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# Glucose sensing in pancreatic islet beta cells: The key role of glucokinase and the glycolytic intermediates

(glucose sensor/insulin gene/phospho*enol*pyruvate carboxykinase/glucose transporter/hexokinase)

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**ABSTRACT** **The beta cells of the pancreatic islets of Langerhans respond to changes in glucose concentration by varying the rate of insulin synthesis and secretion. Beta cells sense glucose concentration by the levels of the products of glucose catabolism. Distinctive beta-cell proteins glucose transporter 2 and glucokinase catalyze the first two steps in beta-cell glucose catabolism. To test whether either protein controls the sensitivity of the beta cell to glucose by controlling the rate of glucose catabolism, we used gene-transfer techniques to express the isoenzymes glucose transporter 1 and hexokinase I in beta cells and measured the response to glucose of the insulin gene promoter. Cells expressing glucose transporter 1 do not differ significantly from control cells, but in cells expressing hexokinase I, insulin promoter activity increases, reaches a maximum by 1 mM glucose, and does not respond to changes in glucose concentration within the physiologic range. We conclude that glucokinase catalyzes the rate-limiting step of glucose catabolism in beta cells and, therefore, acts as the glucose sensor. Pyruvate, the end product of anaerobic glycolysis, is readily oxidized by mitochondria in normal beta cells but cannot substitute for glucose as a stimulator of insulin synthesis and secretion. We found that pyruvate can stimulate the insulin promoter in cells expressing the bacterial gluconeogenic enzyme phospho*enol*pyruvate carboxykinase, which allows the conversion of pyruvate to phospho*enol*pyruvate and the earlier intermediates of glycolysis. We conclude that the intermediates of anaerobic glycolysis between fructose 1,6-diphosphate and phospho*enol*pyruvate are essential for beta-cell glucose sensing.**

In mammals, the beta cells of the pancreatic islets of Langerhans sense changes in the nutritional state of the organism and respond by modulating synthesis and secretion of insulin, the signal for energy storage. Glucose and its metabolite glyceraldehyde 3-phosphate are the most potent nutritional secretagogues of insulin (1, 2). Because glucose analogs that cannot be metabolized by beta cells do not stimulate insulin secretion, whereas glyceraldehyde and other energy sources do stimulate insulin secretion (3, 4), it has been concluded that some final common end product(s) of catabolism (such as ATP, pyridine nucleotides, or acyl CoA derivatives) may be required to stimulate secretion. These high-energy end products alone may not be sufficient, however, because pyruvate is a very poor secretagogue despite evidence that beta cells can efficiently use pyruvate for energy (5). Beta cells lack the gluconeogenic enzyme phospho*enol*pyruvate carboxykinase (PEPCK) and, therefore, cannot produce any of the earlier glycolytic intermediates of glucose catabolism from pyruvate (6, 7) (see Fig. 5), which has led to the conclusion that the intermediates of anaerobic glycolysis between glyceraldehyde 3-phosphate and pyruvate may be involved in glucose sensing (for a recent review, see ref. 8).

If beta cells sense glucose concentration through its catabolic products, then the levels of these products and, therefore, the rate of glucose catabolism, must vary with glucose concentration within the physiologic range. In this respect beta cells differ from many other tissues, such as brain, that reach a maximum limit for glucose catabolism below the physiologic concentration of glucose and do not increase energy production when glucose concentration rises. This ability to vary the rate of glucose catabolism has been proposed to be due to one or both proteins that control the first two steps of glucose catabolism in beta cells: glucose transporter 2 (GT-2) (9), which transports glucose into beta cells, or glucokinase (10), which phosphorylates glucose to glucose 6-phosphate. Both of these proteins are expressed in a restricted set of cell types, and both have a $K_m$ above the physiologic concentration of glucose, which would allow them to increase utilization of glucose as its concentration rises. For either of these proteins to control the rate of glucose catabolism and the level of the products, it must control the rate-limiting step in glucose catabolism.

