Proc. Natl. Acad. Sci. USA
Vol. 88, pp. 7294–7297, August 1991
Medical Sciences

Human liver glucokinase gene: Cloning and sequence determination of two alternatively spliced cDNAs

(human liver glucokinase cDNA/alternative splicing/cassette exon/enzyme isoforms)

YUKIO TANIZAWA, LASZLO I. KORANYI, CRIS M. WELLING, AND M. ALAN PERMUTT

Metabolism Division, Washington University School of Medicine, St. Louis, MO 63110

Communicated by William H. Daughaday, May 17, 1991 (received for review February 20, 1991)

ABSTRACT **A human liver glucokinase (ATP:D-hexose 6-phosphotransferase, EC 2.7.1.1) cDNA was isolated from a liver cDNA library. This cDNA (hLGLK1) appeared to be full length [2548 base pairs (bp) plus additional poly(A) residues], as its size was consistent with a single 2.8-kilobase (kb) glucokinase mRNA on Northern blot analysis of liver poly(A)$^+$ RNA. The cDNA contained an open reading frame of 1392 bp that predicted a protein of 464 amino acids and a molecular mass of 52 kDa; this protein has 97% identity to rat liver glucokinase. Fourteen residues on the amino terminus of the predicted human liver glucokinase, however, differed completely from those of the predicted rat liver enzyme and could be explained by alternative splicing of a 124-bp cassette exon in human cDNA. A second glucokinase cDNA (hLGLK2), missing the 124-bp cassette exon, was isolated by PCR amplification of human liver cDNA. The hLGLK2 cDNA contained an open reading frame of 1398 bp from an ATG codon at position 164, encoding a predicted protein of 466 residues, 98% identical to the rat enzyme, but different from the predicted protein of hLGLK1 cDNA by 16 amino-terminal residues. In contrast, hLGLK1 cDNA contains multiple initiator codons upstream of the predicted initiator codon at position 294 within the cassette exon. Translation of the two mRNAs *in vitro* by a reticulocyte lysate system resulted in proteins of the expected size (52 kDa) for both mRNAs; yet hLGLK2 mRNA was translated four to six times more efficiently. These results suggested that the alternative splicing of a cassette exon in hLGLK1 resulted in an mRNA with an upstream initiator codon and reduced function. The relative biological activity of the two isoforms of human glucokinase and their possible developmental and/or metabolic regulation remain to be determined.**

Glucokinase, found exclusively in liver and pancreatic islet beta cells, is one member of a family of hexokinases (ATP:D-hexose 6-phosphotransferase, EC 2.7.1.1) that appear to have a common evolutionary origin (1–4). Glucokinase, or hexokinase type IV, is distinguished from the other hexokinases by (*i*) its low affinity for glucose, with a K_m in the physiological range of plasma glucose concentration (5–15 mM), such that glucose phosphorylation maintains a gradient for glucose transport, (*ii*) by its lack of inhibition by glucose 6-phosphate, and (*iii*) by its molecular mass of 50 kDa vs. 100 kDa for the other hexokinases. Both tissues that express glucokinase also express a low-affinity, high K_m glucose transporter (Glut-2) (5–7), and thus these tissues play an important role in regulation of glucose metabolism. In the liver, the level of glucokinase activity is regulated by hormonal and nutritional factors (8–11).

Recent cloning and sequencing of a full-length rat liver glucokinase cDNA provided important direction for studying regulation of expression of this enzyme (12). The deduced structure predicted a protein of 465 amino acids and a molecular mass of 52 kDa; this sequence was 53% identical to rat brain hexokinase type I. The rat glucokinase gene was subsequently shown to be encoded by 10 exons in 15.5 kilobases (kb) of DNA (11). Cloning of an islet glucokinase cDNA from rat insulinoma revealed that the mRNA was at least 200 bp longer on the 5′ end and that exon 1 was different, resulting in an amino terminus differing by 15 amino acids between the two tissues (13, 14). Alternative splicing of glucokinase mRNAs has been observed in insulinoma (13), liver cells (15), and pituitary cells (16) from rodents.

