNA synthesis. (B) Selective amplification of type *I*-specific cDNA fragments. PCR1 was performed using external primers able to select type *I* cDNA templates. Products were used as substrates for PCR2 which amplified internal regions. The 22-bp insertion is indicated in brackets as a possible event. The region corresponding to the 30 bp which can be deleted in type *II* cDNA is represented by a "V". Primers 1, 2, 3 and 4 are represented as bold arrows indicating the direction of primer extension, and their sequences are as indicated in Materials and Methods.

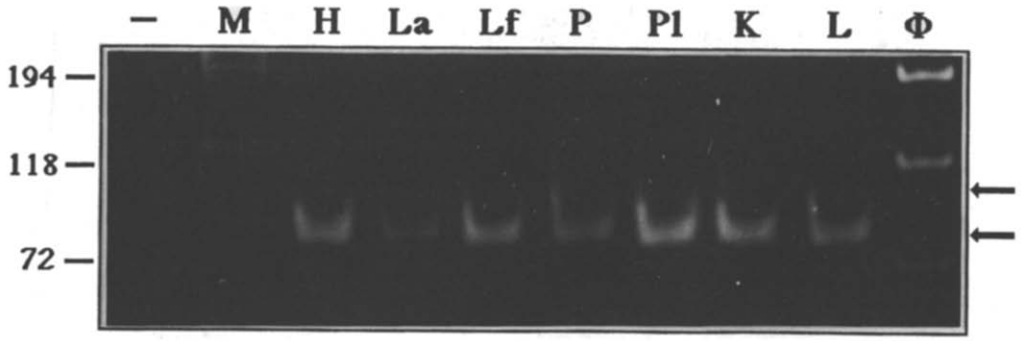

Fig. 4. Analysis of the cDNA species related to GGT in various human samples. PCR2 products, amplified between primers # 3 and 4, were loaded on an 8% (w/v) acrylamide gel. DNA markers were *Hae*III-fragments of the replicative form DNA of ΦX174 (Φ) and plasmid pBR322 (M). Human samples were: H, Hep G2 cells; K, kidney; L, lymphocytes; La, adult liver; Lf, fetal liver; P, fetal pancreas; Pl, placenta. Negative control (–) resulted from the complete three-step experiment performed under identical conditions, but without adding RNA to the initial mixture. cDNA pieces were visualized under UV illumination. Arrows indicate the expected 81- and 103-bp final products which correspond specifically to type *I* GGT mRNA.

a conclusion on the origin (type *I* or *II*) of the signals. In contrast with reported data, our results were obtained from type *I* mRNA specifically, clearing the uncertainty due to the possible presence of type *II* mRNA. They do not allow us to propose any conclusion regarding the expression in different tissues and the relative abundance of each mRNA species. Consequently, by showing the presence of the type *I* GGT mRNA in human tissues, we do not affirm that no other type of GGT mRNA is transcribed in these tissues, nor can we estimate in what ratio to another mRNA species it is transcribed.

However, we can conclude from these observations that type *I* mRNA may be ubiquitously transcribed in human tissues, and translated into an active GGT whose primary structure corresponds to that of the pancreatic enzyme and whose physico-chemical and catalytic properties are identical to those of all GGTs purified from human tissues [2, 16, 25].

Acknowledgements—The authors are thankful to Prof. J. Groffen (Los Angeles, California) for helpful advice and discussions, and to Prof. J. P. Boissel and Dr B. Leheup (Nancy, France) for providing them with human adult and fetal tissues.

CC, TO and MD are recipients of grants from the Ministère de la Recherche et de la Technologie (MRT), the Fondation pour la Recherche Médicale (FRM) and from the Government of Luxembourg (grant BFR 88/013), respectively. This work was partially supported by grants from INSERM (CRE 882015) and Association pour la Recherche contre le Cancer (ARC).

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