Like insulin secretion, insulin synthesis is also regulated by glucose (11). We have shown that the rat insulin I gene 5′ flanking DNA (rat insulin I promoter) can direct glucose-regulated transcription of the linked reporter gene chloramphenicol acetyltransferase (CAT) in beta cells (12). In this paper we used the rat insulin I promoter to study glucose sensing in beta cells, measuring changes in CAT activity as a gauge of the ability of the $\beta$ cell to sense changes in glucose concentration. By expressing glucose transporter 1 (GT-1) and hexokinase I, the low $K_m$ analogs of GT-2 and glucokinase, respectively, in islets, we directly tested whether either protein controls the rate of glucose catabolism within the physiologic range. In addition, we investigated the role of the glycolytic intermediates in glucose sensing by expressing the *Escherichia coli* equivalent of PEPCK [product of the *pckA* gene (13)] in beta cells. Some of these data have been reported in an abstract (14).

## METHODS

**Plasmid Construction.** Construction of Ins-CAT (15) and the cytomegalovirus-promoter-driven expression plasmid pBAT7 (16) has been described. To produce optimal expression levels, we replaced the 5′ untranslated portions of GT-1, hexokinase I, and PEPCK with the $\beta$-globin consensus translation signal and ATG found in pBAT7. The human GT-1 and hexokinase I cDNA clones were provided by Graeme Bell (Howard Hughes Medical Institute, University of Chicago); the *E. coli pckA* DNA was provided by Hughes Goldie (University of Saskatchewan, Canada).

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Abbreviations: GT-1 and GT-2, glucose transporter 1 and 2, respectively; PEPCK, phospho*enol*pyruvate carboxykinase; CAT, chloramphenicol acetyltransferase.

**Islet Transfection.** Twenty-one-day gestation fetal Sprague–Dawley rat islets were isolated and transfected as described (12). Each 1-cm plate of cultured islet cells was transfected with 25 μg of double cesium chloride-purified Ins-CAT plasmid DNA and 10 μg of the cotransfected pBAT7 expression plasmid shown. The transfected cells were grown in RPMI 1640 medium with the nutrient additives shown for ≈36 hr before harvesting and protein extraction. CAT enzyme assays were done with 10 μg of protein for 2 hr (17).

## RESULTS

We transfected dispersed fetal rat islets with the plasmid Ins-CAT, which contains the bacterial CAT gene driven by the 410-bp rat insulin I promoter. After 3 hr growth in 16 mM glucose, these transfected islets had 8-fold higher CAT activity than cells grown in 2 mM glucose (Fig. 1), confirming the effect of glucose on insulin promoter function (12). Addition of 16 mM leucine to the low glucose medium causes a 2.1-fold increase in insulin promoter activity as judged by CAT activity (Fig. 1). Leucine is a ketogenic amino acid: it can be utilized to produce ATP via the tricarboxylic acid cycle, but it cannot normally contribute to glucose production (gluconeogenesis). α-Ketoisocaproic acid (the first intermediate in leucine metabolism) or higher concentrations of leucine give similar stimulation (data not shown). The ratio of response to glucose/response to leucine is roughly equal for all insulin promoter/enhancer constructs we have tested (data not shown), suggesting that both nutrients stimulate transcription through the same cis-acting DNA elements.

Transfected islets grown in low glucose plus 14 mM 2-deoxyglucose lose 97% of CAT activity, whereas 2-deoxyglucose decreases CAT activity by only 20% in islets grown in high glucose (Fig. 1). 2-Deoxyglucose is phosphorylated to 2-deoxyglucose 6-phosphate but is not metabolized further. As a result, it accumulates in cells and inhibits hexokinase but does not inhibit glucokinase (see Fig. 5).

To express GT-1 in the transfected beta cells, we constructed a plasmid with the human GT-1 cDNA downstream from the cytomegalovirus promoter, such that expression of GT-1 in cells transfected with plasmid pBAT7GT-1 is driven by this potent viral promoter. Similar cytomegalovirus expression plasmids were constructed with the human hexokinase I

