

Glucokinase Gene Structure

Functional Implications of Molecular Genetic Studies

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Glucokinase is expressed in both the liver and the pancreatic β -cell and plays a key role in the metabolism of glucose by both tissues. Expression of this enzyme is differentially regulated; hepatic glucokinase is stimulated by insulin and repressed by cAMP, whereas β -cell glucokinase activity is increased by glucose. Recently, the glucokinase gene has been characterized and was found to contain two different transcription control regions. One region regulates transcription of the gene in the liver, whereas the other region, which lies at least 12 kilobases further upstream, controls transcription in the pancreatic β -cell. The finding of two different transcription control regions in a single glucokinase gene provides a genetic basis for the tissue-specific differential regulation of glucokinase and will serve as the basis for further studies to identify and characterize the different regulatory elements and factors in the liver and β -cell, which are presumably involved. Comparison of different glucokinase cDNAs isolated from hepatic, insulinoma, and islet cDNA libraries indicates that at least three glucokinase isoforms are generated by differential RNA processing of the glucokinase gene transcripts. Whether any of these glucokinase isoforms are functionally unique remains to be determined. *Diabetes* 39:523–27, 1990

Information about the tissue-specific differential regulation of glucokinase has been obtained by characterizing the glucokinase gene and its different transcription units in the liver and β -cell. This information offers further support for the concept that independent regulation of glucokinase

in the liver and β -cell is crucial for maintenance of blood glucose homeostasis. These molecular genetic studies also indicate that different glucokinase isoforms are generated in each tissue by an alternate RNA-splicing mechanism. In this article, I briefly review these findings and speculate about their implications for the regulation of blood glucose homeostasis.

DISTINCTIVE CHARACTERISTICS OF GLUCOKINASE

The first step in the metabolism of glucose is phosphorylation at the sixth carbon. This reaction is catalyzed in mammals by a family of hexokinases (types I–IV) that appear to have a common evolutionary origin (1–5). Glucokinase (type IV) differs functionally from the other hexokinases (types I–III) by its higher K_m for glucose (5 mM vs. ~ 20 – $130 \mu\text{M}$), its greater specificity for glucose, and its lack of product inhibition by glucose-6-phosphate (G6P). The enzyme differs structurally from the other hexokinases by its mass. Glucokinase and the yeast hexokinases have molecular masses of $\sim 50,000$ (4), whereas the type I–III hexokinases have molecular masses of $\sim 100,000$. The structure of type I hexokinase was recently determined by cDNA cloning and was found to consist of two halves with considerable sequence homology (4,5). In addition, the amino acid sequence of glucokinase shows extensive homology to yeast hexokinase and both halves of type I hexokinase (4,6). Together, these data provide direct support for the hypothesis that the type I–III hexokinases arose from a gene-duplication and -fusion event involving an ancestral gene similar to the yeast enzymes and present-day glucokinase (1–3).

The functional properties of glucokinase have important consequences for the utilization of glucose by both the liver and the β -cell, the only locations where the enzyme has been detected (7–10). The lower affinity (higher K_m) of glucokinase for glucose, compared with that of the other mammalian hexokinases, allows changes in the rate of glucose phosphorylation to occur at physiological glucose concentrations (4–15 mM). In addition, the lack of end-product inhibition of glucokinase allows glucose phosphorylation to proceed at

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a rate proportional to that of substrate concentration without the inhibitory allosteric effects caused by G6P. These properties of glucokinase, coupled with its position at the beginning of the glycolytic pathway, enable this enzyme to play an important role in both hepatic and β -cell glucose metabolism.

