An Alternate Promoter in the Glucokinase Gene Is Active in the Pancreatic β Cell*

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An alternate promoter in the glucokinase gene is active in the β cell and produces a glucokinase mRNA which is longer and that has a different leader sequence and translation start site than the hepatic glucokinase mRNA. The glucokinase β cell promoter is located at least 12 kilobases upstream from the glucokinase hepatic promoter. Transcription from the glucokinase β cell promoter initiates over a region of 62 bases. The absence of a TATA box homology in the proximal promoter region may account for the diffuse transcriptional initiation. Translation of the β cell glucokinase mRNA predicts a glucokinase isozyme that is different from the hepatic isozyme by 15 amino acids at the N terminus. The use of alternative promoters apparently enables the glucokinase gene to be regulated by insulin in the liver and by glucose in the β cell, thus possibly constituting an important feedback control loop for maintaining glucose homeostasis.

Alternate RNA splicing of the β cell glucokinase mRNA predicts at least two β cell glucokinase isoforms. An alternate splice acceptor site in the 4th exon of the glucokinase gene was identified in two glucokinase cDNAs from rat insulinoma tissue. Use of the alternate splice acceptor site results in a 51-nucleotide in frame deletion in the β cell glucokinase mRNA and removal of 17 amino acids from a region of the protein situated between the putative glucose and ATP binding domains. Analysis of the pattern of RNA splicing in tissues containing β cells indicates that the splice acceptor site utilized in producing hepatic glucokinase mRNA is also utilized in the β cell.

Glucose is the major physiological stimulus for the secretion of insulin by pancreatic β cells. The electrical, ionic, and secretory responses of β cells to glucose are mediated by the metabolism of this sugar (1–5). Glucokinase (ATP: α-D-glucose 6-phosphotransferase, EC 2.7.1.1) catalyzes the initial step in the metabolism of this sugar (1–3). Glucokinase (ATP:D-hexose 6-phosphotransferase, EC 2.7.1.1) catalyzes the initial step in metabolism of this sugar (1–3). Glucokinase (ATP:D-hexose 6-phosphotransferase, EC 2.7.1.1) catalyzes the initial step in metabolism of this sugar (1–3).

Alternate RNA splicing of the glucokinase gene results in a partial restoration of glucokinase activity in the β cell. Furthermore, utilization of an alternate splice acceptor site in the 4th exon of the gene predicts additional amino acids at the N terminus of glucokinase in the liver and the β cell. The different functions of glucokinase in β cells and the liver impose different requirements for the regulation of the enzyme in these two tissues. Indeed, while insulin is the primary stimulant of hepatic glucokinase gene expression, islet glucokinase activity appears dependent upon glucose (6). Bedoya et al. (6) reported glucokinase activity decreased 70% in insulinoma-bearing, hypoglycemic animals. Glucokinase activity recovered to control levels within 24 h of removing the insulinoma from these animals, and a glucose infusion also resulted in a partial restoration of glucokinase activity in animals with an insulinoma. An understanding of how the glucokinase gene is expressed and regulated in the pancreatic β cell is pertinent to both the physiology of glucose homeostasis and β cell-specific gene expression.

The availability of cDNAs encoding hepatic glucokinase (7) has enabled studies on the expression and regulation of the glucokinase gene in the pancreatic β cell. We have characterized the transcription unit of insulinoma glucokinase mRNA and have compared it to the hepatic glucokinase mRNA transcription unit. Interestingly, two different promoters are contained in the glucokinase gene, one being active in β cells and the other in the liver. This finding offers an explanation for the differential regulation of glucokinase in liver and β cells. The promoter/regulatory sequences utilized in the liver apparently enable regulation by insulin while the promoter/regulatory sequences utilized in the β cell may enable regulation by glucose. Translation of glucokinase is initiated within tissue-specific first exon sequences thus leading to 15 different amino acids at the N terminus of glucokinase in the liver and the β cell. Furthermore, utilization of an alternate splice acceptor site in the 4th exon of the gene predicts additional heterogeneity in the glucokinase isozymes present in the β cell.

EXPERIMENTAL PROCEDURES

General Techniques—Standard procedures were used for screening phage libraries, DNA labeling, restriction enzyme mapping, subclone-

\* This work was supported by grants from the American Diabetes Association and the Juvenile Diabetes Foundation and by Vanderbilt Diabetes Research and Training Center Grant GK 07061. The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

The nucleotide sequence(s) reported in this paper has been submitted to the GenBank®/EMBL Data Bank with accession number(s) M25806 and M25807.