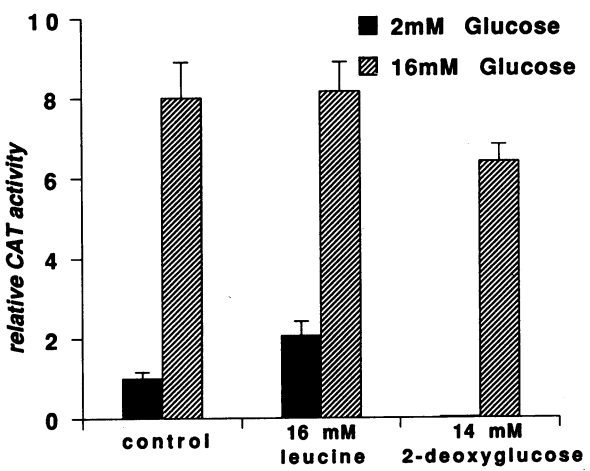


FIG. 1. Effects of leucine and 2-deoxyglucose on insulin promoter activity. Cultured fetal rat islets were transfected with the Ins-CAT plasmid and grown in 2 mM (solid bars) or 16 mM (hatched bars) glucose with the additions shown. CAT enzyme activity was assayed with equal amounts of protein 36 hr after transfection. Each data point represents the mean ± SEM of three independent transfections. CAT activity in control samples grown at 2 mM glucose was arbitrarily set at 1.0. The bar for CAT activity in cells grown in 2 mM glucose and 14 mM 2-deoxyglucose is difficult to see because of the very low level of activity, 0.027 ± 0.006.

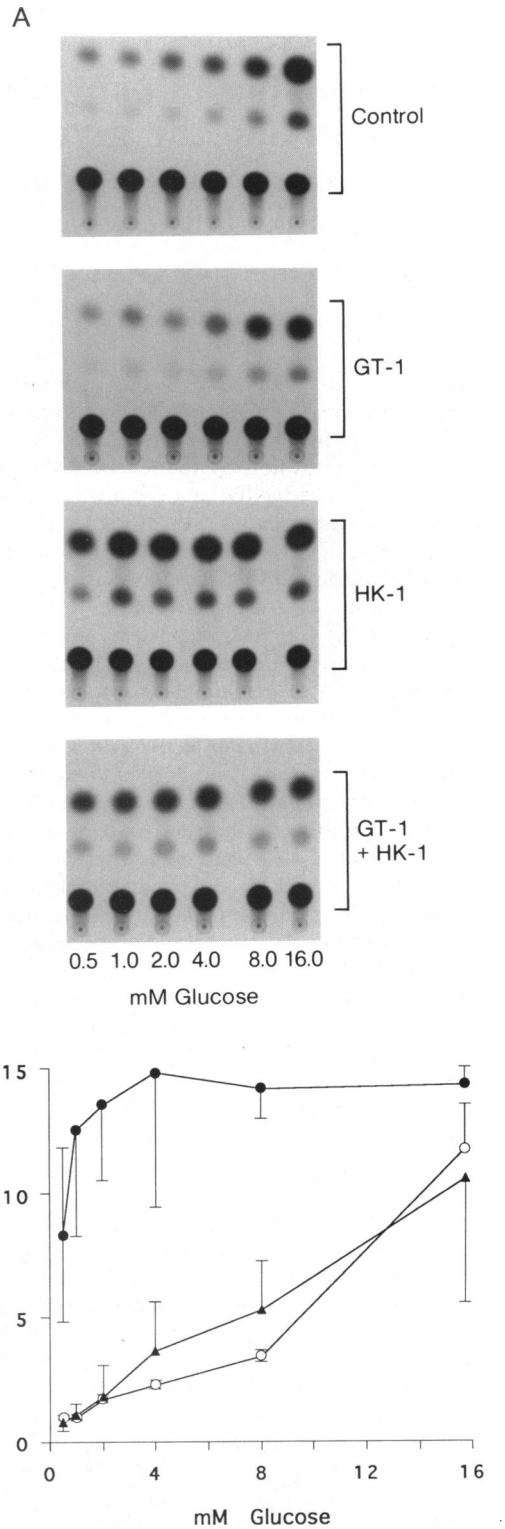


FIG. 2. Effects of hexokinase I and GT-1 expression on glucose sensitivity. Cultured fetal rat islets were transfected with both the Ins-CAT plasmid and either a plasmid expressing hexokinase I (HK-1) or GT-1 or a control expression plasmid without an insert. CAT enzyme activity was assayed after 36-hr growth in the glucose concentrations shown. (*A*) CAT enzyme assay for a representative experiment. The lower spot in each lane is [$^{14}$C]chloramphenicol; the two upper spots are the two monoacetylated forms of [$^{14}$C]chloramphenicol. (*B*) Each data point represents the mean ± SEM of three independent transfections. ●, Cells expressing hexokinase I; ▲, cells expressing GT-1; ○, control cells transfected with control expression plasmid without insert (pBAT7). CAT activity in control samples grown at 0.5 mM glucose was arbitrarily set at 1.0.

cDNA (pBAT7HK-1) and the *E. coli pckA* DNA (pBAT7PCK).