Defects in glucokinase activity have long been suspected as contributors to the aberrant glucose metabolism of non-insulin-dependent diabetes mellitus in human (for review, see ref. 9). Isolation of the rat cDNA (12, 13) gave us the opportunity to screen a human liver cDNA library and isolate glucokinase cDNA clones. We now report the sequence of an apparent full-length clone* and compare its predicted amino acid sequence to that of rat glucokinases. In addition, another liver glucokinase mRNA was isolated with a 124-nucleotide deletion, which predicts a protein differing by 16 amino-terminal residues.

METHODS

cDNA Library Screening, DNA Sequencing, and Data Analysis. A human islet cDNA library (7) was initially screened with a rat-islet glucokinase cDNA (13), and a 2-kb clone (phIGLK) was isolated. This clone was used to screen a human liver oligo(dT)-primed λ ZAP (Stratagene) cDNA library. Inserts were subcloned into Bluescript SK+ (Stratagene) and M13mp18 and M13mp19 RF DNA (BRL), and then sequenced in both strands. Questions of compressions were resolved by sequencing with dITP and terminal deoxynucleotidyltransferase, as described (17, 18). Analysis of DNA and amino acid sequences was performed by programs from DNAStar, Madison, WI.

cDNA Synthesis and PCR Amplification of Human Liver Glucokinase. All human tissues were obtained with institutional approval and informed consent. RNA was extracted (19) from human liver (D. Perlmutter, St. Louis Children's Hospital, S. Giddings, Veterans Administration Medical Center, St. Louis, and National Disease Research Interchange, Philadelphia) poly(A)$^+$ RNA was isolated, and Northern (RNA) blot analysis was done, as described (7, 17). First-strand cDNA was synthesized from 5 μg of human liver total RNA primed by $(dT)_{12-19}$ in a 40-μl reaction mixture (17), and RNA was removed with RNase H (BRL). The PCR was done on cDNA (5 μl) with AmpliTaq DNA polymerase (Perkin–Elmer/Cetus) in 50 μl, as described by the manufacturer, with 2 mM $MgCl_2$ and 50 pmol each of oligonucleotide primers 10635, corresponding to nucleotides 34–59 of hLGLK1 and primer 11133 complimentary to nucleotides

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

*The sequence reported in this paper has been deposited in the GenBank data base (accession no. M69051).

432–456. Cycle conditions were, after 3-min initial denaturation at 94°C, 45 cycles of 94°C for 30 sec, and 71°C for 1 min, with a final extension of 72°C for 9 min.

Construction of phLGLK2 and *in Vitro* Translation. The smaller PCR-amplified glucokinase cDNA fragment (see Fig. 2) was ligated to the *Hin*cII site of M13mp18 and confirmed by sequencing. To construct phLGLK2, the *Xba* I–*Pst* I fragment of the phLGLK1, which contained nucleotides 1–367 of hLGLK1, including the 124-bp insertion (see *Results*), was replaced by the *Xba* I–*Pst* I fragment from the M13 clone, missing the insertion. RNA was synthesized by using T3 RNA polymerase (United States Biochemical) and linearized phLGLK1 or phLGLK2 as templates, as described (17). The RNA product gave a single band of the expected size on 1.2% formaldehyde/agarose gel electrophoresis. *In vitro* translation used 2 μg of synthetic RNA, 20 μM amino acids (except methionine), 40 μCi of [^{35}S]methionine (>1000 Ci/mmol; Amersham; 1 Ci = 37 GBq), 40 units of RNasin, and 35 μl of nuclease-treated rabbit reticulocyte lysate (Promega) in 50-μl volume.

RESULTS

Cloning of Human Liver Glucokinase cDNA. A clone (phLGLK1) was isolated that contained 2548 bp plus additional 3′-poly(A) residues (Fig. 1). A presumptive polyadenylylation signal was seen in the sequence ATTAAA (20), 17 bases upstream of the poly(A) region. An open reading frame from ATG at position 294 to a stop codon at position 1687 encoded a predicted protein of 464 amino acids with an estimated molecular mass of 52 kDa and a pI of 5.07. Northern blot analysis of liver poly(A)$^+$ RNA revealed a single 2.8-kb RNA when hybridized to ^{32}P-labeled glucokinase cDNA (data not shown).

