Adenovirus-mediated Knockout of a Conditional Glucokinase Gene in Isolated Pancreatic Islets Reveals an Essential Role for Proximal Metabolic Coupling Events in Glucose-stimulated Insulin Secretion*

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The relationship between glucokinase (GK) and glucose-stimulated metabolism, and the potential for metabolic coupling between β cells, was examined in isolated mouse islets by using a recombinant adenovirus that expresses Cre recombinase (AdenoCre) to inactivate a conditional GK gene allele (gklox). Analysis of AdenoCre-treated islets indicated that the gklox allele in ~30% of islet cells was converted to a nonexpressing variant (gkdel). This resulted in a heterogeneous population of β cells where GK was absent in some cells. Quantitative two-photon excitation imaging of NAD(P)H autofluorescence was then used to measure glucose-stimulated metabolic responses of individual islet β cells from gklox/gklox mice. In AdenoCre-infected islets, approximately one-third of the β cells showed markedly lower NAD(P)H responses. These cells also exhibited glucose dose responses consistent with the loss of GK. Glucose dose responses of the low-responding cells were not sigmoidal and reached a maximum at ~5 mM glucose. In contrast, the normal response cells showed a sigmoidal response with an $K_{catS0.5}$ of ~8 mM. These data provide direct evidence that GK is essential for glucose-stimulated metabolic responses in β cells within intact islets and that intercellular coupling within the islet plays little or no role in glucose-stimulated metabolic responses.

Glucose-stimulated insulin secretion by pancreatic β cells is a multistep process that depends on increased metabolic flux (1). The rate-determining step in β cell glucose metabolism is widely thought to be glucose phosphorylation, which is catalyzed to a large extent by glucokinase (GK) at physiological glucose concentrations (2). Although many studies have implied an essential role for GK in glucose metabolism, most of this information has been correlative and has not precisely defined the role of this particular hexokinase isoform in β cell glucose usage. GK gene knockout studies in transgenic mice, which offer the most direct and minimally ambiguous route to assessing the function of this enzyme in β cells, have been performed by several different groups. Both global gene knockout studies, and more recent β cell-specific gene knockouts indicate that GK is indispensable for glucose-stimulated insulin secretion (3–6). While these studies clearly demonstrate an essential role for GK in glucose homeostasis, they have been unable to determine the precise role of GK, since studies in β cells from adult animals are prevented by the perinatal mortality that occurs in GK-null mice.

A second, less well studied feature of glucose-stimulated insulin secretion from β cells is its dependence on cell-cell interactions. The amount of insulin secreted from intact islets has long been known to be greater than that secreted from an equivalent number of dispersed β cells (7). Models to explain this behavior generally include cooperative phenomena between islet cells, as suggested by the presence of synchronous electrical responses from clustered β cells and intact islets (8, 9). The pharmacological blocking of gap junctions reduces islet insulin secretion, thereby suggesting that connexins are involved in the intercellular cooperation (10–12). Based on measurements of NAD(P)H in both intact islets, we previously proposed that metabolic uniformity arises from a uniform GK distribution and is not dependent on intercellular coupling (13). The concept of uniform cell-to-cell GK distributions is also supported by a recent immunofluorescence study of intracellular expression patterns in intact islets (14). Given that the role of cell-cell communication within intact islets during glucose-stimulated metabolic flux has never been directly examined, and that glucose-stimulated metabolism in islets does not exhibit the NAD(P)H response heterogeneities observed in isolated β cells (15), it remains possible that intercellular coupling may play a role in generating the metabolic uniformity.

To elucidate the roles of GK and intercellular communication in glucose metabolism within intact pancreatic islets, we utilized three recently developed technologies. First, the Cre/loxP strategy for inducible gene knockouts enables the elimination of specific genes in vitro in tissues from mature animals (16). To use this strategy, mice have been created with a conditional GK gene allele (gklox) in which exons 9 and 10 of the GK gene are flanked by loxP sites, thereby allowing for its inactivation by Cre recombinase (6). Second, specific inactivation of single genes in isolated islets has been enhanced by the development of a recombinant adenovirus expressing Cre (AdenoCre) (17). Adenoviruses have been shown previously to be an efficient means of introducing genes into β cells in isolated islets (18). Third, we used two-photon excitation microscopy (TPEM) to study glucose-stimulated processes within intact islets (19, 20). This quantitative optical sectioning technique has been demonstrated to be useful for assessing glucose-stimulated metabolic responses in intact islets and allows simultaneous determination of the glucose dose response in many individual β cells (13).