Beta cells transfected with pBAT7HK-1 lose the ability to respond to glucose concentration changes within the physiologic range. At 1 mM glucose these cells have already reached maximal CAT activity, and further increases in glucose concentration do not significantly increase CAT activity (Fig. 2). Hexokinase I-expressing cells also demonstrate a marked change in fructose sensitivity (Fig. 3), reflecting the differences in substrate specificity between hexokinase and glucokinase. Glucokinase is highly specific for glucose, whereas hexokinase efficiently phosphorylates most hexoses, although it does show some preference for aldohexoses over ketohexoses.

Unlike the cells expressing hexokinase I, the cells expressing GT-1 do not lose their ability to sense changes in glucose concentration within the physiologic range (Fig. 2). The curve for glucose response in these cells is essentially unchanged from the control curve.

When the transfected beta cells are grown in pyruvate in place of glucose, insulin promoter-driven transcription is markedly reduced; 30 mM pyruvate stimulates only a 1.7-fold increase in CAT activity (Fig. 4). In beta cells expressing bacterial PEPCK, however, pyruvate stimulates a 4.6-fold increase in CAT activity. Those beta cells expressing PEPCK but grown in the absence of pyruvate showed a decrease in CAT activity compared with control cells not expressing PEPCK; this possibly results from the negative effects of the futile cycle created by high level expression of PEPCK (Fig. 5), which requires 1 ATP for each turn of the cycle from pyruvate to phospho*enol*pyruvate to pyruvate. The inability of pyruvate to stimulate insulin promoter activity in beta cells expressing PEPCK and grown in 16 mM glucose (Fig. 4) shows that the signals from PEPCK and glucose are not additive, probably because the signaling intermediates are already at maximum levels in 16 mM glucose. This result reinforces the conclusion that the common products of anaerobic glycolysis and PEPCK—the intermediates between fructose 1,6-diphosphate and phospho*enol*pyruvate and their metabolites—contribute to the stimulation of insulin gene transcription.

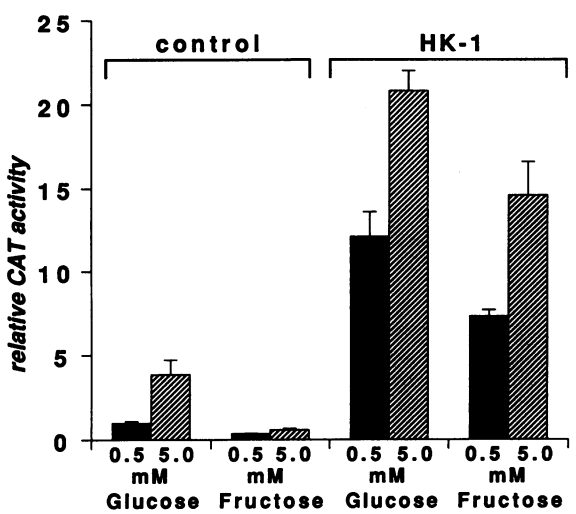


FIG. 3. Sensitivity to fructose in cells expressing hexokinase I (HK-1). Cultured fetal rat islets were transfected with both the Ins-CAT plasmid and either a plasmid expressing hexokinase I or a control expression plasmid without an insert. After 36-hr growth in 0.5 mM (solid bars) or 5.0 mM (hatched bars) concentrations of the hexose, CAT enzyme activity was assayed with equal amounts of protein. Each data point represents the mean ± SEM of three independent transfections. CAT activity in control samples grown at 0.5 mM glucose was arbitrarily set at 1.0.