Identification of a Cassette Exon in Genomic DNA. An overall identity of 89% was observed between human and rat liver glucokinase, except for a region of 124 bp not present in the rat cDNA (see *Discussion*). This 124-bp region in the human cDNA occurred at the junction of exons 1 and 2 in the rat cDNA with 90% identity 3′ to exon 2. The predicted protein has no identity to the rat liver protein for the first 14 amino acids, followed by a large region of high identity. To pursue this 5′ difference, human glucokinase genomic clones were isolated and sequenced with exon-specific oligonucleotide primers. Based on the structure of the rat genomic glucokinase gene, the 124-bp region corresponded to a cassette exon between exons 1 and 2 (data not shown).

Isolation and Sequencing of a Second Human Glucokinase cDNA. In search for alternative forms of human liver glucokinase, liver RNA was reverse transcribed to cDNA, then analyzed by PCR amplification with glucokinase-specific oligonucleotide primers chosen for exons 1 and 2 of the human glucokinase, based on the genomic structure of the rat glucokinase (see Fig. 1, 5′ → 3′, 34–59; 3′ → 5′, 456–432). For cDNA containing the cassette exon a PCR product of 423 bp was predicted, whereas without the cassette exon, a 299-bp product was predicted. Both products were seen (Fig. 2). Southern blot analysis and hybridization with ^{32}P-labeled phLGLK1 confirmed that both PCR products were representative of glucokinase mRNA. Hybridization of the blot with an end-labeled oligonucleotide specific for the 124-bp region revealed only the larger 423-bp product (data not shown).