TISSUE-SPECIFIC REGULATION OF GLUCOKINASE

The regulation of glucokinase in the hepatocyte and β -cell differs in a manner consistent with the different functions of these two cell types. The liver is a major site for uptake and conversion of glucose to other metabolic products during postprandial and hyperglycemic periods, whereas it is a site of glucose production and secretion during starvation and hypoglycemia. When plasma glucose is elevated and glucokinase activity is increased, glucokinase initiates metabolism of glucose by the liver and thereby assures a continued gradient for the inward transport of glucose. However, when plasma glucose is low and glucose is being produced and transported outward by the liver, glucokinase activity is reduced. Insulin and glucagon have opposing effects on hepatic glucokinase gene expression, thus affecting the amount of glucokinase produced. Insulin stimulates glucokinase mRNA expression by increasing transcription of the glucokinase gene (11,12), whereas glucagon inhibits glucokinase mRNA expression (13). Whether the effect of cAMP is mediated at a transcriptional level has not been established. In contrast to the liver, the electrical, ionic, and secretory responses of the β -cell are mediated by the metabolism of glucose (14–16). Glucokinase is considered the rate-limiting step in the generation of a metabolic signal that triggers these responses. The role of glucokinase as the proximal determinant of glucose usage by the β -cell has led this enzyme to be termed the *pancreatic glucose sensor* (17,18). By determining the rate of glucose phosphorylation, glucokinase is thought to control glycolytic flux and hence the cytoplasmic ATP-ADP ratio. An increase in ATP is thought to inhibit opening of ATP-sensitive K^+ channels in the β -cell, leading to plasma membrane depolarization (19–21). Voltage-sensitive Ca^{2+} channels then open, allowing the entry of extracellular Ca^{2+} . The increased cytosolic Ca^{2+} concentration triggers the release of insulin (22).

For glucokinase to function as a stable modulator of glycolysis and insulin secretion by the β -cell, it seems necessary that the enzyme not be regulated by insulin or glucagon. Studies by Bedoya et al. (23) indicate that glucokinase in the β -cell is not affected by changes in plasma insulin but is affected instead by changes in plasma glucose concentration, which supports this notion. A more recent article by lynch et al. (24) shows the differential regulation of hepatic and islet glucokinase mRNAs in response to fasting and refeeding. However, this study leaves unanswered the question of whether the quantity of β -cell glucokinase mRNA is regulated by glucose, because dietary manipulations in a nondiabetic animal do not substantially alter blood glucose concentrations. Nevertheless, both studies point to the importance of understanding how the tissue-specific differential regulation of glucokinase occurs, especially when the role of this enzyme in regulating glucose metabolism of the liver and β -cell is considered (23,24). An important clue to the molecular mechanisms involved in this differential reg-

ulation became apparent by determining the structure of the glucokinase gene and by identifying different transcription units for hepatic and β -cell glucokinase mRNAs.

DUAL TRANSCRIPTION CONTROL REGIONS

Using hepatic glucokinase cDNA as a probe, we performed blot-transfer analysis of RNA from liver and insulinoma tissue (25). Our experiment indicated that the glucokinase mRNA in the β -cell is ~200 nucleotides longer than that in the liver. A glucokinase cDNA from a rat insulinoma cDNA library was then isolated, and its sequence was compared with the sequence of a hepatic glucokinase cDNA (6,25). This comparison revealed that the overall structures of the cDNAs were nearly identical except at the 5'-ends, where the cDNAs were completely different. The point at which the sequences diverged coincided with the location of the splice site between the first and second exons of the hepatic glucokinase transcription unit (12,25). This finding indicated that the hepatic and β -cell glucokinase transcription units utilized different first exons and therefore also utilized different transcription control regions.

Figure 1 illustrates the production of tissue-specific glucokinase mRNAs from the two transcription control regions in the glucokinase gene. The different transcription units in the liver and β -cell give rise to tissue-specific glucokinase mRNAs and to tissue-specific glucokinase isoforms, the structure and origin of which will be discussed separately. Interestingly, the tissue-specific transcription control regions in the glucokinase gene are located at least 12 kilobases (kb) apart, with the β -cell control region located further upstream than the liver control region. The large amount of intervening DNA that separates the two transcription control regions results in a transcription unit for the β -cell glucokinase gene of at least 27.5 kb, whereas that for the hepatic glucokinase gene is only 15.5 kb. Expression of the proximal transcription control region has been detected only in the liver, whereas expression of the upstream transcription control region has been detected only in the β -cell (25). Lynch et al. (24) recently reported finding similar differences in the size of hepatic and islet glucokinase mRNAs but did not determine the basis for this difference.