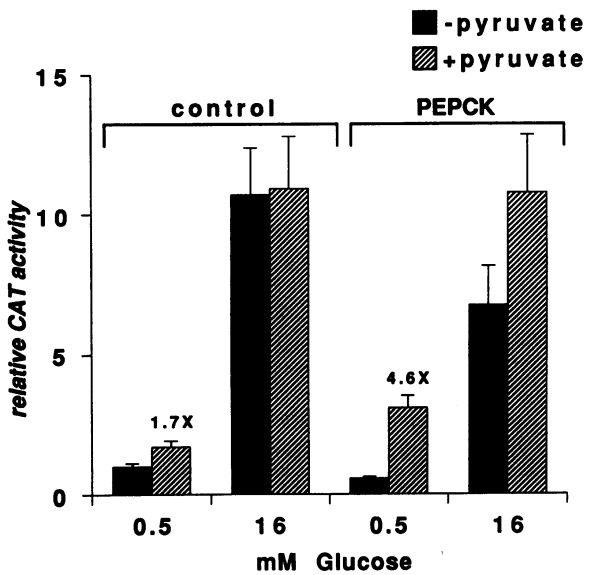


FIG. 4. Sensitivity to pyruvate in cells expressing PEPCK. Cultured fetal rat islets were transfected with both the Ins-CAT plasmid and either a plasmid expressing bacterial PEPCK or a control expression plasmid without an insert. After 36-hr growth in medium with the glucose concentrations shown and either with (hatched bars) or without (solid bars) 30 mM pyruvate, CAT enzyme activity was assayed with equal amounts of protein. Each data point represents the mean ± SEM of seven independent transfections. CAT activity in control samples grown at 0.5 mM glucose without pyruvate was arbitrarily set at 1.0.

## DISCUSSION

**Glucokinase.** The recent cloning and characterization of distinct facultative glucose transporter isoforms led to the proposal that the predominant transporter in beta cells, GT-2, may form a critical part of the "glucose sensor," the glucose-

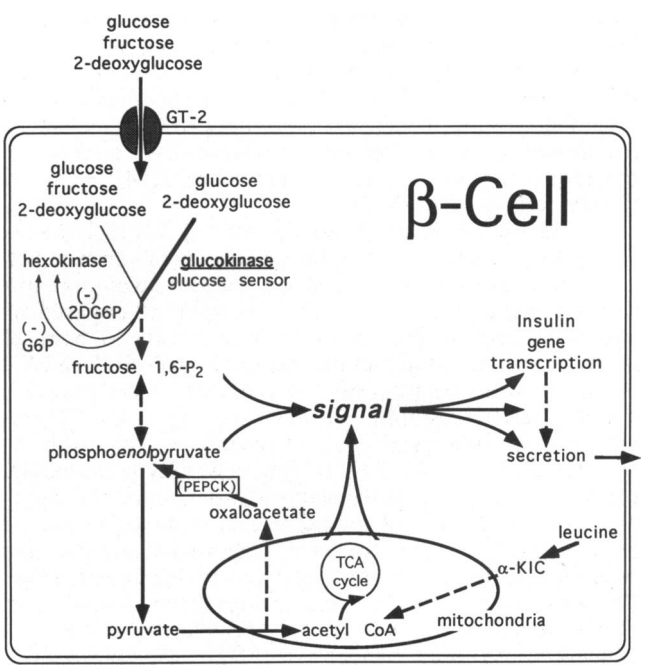


FIG. 5. Metabolic signal production in the beta cell. A proposed schematic outline of beta-cell glucose metabolism as it relates to production of signals for insulin synthesis and secretion is shown. Dashed lines represent pathways with intermediate steps. PEPCK is boxed to indicate that it is not normally present in beta cells. Glut2, GT-2; G6P, glucose 6-phosphate; 2DG6P, 2-deoxyglucose 6-phosphate; α-KIC, α-ketoisocaproic acid; TCA, tricarboxylic acid cycle.

sensing mechanism in beta cells (9, 18, 19). To control glucose sensing in beta cells, however, GT-2 must regulate the rate of glucose catabolism and, therefore, must control the rate-limiting step of glucose catabolism. Estimates of the rate of glucose transport in beta cells suggest that it is at least 100-fold higher than the rate of glucose metabolism (20, 21), leading to the alternate conclusion that glucokinase is the glucose sensor (10, 22).

The GT-1 and hexokinase I expression experiments directly test these opposing hypotheses. The ability of overexpressed hexokinase I to markedly increase the response to low glucose concentrations and to fructose demonstrates that glucokinase is responsible both for the magnitude and the hexose specificity of the glucose response in our transfected beta-cell model system. Therefore, glucokinase is the glucose sensor.

We also performed the inverse experiment, the removal of functional hexokinase by treatment with 2-deoxyglucose. The effect of 2-deoxyglucose at low glucose concentration reflects a large contribution of hexokinase because hexokinase, but not glucokinase, is inhibited by 2-deoxyglucose 6-phosphate; the latter rises to high levels because it cannot be further metabolized by the cells. At high glucose concentrations, however, the addition of 2-deoxyglucose has much less effect, showing that the contribution of hexokinase to glucose phosphorylation is minimal at high glucose concentrations in beta cells (4). The net effect of suppression of hexokinase activity with 2-deoxyglucose is an increase in sensitivity to glucose, as measured by the ratio of CAT activity at 2 and 16 mM glucose.