The smaller PCR-amplified glucokinase cDNA fragment was purified, subcloned into M13mp18, and sequenced. The sequence included the region of flanking primers at nucleotides 34–59 to nucleotides 432–456, but with 124 bp missing relative to the sequence of phLGLK1, as indicated in Fig. 1. Thus hLGLK2 cDNA contained a predicted open reading frame of 1398 bp from an ATG codon at position 164,

```
gcggccgcaa gccctgggct gccagcctca ggcagctctc catccaagca gccgttgctg ccacaggcgg      70
gccttacgct ccaaggctac agcatgtgct aggcctcagc aggcaggagc atctctgcct cccaaagcat     140
ctacctctta gcccctcggg gagatggcga tggatgtcac aaggaggcag gcccagacag ccttgactct     210
gccaaactct cctctaaact caaacctcac atgaccaact actacttgaa acaaatcacc ccttgactag     280

                  Met Pro Arg Pro Arg Ser Gln Leu Pro Gln Pro Asn Ser Gln Val Glu
cagatatatt aac atg ccc aga cca aga tcc cag ctc cca cag ccc aac tcc cag gta gag     341

Gln Ile Leu Ala Glu Phe Gln Leu Gln Glu Glu Asp Leu Lys Lys Val Met Arg Arg
cag atc ctg gca gag ttc cag ctg cag gag gag gac ctg aag aag gtg atg aga cgg       398

Met Gln Lys Glu Met Asp Arg Gly Leu Arg Leu Glu Thr His Glu Glu Ala Ser Val
atg cag aag gag atg gac cgc ggc ctg agg ctg aaa acc cat aaa aag acc aat gtg       455

Lys Met Leu Pro Thr Tyr Val Arg Ser Thr Pro Glu Gly Ser Glu Val Gly Asp Phe
gag atg ctg ccc acc tac gtg cgc tcc acc cca gaa ggc tca gaa gtc ggg gac ttc       512

Leu Ser Leu Asp Leu Gly Gly Thr Asn Phe Arg Val Met Leu Val Lys Val Gly Glu
ctc tcc ctg gac ctg ggt ggc act aac ttc agg gtg atg ctg gtg aag gtg gga gaa       569

Gly Glu Glu Gly Gln Trp Ser Val Lys Thr Lys His Gln Thr Tyr Ser Ile Pro Glu
ggt gag gag ggg cag tgg agc gtg aag acc aaa cac cag acg tac tcc atc ccc gag       626

Asp Ala Met Thr Gly Thr Ala Glu Met Leu Phe Asp Tyr Ile Ser Glu Cys Ile Ser
gac gcc atg acc ggc act gct gag atg ctc ttc gac tac atc tct gag tgc atc tcc       683

Asp Phe Leu Asp Lys His Gln Met Lys His Lys Lys Leu Pro Leu Gly Phe Thr Phe
gac ttc ctg gac aag cat cag atg aaa cac aag aag ctg ccc ctg ggc ttc acc ttc       740

Ser Phe Pro Val Arg His Glu Asp Ile Asp Lys Gly Ile Leu Leu Asn Trp Thr Lys
tcc ttt cct gtg agg cac gaa gac atc gat aag ggc atc ctt ctc aac tgg acc aag       797

Gly Phe Lys Ala Ser Gly Ala Glu Gly Asn Asn Val Val Gly Leu Leu Arg Asp Ala
ggc ttc aag gcc tca gga gca gaa ggg aac aat gtc gtg ggg ctt ctg cga gac gct       854

Ile Lys Arg Arg Gly Asp Phe Glu Met Asp Val Val Ala Met Val Asn Asp Thr Val
atc aaa cgg aga ggg gac ttt gaa atg gat gtg gtg gca atg gtg aat gac acg gtg       911

Ala Thr Met Ile Ser Cys Tyr Tyr Glu Asp His Gln Cys Glu Val Gly Met Ile Val
gcc acg atg atc tcc tgc tac tac gaa gac cat cag tgc gag gtc ggc atg atc gtg       968

Gly Thr Gly Cys Asn Ala Cys Tyr Met Glu Glu Met Gln Asn Val Glu Leu Val Glu
ggc acg ggc tgc aat gcc tgc tac atg gag gag atg cag aat gtg gag ctg gtg gag      1025

Gly Asp Glu Gly Arg Met Cys Val Asn Thr Glu Trp Gly Ala Phe Gly Asp Ser Gly
ggg gac gag ggc cgc atg tgc gtc aat acc gag tgg ggc gcc ttc ggg gac tcc ggc      1082

Glu Leu Asp Glu Phe Leu Leu Glu Tyr Asp Arg Leu Val Asp Glu Ser Ser Ala Asn
gag ctg gac gag ttc ctg ctg gag tat gac cgc ctg gtg gac gag agc tct gca aac      1139

Pro Gly Gln Gln Leu Tyr Glu Lys Leu Ile Gly Gly Lys Tyr Met Gly Glu Leu Val
ccc ggt cag cag ctg tat gag aag ctc ata ggt ggc aag tac atg ggc gag ctg gtg      1196

Arg Leu Val Leu Leu Arg Leu Val Asp Glu Asn Leu Leu Phe His Gly Glu Ala Ser
cgg ctt gtg ctg ctc agg ctc gtg gac gaa aac ctg ctc ttc cac ggg gag gcc tcc      1253

Glu Gln Leu Arg Thr Arg Gly Ala Phe Glu Thr Arg Phe Val Ser Gln Val Glu Ser
gag cag ctg cgc aca cgc gga gcc ttc gag acg cgc ttc gtg tcg cag gtg gag agc      1310

Asp Thr Gly Asp Arg Lys Gln Ile Tyr Asn Ile Leu Ser Thr Leu Gly Leu Arg Pro
gac acg ggc gac cgc aag cag atc tac aac atc ctg agc acg ctg ggg ctg cga ccc      1367

Ser Thr Thr Asp Cys Asp Ile Val Arg Arg Ala Cys Glu Ser Val Ser Thr Arg Ala
tcg acc acc gac tgc gac atc gtg cgc cgc gcc tgc gag agc gtg tct acg cgc gct      1424

Ala His Met Cys Ser Ala Gly Leu Ala Gly Val Ile Asn Arg Met Arg Glu Ser Arg
gcg cac atg tgc tcg gcg ggg ctg gcg ggc gtc atc aac cgc atg cgc gag agc cgc      1481

Ser Glu Asp Val Met Arg Ile Thr Val Gly Val Asp Gly Ser Val Tyr Lys Leu His
agc gag gac gta atg cgc atc act gtg ggc gtg gat ggc tcc gtg tac aag ctg cac      1538

Pro Ser Phe Lys Glu Arg Phe His Ala Ser Val Arg Arg Leu Thr Pro Ser Cys Glu
ccc agc ttc aag gag cgg ttc cat gcc agc gtg cgc agg ctg acg ccc agc tgc gag      1595

Ile Thr Phe Ile Glu Ser Glu Glu Gly Ser Gly Arg Gly Ala Ala Leu Val Ser Ala
atc acc ttc atc gag tcg gag gag ggc agt ggc cgg ggc gcg gcc ctg gtc tcg gcg      1652

Val Ala Cys Lys Lys Ala Cys Met Leu Gly Gln
gtg gcc tgt aag aag gcc tgt atg ctg ggc cag tgagg gcagtggccg caagcgcagg          1710

gaggatgcca cagccccaca gcacccaggc tccatgggga agtgctcccc acacgtgctc gcagcctggc    1780
ggggcaggag gcctggcctt gtcaggaccc aggccgcctg ccataccgct ggggaacaga gcgggcctct    1850
tccctcagtt tttcggtggg acagcccccag ggccctaacg ggggtgcggc aggagcagga acagagactc   1920
tggaagcccc ccacctttct cgctggaatc aattttcccag aaggggagttg ctcactcagg actttgatgc  1990
atttccacac tgtcagagct gttggcctcg cctgggccca ggctctggga aggggtgccc tctggatcct    2060
gctgtggcct cacttccctg ggaactcatc ctgtgtgggg aggcagctcc aacagcttga ccagacctag    2130
acctgggcca aaagggcagg ccaggggctg ctcatcaccc agtcctggcc attttcttgc ctgaggctca    2200
agaggcccag ggagcaatgg gaggggggctc catggaggag gtgtcccaag ctttgaatac cccccagaga   2270
ccttttctct cccataccat cactgagtgg cttgtgattc tgggatggac cctcgcagca ggtgcaagag    2330
acagagcccc caagcctctg ccccaagggg cccacaaagg ggagaagggc cagcccttaca tcttcagctc   2410
ccatagcgct ggctcaggaa gaaaccccaa gcagcattca gcacacccca agggacaacc ccatcatatg    2480
acatgccacc ctctccatgc ccaacctaag attgtgtggg ttttttaatt aaaaatgtta aaagttttaa    2550
aaaaaaaa                                                                       2558
```