The DNA sequences of the two transcription control regions have few features in common. The hepatic glucokinase transcription control region contains a probable TATA box and GC box, elements often found in the promoters of genes that produce mRNA, and initiates transcription over a 5–base pair (bp) region (12). In contrast, the β -cell glucokinase transcription control region does not contain any sequences similar to those usually found in the proximal promoter regions of other genes (e.g., TATA or CCAAT boxes), and transcription initiation from this promoter occurs over a region of at least 62 bp (25). Several sequences that are similar to elements necessary for the expression of other genes in the liver were identified in the hepatic glucokinase transcription control region (12). Although β -cell-specific gene expression is not generally as well studied as liver-specific gene expression, a sequence that is similar at seven of eight bases to an element considered important for expression of the rat insulin genes in insulin-producing cells was identified in the β -cell glucokinase transcription control region (25). Whether this sequence is necessary for the expression of

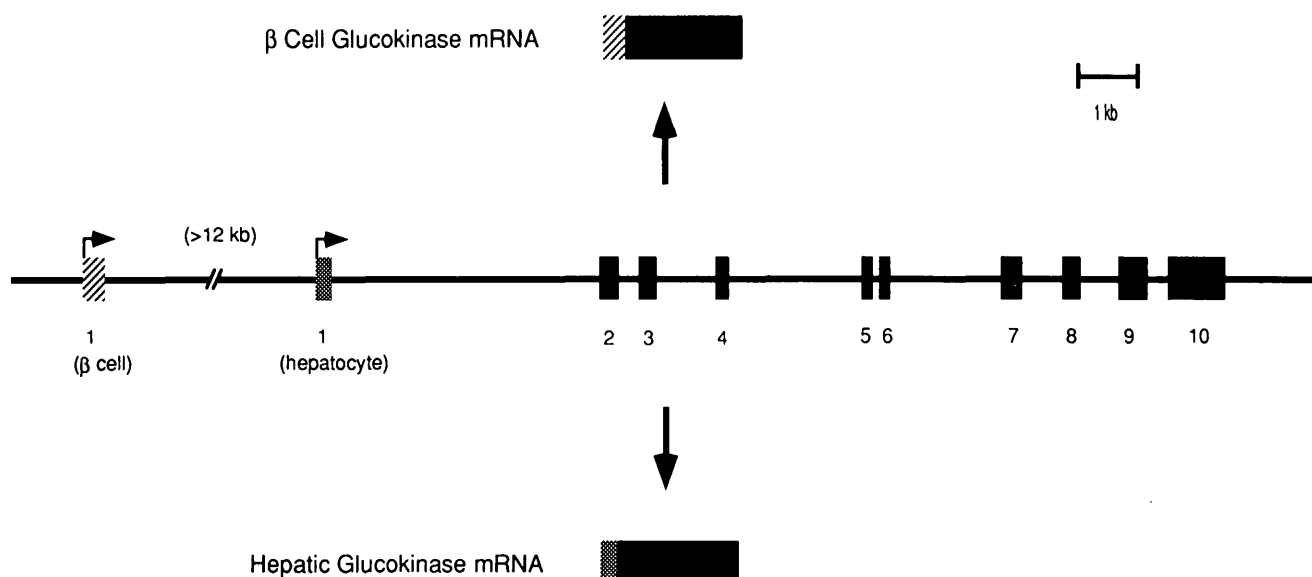


FIG. 1. Use of 2 different transcription control regions in glucokinase gene is illustrated. β -Cell glucokinase promoter is located upstream of hepatic glucokinase promoter. Boxes indicate location of each exon: *hatched boxes*, β -cell-specific sequence; *shaded boxes*, liver-specific sequence; *solid boxes*, constitutive sequence. kb, Kilobases. Adapted from Magnuson and Shelton (25).

the β -cell glucokinase transcription control region in the β -cell, as the similar sequence is for the insulin genes, remains to be proved. In any case, it appears that the different hepatic and β -cell glucokinase transcription control regions are structurally dissimilar, supporting the concept that different sets of transcription factors are likely to interact with each DNA region. This would enable the gene to be regulated differently in the liver and β -cell.