These results demonstrate that even the small decrease in glucose phosphorylation caused by removal of hexokinase activity decreases the total transcriptional (and presumably secretory as well) response to high glucose concentrations; such results provide further evidence of the dominant role of glucose phosphorylation in regulating the rate of glucose catabolism and, thereby, glucose sensing in beta cells. In a similar fashion, any defect that reduces the level of functional glucokinase in beta cells, even modestly, could be expected to increase the glucose concentration required to stimulate insulin synthesis and secretion, thereby increasing fasting blood glucose. This prediction is born out by the recent demonstration that many individuals with the autosomal dominant disorder maturity onset diabetes of the young (MODY), clinically manifested by an elevated fasting glucose, have an inherited mutation in the glucokinase-encoding gene (23, 24). No similar linkage was found to the GT-2 gene (23).

Our data confirm previous evidence (20, 21) that glucose transport is not rate limiting for glucose metabolism in beta cells and do not support the concept that GT-2 plays a central role in glucose sensing. Glucose transport is required for glucose catabolism and, therefore, is permissive for beta-cell glucose sensing—as are all the steps of glycolysis. The GT-2 isoform, however, may not be required: islets grown in culture rapidly shift from GT-2 to GT-1 expression (25) but still respond appropriately to glucose. The explanation for specific expression of GT-2 in beta cells may lie in its high capacity for glucose transport both into and out of cells or, possibly, in features of its expression or function not yet recognized. Some authors have suggested that GT-2 may bind glucokinase and form a glucose-sensing complex (18). The advantage of such a compartmentalized system is not obvious because the intracellular and extracellular glucose concentrations rapidly equalize in beta cells. Furthermore, by immunocytochemistry (26) and cell fractionation (27), glucokinase is localized to the beta-cell cytoplasm, not the cell membrane.

**Glycolysis.** The production of phospho*enol*pyruvate by PEPCK should result in an increase in all the intermediates of anaerobic glycolysis between fructose 1,6-diphosphate and phospho*enol*pyruvate because these intermediate conversion steps are readily reversible and are not energy requiring (Fig. 5). Further conversion of fructose 1,6-diphosphate to the hexose monophosphates requires the gluconeogenic enzyme fructose bisphosphatase, which islets lack. Therefore, the increased ability of pyruvate to stimulate the insulin promoter in the presence of PEPCK probably reflects the role of these middle intermediates of anaerobic glycolysis or their metabolites in glucose signaling.

The intermediates of anaerobic glycolysis may stimulate insulin synthesis and secretion indirectly by activating mitochondrial oxidation or anaplerosis or directly by forming intracellular signaling messengers. Glucose and glyceraldehyde and, therefore, presumably the anaerobic intermediates, stimulate the glycerol phosphate shuttle (28). This cycle shifts reducing equivalents into the mitochondria and may play a role in the glucose-induced stimulation of mitochondrial oxidation of pyruvate through stimulation of pyruvate dehydrogenase (29) and α-ketoglutarate dehydrogenase (30). An additional, possibly complimentary, mechanism of glycolytic intermediate signaling involves the production of acyl CoA derivatives—in particular, the putative messengers fatty acyl CoA and diacylglycerol (31, 32).

In conclusion, these data further reinforce the central role of glucose in beta cell metabolic sensing. We have shown that the intermediates of anaerobic glycolysis are essential for glucose sensing and that the glucose-specific enzyme glucokinase catalyzes the rate-limiting step of glucose catabolism and, therefore, controls sensitivity of the beta cell to glucose.