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reviews see Refs. 4 and 5). The high $K_m$ of glucokinase for glucose, coupled with the lack of end product inhibition compared to other hexokinases, enables glucokinase in the β cell to act as a pacemaker of glycolysis and, hence, of insulin secretion. Inhibition of glucokinase activity by mannose, alloxan, or other inhibitors prevents glucose-stimulated insulin release by β cells (5), observations which are consistent with the view that the enzyme plays an important role in modulating insulin secretion.

The function of glucokinase in the liver is different from that in the β cell. Hepatic glucokinase helps to facilitate the uptake and conversion of glucose by acting as an insulin-sensitive determinant of hepatic glucose usage. An increase in glucose phosphorylation, mediated by glucokinase, helps to maintain a gradient for glucose transport into the liver. The different functions of glucokinase in β cells and the liver impose different requirements for the regulation of the enzyme in these two tissues. Indeed, while insulin is the primary stimulant of hepatic glucokinase gene expression, islet glucokinase activity appears dependent upon glucose (6). Bedoya et al. (6) reported glucokinase activity decreased 70% in insulinoma-bearing, hypoglycemic animals. Glucokinase activity recovered to control levels within 24 h of removing the insulinoma from these animals, and a glucose infusion also resulted in a partial restoration of glucokinase activity in animals with an insulinoma. An understanding of how the glucokinase gene is expressed and regulated in the pancreatic β cell is pertinent to both the physiology of glucose homeostasis and β cell-specific gene expression.

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DNA probes used for library screening and blot transfer hybridization experiments were labeled by random oligonucleotide priming (9).

Isolation of Poly(A)^+ RNAs—Total cellular RNA was isolated by homogenization in 0.32 M sucrose, 10 mM Tris-HCl (pH 7.5), 1 mM EDTA, 0.5 mM PMSF, 1 mM DTT, 0.5 mM spermidine, and 100 μg/ml sheared, denatured salmon testes DNA. 1 mM PVP-40/ml was used to stabilize the DNA and allowed to heat and cool overnight. The filter was then washed three times for 20 min each at 65 °C with 0.2 × SSC (where 1 × SSC is 0.15 M sodium chloride and 0.015 M sodium citrate) followed by autoradiography for 2 days.

Isolation of Glucokinase cDNA and Genomic DNA—Rat insulinoma poly(A)^+ RNA was used to construct a cDNA library in ZAP (Stratagene). Double-stranded cDNA was synthesized (11) and inserted into the EcoRI site of XZAP DNA using EcoRI linkers. The ligated DNAs were packaged yielding a library of approximately 1.5 × 10^8 recombinant bacteriophage. The library was plated on a lawn of Escherichia coli B/4 for screening. After purification of individual plaques, the bacteriophage clones were converted to plasmid clones in Bluescript M13+ by infection with R407 helper phage according to the supplier's protocol (Stratagene). The rat genomic DNA library in EMBL 3 from which XGK7 was isolated was obtained from G. Scherer (Institute for Human Genetics, Freiberg, West Germany) (13). The restriction map of XGK7 was determined using both multiple restriction enzyme digests and the indirect end-labeling method of Rackwitz et al. (13).

DNA Sequence Analysis—Fragments of the GK.Z9 cDNA and AGKT genomic DNA were subcloned into pEMBL 18 and pEMBL 19 (16) using cohesive end ligations. Single-stranded DNA was produced by superinfection with M13K07 helper phage. DNA sequence analysis of the single-stranded DNA templates was accomplished using a modified T7 polymerase (Sequenase II, United States Biochemical Corp.) and dyeoxynucleotide chain termination reactions (15). The reaction products were separated on 6% acrylamide, 7 M urea sequencing gels and visualized by autoradiography. The sequences of the 18-mer oligonucleotide primers (5′ to 3′) used are: GK768: CTCTTTGACTACATCTCT; GK806: GGCTATGGATACCCG; GK1276: TTTCTTCGTCGCTGCT; and GK1351: CAGCTGACTGACCTCGG.

RESULTS

Analysis and Cloning of the Glucokinase mRNA in Insulinoma Tissue—The size of the glucokinase mRNA in rat insulinoma tissue was estimated by Northern blot analysis. A single glucokinase mRNA species was detected in both rat insulinoma and liver poly(A)^+ RNA using the hepatic glucokinase GK.Z2 cDNA as a probe. The size of the glucokinase mRNA in insulinoma was, however, larger than that from liver (Fig. 1, compare lanes 1 and 2). The hepatic glucokinase mRNA is 2346 nucleotides long, not including the poly(A) tail (18). The glucokinase mRNA in insulinoma tissue appeared to be approximately 2600 nucleotides in length.