POTENTIAL FEEDBACK LOOP INVOLVING TWO PROMOTERS IN THE GLUCOKINASE GENE

The possibility of a feedback loop involving hepatic and β -cell glucokinase has been proposed (23). Identification of two different promoters in the glucokinase gene therefore provides a genetic basis for the differential regulation of this enzyme in the liver and β -cell. Hepatic glucokinase, which is stimulated by an increase in the plasma insulin concentration, affects the rate of glucose usage by the liver, thereby helping to lower the plasma glucose concentration (Fig. 2). The plasma glucose concentration, on the other hand, affects glucokinase expression in the β -cell. Increased plasma glucose would induce expression of glucokinase in the β -cell, thus causing increased β -cell glycolysis and greater insulin secretion. Whether this feedback loop is of any physiological consequence remains to be determined. A first step in this direction is to determine how glucose regulates expression of glucokinase in the β -cell. Assuming that some aspect of glucokinase gene structure is important for the regulation of glucokinase by glucose in the β -cell, one of two mechanisms seems likely to be involved. Glucose could alter glucokinase synthesis in the β -cell by a transcriptional mechanism involving the upstream glucokinase transcription control region or by a translational mechanism involving the different 5'-noncoding mRNA sequences.

The existence of two different transcription control regions in the gene for glucokinase is not without precedent. Other genes are known to utilize different transcription control re-

gions that, by alternative splicing of the RNA transcripts, produce mRNAs that code for identical proteins. Two examples of this are the α -amylase and α_1 -antitrypsin genes. The α -amylase gene, which is expressed in both the liver and salivary glands, utilizes a salivary gland-specific promoter and another promoter that is active in both tissues (26). The α_1 -antitrypsin gene is expressed in the hepatocyte and the macrophage and utilizes different promoters in each cell type (27). The glucokinase gene is similar to that of the α_1 -antitrypsin gene in that it contains two tissue-specific transcription control regions. However, whereas the same protein is produced by the α_1 -antitrypsin gene in the macrophage and liver, this is not the case with the glucokinase gene, because the splice junction between the first and second exons occurs within the reading frame of the enzyme. The alternate first exons of the glucokinase gene contain

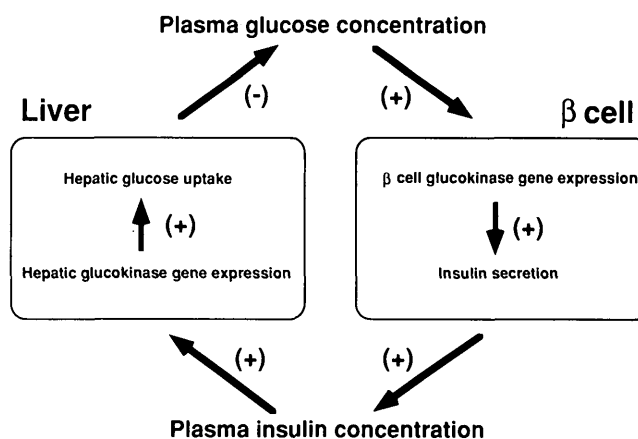


FIG. 2. Potential feedback loop involving dual glucokinase promoters is illustrated. Alternate transcription control regions in glucokinase gene provide physical basis by which this enzyme can be regulated independently in liver and β -cell. This may allow feedback loop involving 2 different transcription control regions as shown.

alternate translation-initiation codons, which result in different glucokinase isozymes in the liver and β -cell.