FIG. 1. Nucleotide and predicted amino acid sequence of human liver glucokinase 1 cDNA (hLGLK1) and enzyme. The 124 bases deleted in liver glucokinase 2 cDNA (hLGLK2) are underlined. Forward and reverse oligonucleotide primers used to amplify liver cDNA by PCR as illustrated in Fig. 2 are indicated.

encoding a protein of 466 residues, 98% identical to the rat enzyme, but different from the predicted protein of hLGLK1 cDNA by 16 amino-terminal residues.

***In Vitro* Translation of the Two Glucokinase mRNAs.** Each cDNA was subcloned into RNA transcription vectors, and synthetic mRNAs translated in a reticulocyte lysate cell-free system resulted in proteins the predicted size for glucokinase (52 kDa), as well as 48-kDa proteins, and less abundant

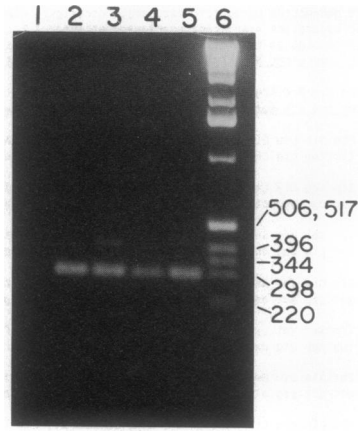

FIG. 2. Analysis of human liver mRNA by PCR amplification with glucokinase-specific oligonucleotide primers (indicated in Fig. 1). Total liver RNA was reverse transcribed, and equal aliquots of cDNA were amplified. Products were separated on a 2.0% agarose gel and stained with ethidium bromide. Lanes: 1, control without RNA; 2–5, human liver cDNA from four different individuals; 6, DNA size markers, 1-kb ladder (BRL; 1 μg).