To define the structural differences between the β cell and liver glucokinase mRNAs, a glucokinase cDNA from insulinoma tissue was isolated. A 740-bp GK cDNA fragment (EcoRI-BalI) from the 5′ end of the hepatic glucokinase cDNA GK.Z1 was used to screen a rat insulinoma cDNA library. Three glucokinase cDNAs were identified; two of these hybridized to an oligonucleotide probe complementary to sequences within exon 2 of the hepatic glucokinase transcription unit (GK.Z9 and GK.Z17). The restriction map of the longer of the two insulinoma glucokinase cDNAs, GK.Z9, was determined and is shown in Fig. 2. The map was similar to that of the hepatic glucokinase cDNA GK.Z2 (shown in Fig. 2 for comparison) with one exception: the internal Pol-Bal fragment was approximately 50 bp shorter.

Comparison of Insulinoma and Hepatic Glucokinase cDNAs—The DNA sequence of GK.Z9 was determined and found to contain 2216 bases which, compared to the estimated length of the insulinoma glucokinase mRNA, was approximately 400 bp short of full length. Comparison of the DNA sequence of GK.Z9 with that of GK.Z2 revealed the 3′ ends of the cDNAs were identical except at two bases. The entire reading frame for insulinoma glucokinase was conserved in GK.Z9, indicating that the only portion of the glucokinase

2 The abbreviations used are: MOFS, 4-morpholinepropanesulfonic acid; kb, kilobase pairs; bp, base pairs; PCR, polymerase chain reaction.

1 The differences between the cDNA sequences of GK.Z9 and GK.Z2, according to the numbering in Ref. 5, are 1) a C instead of an A at position 1466 (also predicts an alanine) and 2) an A instead of a G at position 1937 (noncoding).
mRNA not represented in this cDNA were 5'-noncoding sequences. Further comparison of the sequences of the hepatic and insulinoma glucokinase cDNAs revealed two additional differences. First, 81 bp at the 5' end of GK.Z9 differed completely with corresponding sequence in GK.Z2. Second, a 51 nucleotide deletion was present with GK.Z9 as compared to GK.Z2. The exact sequence differences are shown in Fig. 3. The location of the sequence differences coincided exactly with the locations of two splice junctions in the glucokinase gene product. The cDNA sequences were identical on the 5' end of the first splice junction in the hepatic transcription unit (Fig. 3, panel A); however, they were different on the 5' side. Similarly, a sequence deletion in the pancreatic glucokinase cDNA was found to coincide with the splice junction between the 3rd and 4th exon in the hepatic transcription unit (Fig. 3, panel B). The 51-nucleotide deletion in GK.Z9 is not a cloning artifact because a second insulinoma cDNA, GK.Z17, also contained a PstI-BalI fragment of the same size (data not shown).

The differences in the nucleotide sequence of the insulinoma and hepatic glucokinase cDNAs predict glucokinase isozymes with different amino acid sequences. The insulinoma glucokinase mRNA contains a different translation initiation codon resulting in 15 different amino acids at the N terminus of pancreatic glucokinase (Fig. 3, panel A). Alternately, use of a second potential translation initiation site located immediately 3' of the first in-frame initiation codon would predict 8 different amino acids at the N terminus of pancreatic glucokinase. When translated to protein, the 51-nucleotide deletion in the insulinoma GK.Z9 cDNA predicts an in-frame deletion of 17 amino acids (Fig. 3, panel B). The amino acids missing in β cell glucokinase are located in a region of the protein between the putative ATP and glucose binding domains.

The finding of alternate RNA splicing within the reading frame of β cell glucokinase was unexpected. Both insulinoma cDNAs isolated were generated from mRNAs that utilized a distal splice acceptor site in exon 4. Previously, two hepatic glucokinase cDNAs were characterized (7), both of which utilized a splice acceptor site 51 nucleotides more proximal. On the basis of these different glucokinase cDNAs, it appeared the distal splice acceptor site might be utilized specifically in the β cell. To investigate this possibility, an experiment utilizing the PCR to amplify glucokinase cDNA sequences from several tissues was done. Two primers, one of which contained DNA sequence from the 51-nucleotide deletion found in GK.Z9 (GK768) and another containing sequence from a common region (GK357), were used to amplify glucokinase first strand cDNA sequences (the location of the primers is illustrated in Fig. 2). Splicing of the glucokinase gene product to the proximal acceptor site in exon 4 would be detected as a 487-bp cDNA product. The products of PCR using the GK768 and GK357 primers are shown in Fig. 4, panel A. A product of the predicted size was observed from reactions containing liver (lane 1), insulinoma (lane 2), and pancreatic cDNAs (lane 3). The finding of a PCR product of 487 bp from insulinoma and pancreas cDNA indicates that mRNAs are indeed present in these tissues which utilize the proximal splice acceptor site in exon 4. The use of the proximal exon 4 splice in glucokinase mRNA from insulinoma tissue was also detected in a separate RNase protection experiment (data not shown).