MULTIPLE GLUCOKINASE ISOFORMS

Comparison of glucokinase cDNAs isolated from hepatic, islet, and insulinoma cDNA libraries indicates that different glucokinase isoforms are generated through alternate splicing of the glucokinase gene product. One of these isoforms is specific for the liver, whereas two others are specific for insulin-producing cells. To clarify future discussion of these different glucokinase isoforms, herein, the liver glucokinase isoform will be referred to as glucokinase L1, and the two glucokinase isoforms identified in insulin-producing tissues will be referred to as glucokinase B1 and B2. The structure of these isoforms is illustrated in Fig. 3. The glucokinase isoform generated in the liver (L1) differs from those generated in insulin-producing cells (B1 and B2) by sequences at the NH₂-terminal. This difference accounts for no more than 15 amino acids and does not greatly alter either the overall mass or charge of the enzyme (Fig. 3). However, it is possible that this difference is functionally significant. The hydrophobic amino acids at the NH₂-terminal of hexokinase I, for instance, anchor the enzyme to the outer mitochondrial membrane (28). It is doubtful that the residues at the NH₂-terminal of β -cell glucokinase perform the same role, because they are less hydrophobic than the hexokinase I sequence (25). Note also that the exact terminal NH₂ residue shared by the β -cell glucokinase isoforms remains uncertain, because there are two potential translation-initiation codons in the β -cell glucokinase mRNAs and it is not known whether one or both of these is utilized. The predicted sizes for the β -cell glucokinase isoforms indicated in Fig. 3 are based on use of the first AUG (25). Translation initiation at the second AUG would shorten the B1 and B2 isoforms by 7 amino acids.

The structure of the glucokinase L1 isoform is based on the sequence of a cDNA clone isolated from a rat liver cDNA library (6). The structure of the B1 isoform is based on reports from my laboratory and that of Newgard (25,29). Whereas we predicted the existence of the B1 isoform from polymerase chain-reaction–amplification experiments (25), Milburn

et al. (29) isolated cDNA clones from a rat islet cDNA library encoding a B1 isoform. In addition, cDNA clones isolated from a rat insulinoma tissue cDNA library indicate the existence of the glucokinase B2 isoform (25). This isoform is generated by the use of an alternate splice-acceptor site in the fourth exon of the gene. Deletion of 51 nucleotides from the mRNA results in an isoform that is missing 17 amino acids in a region of the protein situated between the putative ATP- and glucose-binding domains. There is no evidence for an L2 isoform (analogous to B2 but with the liver-specific 5'-end).

It is not known whether these three glucokinase isoforms have the same or different enzymatic properties. Although the single hepatic glucokinase isoform (L1) accounts for the glucokinase activity found in the liver, it has not been established whether one or both of the B1 and B2 isoforms generate the glucokinase activity measured in insulin-producing cells. It would be surprising if activity of the B1 isoform were dramatically affected by changing a few amino acids at the NH₂-terminal. However, deletion of 17 amino acids in a region of the B2 isoform near the putative glucose-binding domain seems more likely to have some effect. Exactly what impact this might have on the enzyme (for instance on the V_{max} , K_m , or substrate specificity) cannot be predicted and must be determined by expressing the B2 isoform and measuring these parameters. Although the relative abundance of the B1 and B2 isoforms in the β -cell has not been directly established, it may be of some consequence that two cDNAs for the B1 isoform were isolated from nondiabetic islets (29) and two cDNAs for the B2 isoform were isolated from insulinoma tissue (25). This suggests there is a different ratio of the B1 and B2 isoforms in nondiabetic and transformed β -cells. If this is found to be the case with assays that directly quantitate the ratio of the respective RNAs, and if these isoforms were to differ dramatically in their enzymatic properties, then the alternate RNA processing of the glucokinase gene product could affect glucose-signal–insulin-secretion coupling in the β -cell by altering the structure of glucokinase. This possibility, although speculative, needs to be studied further by characterizing the activity and abundance of each glucokinase isoform in the β -cell.