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1. Hellman, B., Idahl, L. A., Lernmark, A., Sehlin, H. & Taljedal, I. B. (1974) *Arch. Biochem. Biophys.* **162**, 448–457.
2. MacDonald, M. & Fahein, L. (1988) *Diabetes* **37**, 997–999.
3. Meglasson, M. D. & Matschinsky, F. M. (1986) *Diabetes Metab. Rev.* **2**, 163–214.
4. Giroix, M.-H., Sener, A., Pipeleers, D. G. & Malaisse, W. J. (1984) *Biochem. J.* **223**, 447–453.
5. Sener, A., Kawazu, S., Hutton, J. C., Boschero, A. C., Devis, G., Somers, G., Herchuelz, A. & Malaisse, W. J. (1978) *Biochem. J.* **176**, 217–232.
6. MacDonald, M. J. & Chang, C.-M. (1985) *Diabetes* **34**, 246–250.
7. MacDonald, M. J., McKenzie, D. I., Walker, T. M. & Kaysen, J. H. (1992) *Horm. Metab. Res.* **24**, 158–160.
8. MacDonald, M. (1990) *Diabetes* **39**, 1461–1466.
9. Thorens, B., Sarkar, H., Kaback, H. R. & Lodish, H. F. (1988) *Cell* **55**, 281–290.
10. Matschinsky, F. M. (1990) *Diabetes* **39**, 647–652.
11. Steiner, D. F., Chan, S. J., Welsh, J. M. & Kwok, S. C. M. (1985) *Annu. Rev. Genet.* **19**, 463–484.
12. German, M. S., Moss, L. G. & Rutter, W. J. (1990) *J. Biol. Chem.* **265**, 22063–22066.
13. Medina, V., Pontarollo, R., Glaeske, D., Tabel, H. & Goldie, H. (1990) *J. Bacteriol.* **172**, 7151–7156.
14. German, M. S. & Rutter, W. J. (1991) *Diabetes* **40**, Suppl. 1, 163A (abstr.).
15. Karlsson, O., Edlund, T., Moss, J. B., Rutter, W. J. & Walker, M. D. (1987) *Proc. Natl. Acad. Sci. USA* **84**, 8819–8823.
16. German, M. S., Wang, J., Chadwick, R. B. & Rutter, W. J. (1993) *Genes Dev.*, in press.
17. Edlund, T., Walker, M. D., Barr, P. J. & Rutter, W. J. (1985) *Science* **230**, 912–916.
18. Johnson, J. H., Newgard, C. B., Milburn, J. L., Lodish, H. F. & Thorens, B. (1990) *J. Biol. Chem.* **265**, 6548–6551.
19. Unger, R. H. (1991) *Science* **251**, 1200–1205.
20. Matschinsky, F. M. & Ellerman, J. E. (1968) *J. Biol. Chem.* **243**, 2730–2736.

21. Sehlin, J. (1981) in *The Islets of Langerhans*, eds. Cooperstein, S. J. & Watkins, D. (Academic, New York), pp. 53–74.
22. Meglasson, M. D. & Matschinsky, F. M. (1984) *Am. J. Physiol.* **246**, E1–13.
23. Froguel, P., Vaxillaire, M., Sun, F., Velho, G., Zouali, H., Butei, M. O., Lesage, S., Vionnet, N., Clement, K., Fougerousse, F., Tanizawa, Y., Weissenbach, J., Beckmann, J. S., Lanthrop, G. M., Passa, P., Permutt, M. A. & Cohen, D. (1992) *Nature (London)* **356**, 162–164.
24. Vionnet, N., Stoffel, M., Takeda, J., Yasuda, K., Bell, G. I., Zouali, H., Lesage, S., Velho, G., Iris, F., Passa, P., Froguel, P. & Cohen, D. (1992) *Nature (London)* **356**, 721–722.
25. Tal, M., Thorens, B., Surana, M., Fleischer, N., Lodish, H., Hanahan, D. & Efrat, S. (1992) *Mol. Cell. Biol.* **12**, 422–432.
26. Jetton, T. L. & Magnuson, M. A. (1992) *Proc. Natl. Acad. Sci. USA* **89**, 2619–2623.
27. Vischer, U., Blondel, B., Wollheim, C. B., Hoppner, W., Seitz, H. J. & Iynedjian, P. B. (1987) *Biochem. J.* **241**, 249–255.
28. Sener, A. & Malaisse, W. J. (1992) *J. Biol. Chem.* **267**, 13251–13256.
29. McCormack, J. G., Longo, E. A. & Corkey, B. E. (1990) *Biochem. J.* **267**, 527–530.
30. Sener, A., Rasschaert, J. & Malaisse, W. J. (1990) *Biochim. Biophys. Acta* **1019**, 42–50.
31. Prentki, M., Vischer, S., Glennon, M. C., Regazzi, R., Deeney, J. T. & Corkey, B. E. (1992) *J. Biol. Chem.* **9**, 5802–5810.
32. Liang, Y. & Matschinsky, F. M. (1991) *Diabetes* **40**, 327–333.