**Location of the Glucokinase β Cell Promoter:** The 81 nucleotides at the 5' end of the β cell glucokinase mRNA were used

**Fig. 1.** Northern blot analysis of glucokinase mRNAs. 10 µg of poly(A)+ RNA from rat insulinoma tissue (lane A) and the livers of diabetic rats treated with insulin for 4 h (lane B) was size-fractionated in a 1.4% agarose gel containing 6.3% formaldehyde. The RNA was blot transferred to nitrocellulose and probed with a 32P-labeled hepatic cDNA fragment. 18 and 28 S RNA standards were run in an adjacent lane.

**Fig. 2.** Restriction map sequencing strategy for GK.Z9. The restriction map of a glucokinase cDNA isolated from insulinoma tissue (GK.Z9) is shown. The map of a liver glucokinase cDNA (GK.Z2) is shown for comparison. The arrows indicate sequencing reactions. Sequence across the PstI site in GK.Z9 has been obtained previously for both the liver cDNA and the glucokinase gene. The hatched boxes in the cDNA indicate β cell and liver-specific sequences and the locations of the putative ATP and glucose binding domains. The location of the 51 nucleotides present in GK.Z2, not found in GK.Z9, is shown by the box under "GK768," one of the primers used in PCR amplification experiments.
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Fig. 3. Nucleotide and predicted amino acid sequence differences between the β cell and hepatic glucokinase cDNAs and isoforms. Panel A, the nucleotide sequence of the 5' end of GK.Z9 and the translated open reading frame from the first in-frame ATG are shown. The nucleotide and amino acid sequences are compared to those from the hepatic glucokinase cDNA GK.Z2. The location of the splice junctions is indicated below the sequences. Panel B, the nucleotide and translated amino acid sequences flanking the internal deletion of the insulinoma glucokinase cDNA GK.Z9 is shown. The splice junction separating sequences derived from the 3rd and 4th exons is indicated below the sequences. a.a., amino acids.

Fig. 4. Amplification of glucokinase cDNA fragments using the polymerase chain reaction. First strand cDNA was prepared from poly(A)+ RNAs isolated from rat insulinoma, rat pancreas, and insulin-treated diabetic rat liver. PCR amplifications were performed as described under "Experimental Procedures." The products were separated in a 1.2% agarose gel containing ethidium bromide and visualized by fluorescence under UV illumination. The products hybridized to glucokinase DNA probes in a separate Southern blot hybridization experiment (not shown). Panel A, PCR amplification products generated using GK768 as the 5' primer and GK357 as the 3' primer are shown: lane 1, liver cDNA; lane 2, rat insulinoma cDNA; and lane 3, pancreas cDNA. Panel B, PCR amplification products generated using GK1351 as the 5' primer and GK1276 as the 3' primer are shown: lane 1, pGK7.B2 DNA; and lane 2, insulinoma cDNA. Panel C, PCR amplification products generated using GK806 as the 5' primer and GK357 as the 3' primer are shown: lane 1, liver cDNA; lane 2, insulinoma cDNA; and lane 3, pancreas cDNA.

to identify the promoter and first exon sequences in the glucokinase gene which are expressed in the β cell. These sequences were not detected in λGK5 (18), the genomic DNA clone containing the hepatic glucokinase promoter (data not shown). The glucokinase β cell promoter and first exon sequences were, therefore, located further upstream in the gene. To identify a genomic DNA fragment containing the β cell-specific glucokinase sequences, a rat genomic DNA library was screened using the EcoRI-PstI fragment from GK.Z9 as a probe. From approximately 1 x 10^6 plaques, a single clone, λGK7, was isolated which hybridized with the pancreas-specific portion of the cDNA probe. Analysis of the genomic DNA contained in this recombinant bacteriophage indicated the sequences hybridizing with the GK.Z9 fragment were located near the 3' end of the DNA fragment (Fig. 5). Unfortunately, the λGK7 DNA insert did not overlap the λGK5 DNA insert, thus the distance between the hepatic and β cell glucokinase promoters or the size of the first intron in the glucokinase β cell transcription unit were not determined.

However, based on the amount of DNA downstream of the β cell glucokinase promoter and upstream of the hepatic glucokinase promoter, this distance is greater than 12 kb. A 4-kb BamHI fragment which hybridized with GK.Z9 was subcloned and analyzed further (see pGK7.B2 in Fig. 5).