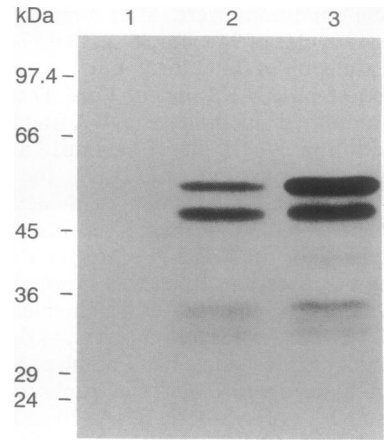

FIG. 3. *In vitro* translation of synthetic hLGLK1 and -2 mRNAs by rabbit reticulocyte lysate, as described. Aliquots (2.5 μl) of the reaction mixture were subjected to SDS/10% PAGE (20). Fluorography was for 1 hr at −80°C with Entensify (NEN). Lanes: 1, no RNA; 2, RNA transcribed from phLGLK1; 3, RNA transcribed from phLGLK2. Positions of molecular mass markers (Sigma) are indicated.

smaller proteins (Fig. 3). Densitometric analysis of the translation product revealed that hLGLK2 mRNA was translated four to six times more efficiently than hLGLK1 mRNA for the predicted 52-kDa glucokinase protein.

DISCUSSION

We isolated a human liver glucokinase cDNA (hLGLK1) of 2548 bases exclusive of poly(A) residues, which appeared to be full length as this size was consistent with the single 2.8-kb band seen on Northern blot analysis. If the initiator codon at 294 is the preferred translation start site (see below), this mRNA would encode a protein of 464 amino acids and an estimated molecular mass of 52 kDa. Of note is the fact that a fusion protein of hLGLK1 with glutathione *S*-transferase, expressed in bacteria, was a functional glucokinase enzyme (Y.T., unpublished work). A second form of glucokinase mRNA with a 124-nucleotide deletion was observed by PCR amplification of human liver cDNA. The hLGLK2 cDNA contained a predicted open reading frame of 1398 bp from an ATG codon at position 164, encoding a predicted protein of 466 residues, 98% identical to the rat enzyme, but different from the predicted protein of hLGLK1 cDNA by 16 amino-terminal residues. This mRNA is thought to occur by alternative splicing of a cassette exon between exons 1 and 2 in genomic DNA.

The rat liver glucokinase cDNA has been shown to be encoded by 10 exons within 15.5 kb of genomic DNA (11). Comparisons of the nucleotide sequences of the 5′ ends of the human liver and rat glucokinase cDNAs revealed a region from 30–211 bp of hLGLK1 that shared 66% sequence identity with a comparable region (1–172 bp) of the rat cDNA (Fig. 4). The next 124 bp in the human hLGLK1 cDNA have no homology with the rat cDNA. For human cDNAs the region comparable to that encoded by rat exons 2–10 has 88% sequence identity, and the 3′-untranslated region has 68% identity.

An isoform of glucokinase cDNA was also described in rat liver with 151 bp of DNA between exons 1 and 2 shown to be due to alternative splicing of an additional cassette exon (15). Although insertion of the 124-bp cassette exon in the human glucokinase (hLGLK1) cDNA occurred at the same site as insertion in the alternatively spliced rat liver enzyme, there was no sequence identity seen between the two insertions. Although hLGLK1 was the only clone we isolated from the cDNA library of 5×10^5 plaques, hLGLK2 seemed to be more abundant according to the result of the PCR amplification (Fig. 2). In addition, the relative amount of the two forms seemed to vary among the individuals. Nevertheless, because of the difficulty in the quantification by PCR amplification, we cannot be certain about the relative abundance of these mRNAs. Whether nutritional and/or hormonal factors alter levels of the two forms of human glucokinase mRNA is also unknown. This question could be important for future studies, considering the potential physiological and pathological consequences, were the translated forms of glucokinase different in catalytic properties or stability.