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The abbreviations used are: GK, glucokinase; AdenoCre, recombinant adenovirus that expresses Cre recombinase; TPEM, two-photon excitation microscopy; PCR, polymerase chain reaction.
The combination of these three recent methodological advances enabled us to directly examine the role of GK in glucose-stimulated metabolism in single β cells within intact pancreatic islets. By exposing cultured islets isolated from mice that are homozygous for the conditional gklox allele to AdenoCre, we have been able to eliminate GK in a sizable fraction of β cells and to examine interactions between cells with normal GK levels and those that are not expressing GK. These data provide direct evidence that GK is an important component of the β cell glucose sensor. However, even cells that are presumed to fully lack GK retain an attenuated glucose response, thus suggesting that the glucose sensor is multicomponent and likely involves hexokinases other than GK. In addition, these data provide compelling evidence that individual β cells function as independent glucose-sensing units at the metabolic level.

**EXPERIMENTAL PROCEDURES**

**Animals**—Mice that contain a conditional GK gene allele (gklox+) have been described previously (6). All animals used in this study were homozygous at this allele (gkloxloxdel) in a mixed C57Bl/6-129SvEvTac genetic background. This conditional allele is identical to the wild-type allele, but contains loxP sites flanking exons 9 and 10 of the gene. The neomycin resistance cassette used to perform the gene targeting is absent in the gklox allele. PCR primer pairs for distinguishing the gklox and gkdel alleles have been described previously (6).

**Preparation of AdenoCre**—293 cells (21) were cultured in Dulbecco’s modified Eagle’s medium containing 10% fetal calf serum. AdenoCre (obtained from F. Graham, McMaster University, Ontario, Canada) expresses Cre under the control of the cytomegalovirus immediate early promoter (17). This virus was grown and purified according to Becker et al. (22). High titer stocks of adenovirus (3–7 × 10^12 plaque-forming units/ml, determined as 1 A_{260} = 10^{12} particles/ml) were prepared by equilibrium centrifugation in CsCl, stored in small aliquots at −80 °C, and used immediately after thawing.

**Isolation and Culture**—Islets were isolated from gkloxlox mice by distention of the splenic portion of the pancreas followed by collagenase digestion (23, 24). Islets were cultured at 37 °C in RPMI 1640 medium containing 20 mM glucose, 10% fetal bovine serum, 100 IU/ml penicillin, and 100 µg/ml streptomycin (Life Technologies, Inc.) with 5% CO₂ atmosphere. For each experiment islets were divided into two groups. The first group was incubated in 2 ml of culture medium containing recombinant adenovirus at a concentration of 1.5 × 10^8 plaque-forming units/ml for 1 h at 37 °C prior to culturing (18). Higher concentrations of adenovirus were also tried, but did not result in greater infection efficiency. The second group was not exposed to adenovirus. After infection islets were washed three times in culture medium. Both groups of islets were maintained in culture for 4 days before imaging. For the AdenoCre treated islets, culture in 20 mM glucose maintained their viability and morphological integrity better than those cultured at lower glucose levels.

**Analysis of GK Protein Levels**—Western blot analysis performed on preparations from isolated islets and GK activity was measured in isolated islets as described previously (6). Immunofluorescence staining of GK was also performed on isolated islets as described previously (13), except that an affinity-purified sheep anti-GK-IgG was used at a 1:10 dilution, and preimmune sheep serum was used as the control. The resulting autoradiogram was quantified by densitometry.

**Measurement of NAD(P)H Autofluorescence**—NAD(P)H imaging was performed by TPEM as described previously (13). For imaging, two islets (one from each group) were adjacent attached to a coverslip bottom dish (Mat-Tek Corp.). A 0.5-µl drop of Cell-Tak (Collaborative Biomedical Products) was placed in the center of the dish and dried for 30 s at 42 °C; the dish was rinsed with Hanks’ balanced salt solution (Life Technologies, Inc.) and the islets placed on the Cell-Tak. During imaging, the islets were perfused at 1 ml/min with BMHH buffer (125 mM NaCl, 5.7 mM KCl, 2.5 mM CaCl₂, 1.2 mM MgCl₂, and 10 mM HEPES, and 0.1% bovine serum albumin [pH 7.4]). The sample was held at 37 °C with a microincubator (TLC-MI, Adams & List Associates, Westbury, NY) that heated both the sample dish and incoming perfusate. An air stream incubator (Nicholson Precision Instruments, Gaithersburg, MD) heated the objective to eliminate heat transfer through the glass-oil-objective interface. All NAD(P)H autofluorescence measurements were made after a 15-min equilibration period on the microscope stage at 1 µm (basal) glucose. NAD(P)H glucose dose response images were acquired after a 5-min equilibration at each glucose concentration. Three consecutive 3-s scans were averaged to form the image for each concentration.