Identification of Transcription Initiation Sites in the β Cell—To determine whether the β cell glucokinase mRNA leader sequences were contained in a single exon, another PCR experiment was performed. A 3' primer (GK1276), complementary to pancreas-specific sequences found in GK.Z9, and a 5' primer (GK1351), complementary to genomic DNA sequences located further upstream in the gene, were used. The location of the sequences complimentary to the primers is indicated in Fig. 5. A 472-bp DNA fragment was amplified from both pGK7.B2 (Fig. 4, panel B, lane 1) and insulinoma cDNA (Fig. 4, panel B, lane 2). This result indicates that sequences from the glucokinase gene and cDNA are co-linear between the locations of the two oligonucleotide primers and that the 5'-noncoding sequence of the glucokinase mRNA, not fully represented in GK.Z9, is present as a single exon. Thus, sequences from the 5' end of the glucokinase β cell mRNA can accurately be deduced from the genomic DNA sequences between the primers GK1276 and GK1351.

An RNase protection experiment was done to determine whether DNA sequences in GK7 upstream of the GK.Z9 homology contained the glucokinase β cell promoter. An antisense RNA probe was generated from DNA sequences between the HindIII-XbaI sites of pGK7.B2 (see Fig. 5). Poly(A)+ RNAs from rat insulinoma, insulin-treated diabetic rat liver, and rat brain were tested. Only the insulinoma poly(A)+ RNA probed the antisense RNA probe from digestion (Fig. 6, panel A, lane 2) indicating sequences corresponding to the 5' end of the glucokinase mRNA in the β cell were present in this genomic DNA fragment. Interestingly, many different sized fragments were protected from RNase digestion by the insulinoma poly(A)+ RNA, indicating that transcription initiation from the glucokinase β cell promoter spans a 62-base region.

A primer extension assay was used to confirm the multiple transcription initiation sites from the glucokinase β cell promoter. Fig. 6, panel B, shows the products synthesized from a 30-mer oligonucleotide primer complementary to sequences near the 5' end of the pancreatic glucokinase mRNA. Poly(A)+ RNAs from rat insulinoma, insulin-treated diabetic rat liver, and rat brain were tested again. Results similar to
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Fig. 6. Identification of β cell glucokinase transcription initiation sites. Panel A, RNase protection analysis. An RNase protection assay was performed as described under “Experimental Procedures.” 10 µg of RNA was used in each assay: lane 1, tRNA; lane 2, rat insulinoma poly(A)+ RNA; lane 3, insulin-treated diabetic rat liver poly(A)+ RNA; and lane 4, rat brain poly(A)+ RNA. Size markers were run in an adjacent lane. Panel B, primer extension analysis. A primer extension assay was performed as described under “Experimental Procedures.” 10 µg of RNA was used in each assay: lane 1, tRNA; lane 2, rat insulinoma poly(A)+ RNA; lane 3, insulin-treated diabetic rat liver poly(A)+ RNA; and lane 4, rat brain poly(A)+ RNA. Size markers were run in an adjacent lane. Panel C, location of the multiple transcription initiation sites. The nucleotides corresponding to the bands observed in both the RNase protection assay (top) and primer extension assay (bottom) are shown. The large and small dots correspond to strong and weak bands observed from each assay, respectively.

those for the RNase protection assay were obtained. Primer extension products were detected only from insulinoma poly(A)+ RNA (lane 2) and not from the other RNAs tested (lanes 1, 3, and 4). In addition, the transcription initiation sites indicated by the primer extension experiment mapped to the same region on the glucokinase gene as those determined by the RNase protection assay. The location of the transcription initiation sites identified by the two experiments corresponded well (Fig. 6, panel C), although the RNase protection assay, which used a probe of higher specific activity, detected two sites further upstream. The wide region of transcription initiation makes the numbering of upstream DNA bases rather arbitrary. However, for this purpose we designated an adenine, which lies between the most proximal initiation sites detected in the RNase protection and primer extension assays, as +1.

DNA Sequence of the Glucokinase β Cell First Exon and Promoter—The sequence of the DNA flanking the transcription initiation sites was determined. The limit for sequencing in the 5’ direction was a BamHI site (−2299). A portion of that sequence is shown in Fig. 7 and contains 1000 bp of DNA upstream of the designated transcription initiation site, the 473-bp first exon in the β cell glucokinase transcription unit, and 27 bp from the first intron of the glucokinase gene.