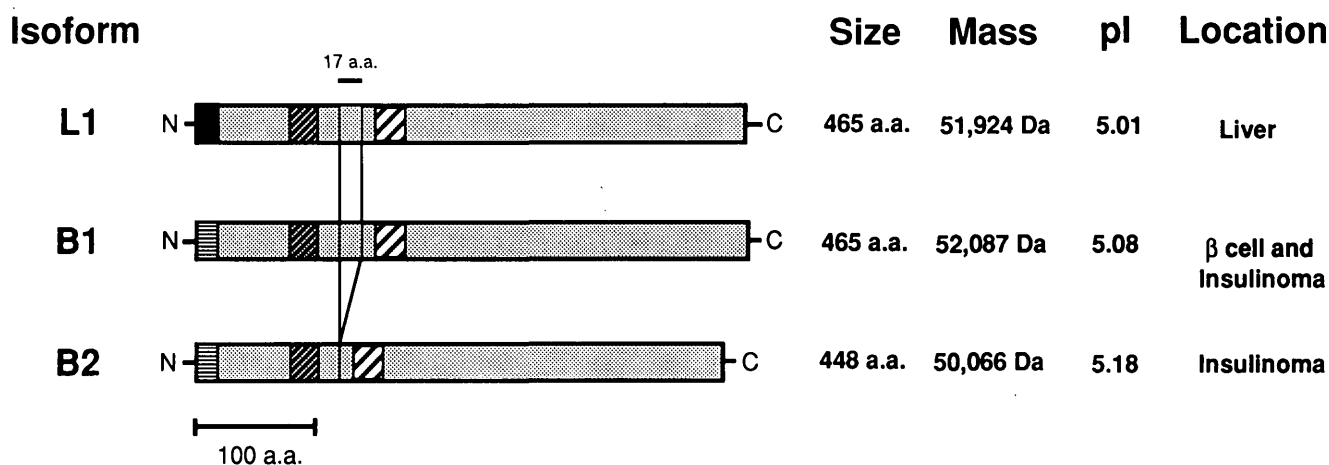


FIG. 3. Different glucokinase isoforms have been identified. Three different glucokinase isoforms identified by cDNA cloning are shown. Predicted masses and charges of different isoforms of glucokinase and locations at which their sequences differ are indicated. Origin of each specific isoform is discussed in text. Closely hatched boxes, putative ATP-binding domain; broadly hatched boxes, putative glucose-binding domain; horizontally striped boxes, β -cell-specific sequence; solid boxes, liver-specific sequence. a.a., Amino acid.

POTENTIAL EFFECT OF DIFFERENT mRNA 5'-NONCODING SEQUENCES

In addition to generating glucokinase isoforms with different NH₂-terminals, the use of alternate first exons in the glucokinase gene results in different 5'-noncoding sequences in the liver and β -cell glucokinase mRNAs. The β -cell glucokinase mRNA 5'-noncoding sequence is ~400 nucleotides long, 200 nucleotides longer than that in the hepatic glucokinase mRNA, and also contains two short open reading frames, each followed by an in-frame termination codon (25). These differences in the sequences preceding the reading frame for the enzyme may affect the translational efficiency of the different glucokinase mRNA templates. For instance, ornithine decarboxylase mRNA, which is translated poorly in vitro, is characterized by a long 5'-noncoding sequence that has four AUG codons upstream of the long open reading frame (30,31). It is possible therefore that the different 5'-noncoding sequences in the β -cell glucokinase mRNA result in a template that is translated less efficiently than the hepatic glucokinase mRNA. This possibility remains to be tested.

FUTURE DIRECTIONS

Many questions about the function and regulation of glucokinase in the liver and β -cell remain to be answered. The *cis*-acting elements and *trans*-acting factors involved in the expression of β -cell glucokinase need to be identified, characterized, and compared with those necessary for expression of the insulin genes. Likewise, the hepatic glucokinase promoter should be studied in detail to learn what elements and factors mediate the regulatory effects of insulin and cAMP. In addition, further characterization of the different glucokinase isoforms is important for understanding the function of this enzyme. Thus, studies that characterize the tissue-specific expression and regulation of the glucokinase gene and the function of glucokinase itself should provide a deeper understanding of the role of this enzyme in maintaining glucose homeostasis.

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