Rat glucokinase has a single initiator codon followed by a long open reading frame (21). Upstream of the ATG at 294 for hLGLK1 are six other ATG codons (Fig. 1, positions 94, 164, 170, 174, 241, and 284). Initiator ATG codons at positions 94,

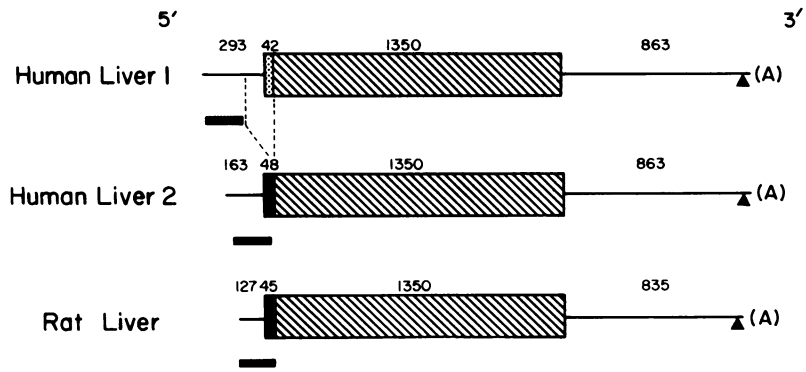

FIG. 4. Comparison of human hLGLK1 and -2 and rat liver (12) glucokinase cDNAs. Heavy lines under the 5′ regions refer to conserved regions, and the 124 deletion in hLGLK2 relative to hLGLK1 is indicated by dotted lines. Boxes refer to predicted coding regions, and lines represent 5′- and 3′-untranslated regions, respectively; hatched areas represent the highly conserved coding regions, and the other areas refer to differences in amino-terminal coding regions, as described. Termination signals ATTAAA are indicated by closed triangles.

```
                    10v       20v
HLGLK1      MPRPRSQLPQPNSQVEQILAEFQLQEED
                              ||||||||||||||
-RATGLK MAMDTTRCGAQL-LTLVEQILAEFQLQEED
          ||||  ||   ||  ||||||||||||||||
HLGLK2   MAMDVTRSQAQTALTLVEQILAEFQLQEED
```

FIG. 5. Comparison of the NH_2 termini of human and rat liver glucokinases.

164, 170, 174, and 284 predict small proteins that terminate before the initiation ATG at 294. Furthermore, the ATG codons at 94, 164, and 170 are potential "strong" initiator codons, according to the rules of Kozak (20). Thus, hLGLK1 might be a transcriptional unit encoding small upstream peptides, and these upstream ATGs might markedly affect the translation rate of hLGLK1 at ATG 294, which encodes the predicted protein of 464 residues. The results of *in vitro* translation of synthetic mRNAs for hLGLK1 and -2 indicated that both are functional mRNAs encoding full-length glucokinase-predicted protein of 52 kDa. The smaller (48-kDa) protein observed could represent either partially degraded mRNA or protein, partially translated protein, or the products of initiation at downstream ATG codons 390 and 411, which would encode predicted proteins of 48.3 and 47.3 kDa, respectively. The translation of 52-kDa protein with hLGLK2 mRNA was four to six times greater (by densitometric analysis) relative to that of hLGLK1 (Fig. 3). Because these results were obtained in an *in vitro* system, the physiological relevance of this finding cannot be determined. Further, a full-length hLGLK2 cDNA clone was not isolated. Thus the determination of the relative level of expression of the two glucokinase enzymes *in vivo* awaits the development of specific antibodies and immunoblot analysis of liver protein.

The amino acid identities between rat and the human liver glucokinases were compared (Fig. 5). For hLGLK1 there is almost no identity in the first 14 amino acids with the rat, whereas for hLGLK2 11/16 (69%) of the amino-terminal residues are identical; this is followed by an area with 97% amino acid identity in a region of 450-amino acid overlap. This comparison contrasts to 53% amino acid identity between rat glucokinase and the carboxyl-terminal region of rat brain hexokinase. The putative glucose- and ATP-binding domains were also highly conserved (12).

As a consequence of isolating two human liver glucokinase cDNAs, the contribution of this gene to genetic susceptibility to non-insulin-dependent diabetes mellitus can now be assessed. The potential use of alternative promoters, as well as alternative splicing of glucokinase mRNAs, makes the search for defects in the glucokinase genes of diabetic subjects an interesting one.