Expression of the Hepatic Glucokinase Promoter—The downstream glucokinase promoter has been shown to be active in the liver (18). To determine whether it might also be active in the β cell, a third PCR experiment was performed. Primers were chosen such that the 3’ primer (GK357) would anneal to sequences common to both liver and β cell glucokinase cDNA sequences, but the 5’ primer (GK806) would anneal only to hepatic glucokinase sequences. Synthesis of a 847-bp product would, therefore, be specific for expression of the downstream glucokinase promoter. A product of this size was observed only when cDNA from insulin-treated rat liver was amplified (Fig. 4, panel C). Products were not seen from amplification reactions using insulinoma (lane 2) or pancreas cDNAs (lane 3) indicating the downstream glucokinase promoter is active in the liver but not in the β cell.

Fig. 7. Genomic DNA sequences flanking the β cell glucokinase promoter. The sequence of DNA flanking the transcription initiation sites of the glucokinase β cell promoter is shown. Noteworthy features are underlined and numbered: 1, the predicted translation initiation codon; 2, the region of transcription initiation; 3, eight nucleotides similar to an enhancer in the insulin gene which is discussed in the text; and 4, a repetitive purine/pyrimidine tract with potential to form Z-DNA. In addition, two short reading frames are indicated which lie upstream of the reading frame for glucokinase.

DISCUSSION

Alternate Promoters in the Glucokinase Gene—The existence of two different promoters in a single glucokinase gene leads to the production of tissue-specific glucokinase mRNAs as illustrated in Fig. 8. The glucokinase promoter active in β cells is located at least 12 kb upstream of the promoter active in the liver resulting in a transcription unit of greater than 27.5 kb compared to the hepatic glucokinase transcription unit of 15.5 kb. The use of alternative promoters is a means of regulating the tissue- and developmental state-specific expression of proteins (19). Several genes have been described that use multiple promoters active in different tissues or at different developmental stages (19). For instance, the α-amylase gene is expressed in both the liver and the salivary gland and contains two promoters, one of which is active only in the salivary gland while the other is active in both the liver and salivary gland (20). The α1-antitrypsin gene, however, utilizes two promoters which are specifically expressed in single tissues: one promoter is active in the liver, the other in the macrophage (21). The glucokinase gene is similar to the α1-antitrypsin gene because the two promoters it contains each appear to be active in a specific tissue.

Glucokinase Isozymes in the β Cell—Hepatic and islet glucokinase have been shown to be indistinguishable by any chromatographic, enzymatic, or immunologic criteria (22, 23). The cloning of an insulinoma glucokinase cDNA, and comparison of its sequence to a hepatic glucokinase cDNA, indicates that the enzymes in these tissues are indeed structurally
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**Fig. 8. Alternate promoters in the glucokinase gene.** The use of alternate promoters in the rat glucokinase gene is diagrammatically illustrated.

Quite similar. An important difference, however, is the 15 amino acids at N-terminal ends of the proteins. Initiation of translation from different codons in the glucokinase mRNAs in the liver and $\beta$ cell, due to the use of tissue-specific first exons, gives rise to the different terminal amino acids. The tissue-specific N-terminal amino acids have little effect on either the mass or charge of the predicted glucokinase isoforms. Furthermore, the identical enzymatic properties of glucokinase from the liver and $\beta$ cell suggests altering these N-terminal amino acids has little functional impact.

Alternative RNA splicing at the splice acceptor site in the 4th exon appears to generate additional diversity in glucokinase isoforms in the $\beta$ cell. The masses of the $\beta$ cell glucokinase isoforms are predicted to differ by less than 4%: 52,087 versus 50,066 Da, respectively. The isoelectric points of the isoforms differ by a similar amount: the larger has a calculated pI of 5.08 whereas the smaller has a pI of 5.18. The portion of the protein affected lies between the putative ATP and glucose binding domains. Whether deletion of 17 amino acids in this region has an effect on enzymatic behavior is not known and can be determined only by functional expression of the different cDNAs.

The Glucokinase $\beta$ Cell Promoter—The glucokinase $\beta$ cell promoter initiates transcription from multiple transcription initiation sites. Interestingly, examination of the proximal promoter sequence did not reveal either a TATA or CAAT homology, features usually found in the proximal promoter regions of tissue-specific promoters. The absence of the TATA homology may explain the wide range of transcription initiation from this promoter since this element is thought to be important for the precise positioning of transcription initiation sites (24).

An important consequence of these studies is the finding of a promoter whose activity appears to be restricted to $\beta$ cells. The only other promoters described whose activity is limited primarily to $\beta$ cells are those for insulin. Genes which are expressed specifically in a given tissue might use the same transcription factors as a basis for coordinated, tissue-specific control of gene transcription. For example, the same liver-specific factors interact with the promoters of the fibrinogen and $\alpha_1$-antitrypsin genes (25), two genes that have promoters active only in the liver. It is possible, therefore, that $\beta$ cell-specific nuclear factors recognize elements in genes expressed specifically in the $\beta$ cell. The insulin promoter has been studied extensively in order to locate $\beta$ cell-specific enhancer elements, and at least one sequence appears to be important in this regard (26–28). As a first step in locating $\beta$ cell-specific enhancer elements in the glucokinase $\beta$ cell promoter, we compared the 5′-flanking DNA sequences of the glucokinase $\beta$ cell and rat insulin I genes for conserved DNA sequences that might suggest such conserved functional elements. Although such a simple analysis does not establish whether conserved sequences are functionally important, it might suggest a starting point for functional studies. The sequence GCCATCAG, located at positions −126 to −133 upstream of the designated initiation site in the glucokinase gene, is a 7 out of 8 bp match for the sequence GCCATCTG, an element crucial for the expression of the rat insulin I promoter in insulinoma cells (26–28). The homology within this region actually extends further 5′ and, in total, includes a 12 out of 14 bp match with rat insulin I sequences. It is possible this sequence is important for expression of glucokinase in the $\beta$ cell.

Tissue Expression of the Dual Glucokinase Promoters—Based on existing reports and our data, it appears that the upstream glucokinase promoter is expressed primarily or solely in $\beta$ cells while the downstream promoter is expressed primarily or solely in the liver. Glucokinase immunoreactivity has been detected in cytoplasmic protein from liver and islets but not from intestinal mucosa, exocrine pancreas, epididymal adipose, kidney, brain, or spleen (29). Our studies indicate the upstream glucokinase promoter is active in insulinoma tissue and not in the liver or brain, and the downstream glucokinase promoter is active in the liver and not in tissues containing $\beta$ cells. Expression of the hepatic glucokinase promoter was detected only in the liver and not in insulinoma or pancreatic tissues. Furthermore, identification of sequences in the hepatic glucokinase promoter which are similar to elements known to be important for liver-specific gene expression (18) lends further support to the notion that the downstream glucokinase promoter is expressed specifically in liver. Similar arguments also apply to the upstream glucokinase promoter which appears to be expressed specifically in $\beta$ cells.

Functional Implications of Multiple Promoters in the Glucokinase Gene—Our finding of alternate promoters in the glucokinase gene is not entirely surprising. Liver and pancreatic islets are distantly related during development, and expression of genes in these different tissues probably requires different transcription factors. More importantly, glucokinase is regulated differently in the liver and $\beta$ cell, and the proposed functional roles for the enzyme are different in the two tissues. The finding of alternate promoters in the glucokinase gene offers a likely mechanism for the differential regulation of hepatic and $\beta$ cell glucokinase. Regulation of hepatic glucokinase gene transcription by insulin (18, 30) suggests that factors, probably modulated by changes in plasma insulin concentration, act to affect the rate of RNA transcription from the hepatic glucokinase promoter. However, for glucokinase to function as a moderator of $\beta$ cell glycolysis and insulin secretion, the enzyme cannot, a priori, be regulated by
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insulin. Therefore, it is reasonable to think there are factors in \( \beta \) cells that regulate activity of the \( \beta \) cell glucokinase promoter in response to plasma glucose. Although it needs to be established whether this is indeed the case, characterization of the glucokinase transcription unit and identification of an alternate promoter which is active in \( \beta \) cells now enables such experiments to be undertaken.

A Possible Feedback Control Loop—Dual promoters in the glucokinase gene may constitute an important feedback control loop. Hepatic glucokinase is regulated by insulin which, by affecting glucose usage, alters the concentration of blood glucose. \( \beta \) cell glucokinase, on the other hand, may be regulated by glucose. If so, then alterations in glucokinase activity would affect the rate of glycolysis and, hence, insulin secretion by the \( \beta \) cell. The regulation of glucokinase by insulin in the liver and by glucose in the \( \beta \) cell may constitute an important feedback loop for maintaining glucose homeostasis as suggested previously (6). Alternate promoters in the glucokinase gene provide a physical explanation by which differential regulation could occur, thus, providing a basis for feedback regulation to occur within a single glucokinase gene expressed in the liver and pancreatic \( \beta \) cells.

Concluding Remarks—Characterization of the glucokinase gene \( \beta \) cell transcription unit is an important step toward understanding the expression of glucokinase in the \( \beta \) cell. Identification of \( \beta \) cell-specific glucokinase isozymes and an alternate glucokinase promoter active in \( \beta \) cells provides the necessary framework and reagents for the study of the expression and regulation of glucokinase in normal and diabetic animals. Studies to characterize both the factors regulating expression of the glucokinase \( \beta \) cell promoter and the function of the enzyme in the \( \beta \) cell are now possible.

Acknowledgments—We thank Dr. W. Chick for providing rat insulinoma tissue, Dru. M. Tamkun and P. A. Weil for critical reading of the manuscript, and V. Kim for her energetic involvement in early stages of this work.