We thank Drs. Rick Wetsel and Harvey Colten, St. Louis Children's Hospital, for providing the human liver cDNA library, Drs. Stuart Adler and Mike Mueckler for helpful discussions and review of the manuscript, and Jeannie Wokurka for help in preparation of the manuscript. Dr. Mark Magnuson provided the rat glucokinase cDNA, a preprint of unpublished data, and helpful advice. This work was supported, in part, by Grant DK16746 (M.A.P.) from the National Institutes of Health. L.I.K. was a recipient of a Juvenile Diabetes Foundation Fellowship Award. Y.T. was the recipient of a Mentor Based Fellowship Award of the American Diabetes Association.

1. Colowick, S. P. (1973) in *The Enzymes*, ed. Boyer, P. D. (Academic, New York), Vol. 9, pp. 1–48.
2. Schwab, D. A. & Wilson, J. E. (1989) *Proc. Natl. Acad. Sci. USA* **86**, 2563–2567.
3. Middleton, R. J. (1990) *Biochem. Soc. Transact.* **18**, 180–183.
4. Nishi, S., Susumu, S. & Bell, G. I. (1988) *Biochem. Biophys. Res. Commun.* **157**, 937–943.
5. Fukumoto, H., Seino, S., Imura, H., Seino, Y., Eddy, R. L., Fukushima, Y., Byers, M. G., Shows, T. B. & Bell, G. I. (1988) *Proc. Natl. Acad. Sci. USA* **85**, 5434–5438.
6. Thorens, B., Sarkar, H. K., Kaback, H. R. & Lodish, H. F. (1988) *Cell* **55**, 281–290.
7. Permutt, M. A., Koranyi, L., Keller, K., Lacy, P. E., Scharp, D. W. & Mueckler, M. (1989) *Proc. Natl. Acad. Sci. USA* **86**, 8688–8692.
8. Minderop, R. H., Hoeppner, W. & Seitz, H. J. (1987) *Eur. J. Biochem.* **164**, 181–187.
9. Matschinsky, F. M. (1990) *Diabetes* **39**, 647–652.
10. Iynedjian, P. B., Pilot, P.-R., Nouspikel, T., Milburn, J. L., Quaade, C., Hughes, S., Ucla, C. & Newgard, C. B. (1989) *Proc. Natl. Acad. Sci. USA* **86**, 7838–7842.
11. Magnuson, M. A., Andreone, T. L., Printz, R. L., Koch, S. & Granner, D. K. (1989) *Proc. Natl. Acad. Sci. USA* **86**, 4838–4842.
12. Andreone, T. L., Printz, R. L., Pilkis, S. J., Magnuson, M. A. & Granner, D. K. (1989) *J. Biol. Chem.* **264**, 363–369.
13. Magnuson, M. A. & Shelton, K. D. (1989) *J. Biol. Chem.* **264**, 15936–15942.
14. Magnuson, M. A. (1990) *Diabetes* **39**, 523–527.
15. Hayzer, D. J. & Iynedjian, P. G. (1990) *Biochem. J.* **270**, 261–263.
16. Hughes, S. D., Quaade, C., Milburn, J. L., Cassidy, L. & Newgard, C. B. (1991) *J. Biol. Chem.* **266**, 4521–4530.
17. Maniatis, T., Fritsch, E. F. & Sambrook, J. (1989) in *Molecular Cloning: A Laboratory Manual* (Cold Spring Harbor Lab., Cold Spring Harbor, NY), 2nd Ed.
18. Fawcett, T. W. & Bartlett, S. G. (1990) *Biotechniques* **9**, 46–48.
19. Chomczynski, P. & Sacchi, N. (1987) *Anal. Biochem.* **162**, 156–159.
20. Kozak, M. (1984) *Nucleic Acids Res.* **12**, 857–872.
21. Liang, Y., Jetton, T. L., Zimmerman, E. C., Najafi, H., Matschinsky, F. M. & Magnuson, M. A. (1991) *J. Biol. Chem.* **266**, 6999–7007.