REFERENCES

1. Ashcroft, S. J. H. (1980) Diabetologia 18, 5–15
2. Taljedal, I.-B. (1981) Diabetologia 21, 1–17
3. Ashcroft, F. M., Harrison, D. E., and Ashcroft, S. J. H. (1984) Nature 312, 446–448
4. Weinhouse, S. (1976) Curr. Top. Cell. Regul. 11, 1–50
5. Meglasson, M. D., and Matschinsky, F. M. (1986) Diabetes Metabolism Rev. 2, 165–214
6. Bedoya, F. J., Matschinsky, F. M., Shimizu, T., O’Neil, J. J., and Appel, M. C. (1986) J. Biol. Chem. 261, 10760–10764
7. Andreone, T. L., Printz, R. L., Pilkis, S. J., Magnuson, M. A., and Granner, D. K. (1989) J. Biol. Chem. 264, 363–369
8. Maniatis, T., Fritsch, E. F., and Sambrook, J. (1982) Molecular Cloning: A Laboratory Manual, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY
9. Feinberg, A. P., and Vogelstein, B. (1983) Anal. Biochem. 132, 6–13
10. Chick, W. L., Warren, S., Chute, R. N., Like, A. A., Lurie, V., and Kitchen, K. C. (1977) Proc. Natl. Acad. Sci. U. S. A. 74, 628–632
11. Gubler, U., and Hoffman, B. J. (1983) Gene (Amst.) 25, 263–269
12. Shinomiya, T., Scherer, G., Schmid, W., Zentgraf, H., and Schutz, G. (1984) Proc. Natl. Acad. Sci. U. S. A. 81, 1346–1350
13. Rachwitz, H.-R., Zehetner, G., Frischau, A.-M., and Lehrach, H. (1984) Gene (Amst.) 30, 196–200
14. Dente, L., Cesareni, G., and Cortese, R. (1983) Nucleic Acids Res. 11, 1645–1655
15. Sanger, R., Nicklen, S., and Coulson, A. R. (1977) Proc. Natl. Acad. Sci. U. S. A. 74, 5463–5467
16. Melton, D. A., Kreig, P. A., Rebagliati, M. R., Maniatis, T., Zinn, K., and Green, M. R. (1984) Nucleic Acids Res. 12, 7053–7066
17. Saki, R. K., Gelfand, D. H., Stoffel, S., Scharf, S. J., Higuchi, R., Horn, G. T., Mullis, K. B., and Erlich, H. A. (1988) Science 239, 487–491
18. Magnuson, M. A., Andreone, T. L., Printz, R. L., Kock, S., Granner, D. K. (1989) Proc. Natl. Acad. Sci. U. S. A., 86, 4858–4862
19. Breitbart, R. E., Andreadis, A., and Nadal-Ginard, B. (1987) Annu. Rev. Biochem. 56, 467–95
20. Young, R. A., Hagenbuchle, O., and Schibler, U. (1981) Cell 23, 451–458
21. Perlino, E., Cortese, R., and Ciliberto, G. (1987) EMBO J. 6, 2767–2771
22. Meglasson, M. D., Burch, P. T., Berner, D. K., Najafi, H., Vogin, A. P., and Matschinsky, F. M. (1983) Proc. Natl. Acad. Sci. U. S. A. 80, 85–89
23. Vischer, U., Blondel, B., Wollheim, C. B., Hoppner, W., Seitz, H. J., and Iynedjian, P. B. (1987) Biochem. J. 241, 249–255
24. Breathnach, R., and Chambon, P. (1981) Annu. Rev. Biochem. 50, 349–383
25. Courtois, G., Morgan, J. G., Campbell, L. A., Fourel, G., and Crabtree, G. R. (1987) Science 238, 688–692
26. Ohlsson, H., Karlsson, O., and Edlund, T. (1988) Proc. Natl. Acad. Sci. U. S. A. 85, 4228–4231
27. Karlsson, O., Edlund, T., Moss, J. B., Rutter, W. J., and Walker, M. D. (1987) Proc. Natl. Acad. Sci. U. S. A. 84, 8819–8823
28. Moss, L. G., Moss, J. B., and Rutter, W. J. (1983) Mol. Cell. Biol. 8, 2620–2627
29. Iynedjian, P. B., Mobius, G., Seitz, H. J., Wollheim, C. B., and Renold, A. E. (1986) Proc. Natl. Acad. Sci. U. S. A. 83, 1989–2001
30. Iynedjian, P. B., Gjinovci, A., and Renold, A. E. (1988) J. Biol. Chem. 263, 740–744