**Image Analysis and Quantitation**—To quantify the glucose response of NAD(P)H autofluorescence, digital image analysis was performed on Macintosh Power PC computers running NIH Image 1.61 (Bethesda, MD). Single cell data were taken in 25-pixel circular regions of interest that did not include the cell nucleus; the same regions of interest was used for all measurements (in images acquired with different glucose perfusion concentrations) on that cell. Only cells that remained in the same location within the image and maintained its same total area (to within 3%) were used in the analysis. This excluded cells that might have shifted into or out of the focal plane during the experimental procedures.

**RESULTS**

Both the ability and efficiency of AdenoCre to cause recombination in islets from mice that were homozygous for the gklox allele was determined. PCR analysis of AdenoCre-treated islets revealed the conversion of the gklox allele to the gkdel allele only.
after virus treatment (Fig. 1A). To assess the efficiency of AdenoCre-mediated recombination, DNA was isolated from ~500 each of AdenoCre-treated and control islets and analyzed by Southern blot analysis. Densitometry of the resulting autoradiograms revealed that AdenoCre had converted ~30% (shown in Fig. 1B) of the $g^k_{\text{lox/lox}}$ alleles to the $g^k_{\text{del}}$ allele (lane 2). Similar analysis of DNA from untreated $g^k_{\text{lox/lox}}$ islets did not indicate any $g^k_{\text{del}}$ allele (lane 1).

While the appearance of the $g^k_{\text{del}}$ allele predicted a partial loss of GK expression in AdenoCre-treated islets, the actual reduction of GK levels was assessed by Western blot, GK activity measurements, and GK immunofluorescence. Western blot analysis yielded a 29.7 ± 4.4% reduction in GK levels from AdenoCre-treated islets ($n = 3$; shown in Fig. 1C), and activity measurements ($n = 3$, data not shown) also showed reduced GK activity in AdenoCre-treated islets. Because adenovirus treatment disrupts islet integrity, GK immunofluorescence could not be accurately determined since none of the islets which were imaged for NAD(P)H autofluorescence responses survived the fixation and staining procedures ($n = 28$ islets). However, four GK immunostained islets each showed reduced GK levels and exhibited heterogeneous GK immunofluorescence, similar to the NAD(P)H results presented below (data not shown).

To determine whether AdenoCre-mediated elimination of GK caused differences in glucose-induced NAD(P)H responses, control and treated $g^k_{\text{lox/lox}}$ islets were examined side-by-side using TPEM. Representative NAD(P)H autofluorescence images of control and treated islet pairs are shown in Fig. 2. Control islets showed a very uniform autofluorescence signal during the 1 mM glucose perifusion, which was enhanced by perifusion with the higher glucose. In contrast, AdenoCre-treated islets exhibited significant heterogeneity of NAD(P)H response. Under low glucose perifusion, the latter group of islets showed low but fairly uniform autofluorescence patterns, but in response to high glucose, many cells failed to show the expected rise in NAD(P)H.

To assess quantitatively the metabolic response of individual β cells within whole islets, NAD(P)H response ratios (defined as NAD(P)H signal at 25 mM glucose perifusion)/(NAD(P)H signal at 1 mM glucose perifusion) were determined for 220 nonperipheral β cells from 5 AdenoCre-infected islets. A histogram of the resulting ratios (Fig. 3) shows two separate distributions of cellular response. Approximately two-thirds of the analyzed β cells showed a response similar to that observed in wild-type mice. The other approximately one-third of the cells formed a population with a lower NAD(P)H response. The solid line in Fig. 3 indicates the response ratios of >1000 β cells in islets isolated from wild-type mice. The right-hand peak of the histogram shows that β cells from $g^k_{\text{lox/lox}}$ islets are ~5% less responsive than cells from wild-type mice. The one-third AdenoCre infection rate is consistent with the 35% of islet cells that were infected with another adenovirus, which expresses β-galactosidase.

Measurements of NAD(P)H levels over a range of different glucose concentrations were performed to further define the metabolic responses of single β cells in AdenoCre-treated islets. Two resulting glucose dose response curves are shown in Fig. 4. The first curve (solid circles) was generated from 10 normal response β cells in two islets (cells that fall near the right-hand peak of the histogram in Fig. 3). The second curve (open circles) was generated from eight low response β cells in the same two islets (these cells fall near the left-hand peak of the histogram in Fig. 3). This curve is not noticeably sigmoidal and reaches a maxi-

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**Fig. 2. NAD(P)H response to glucose of control and AdenoCre-treated $g^k_{\text{lox/lox}}$ islets.** A, NAD(P)H autofluorescence images of both islets (control islet (left) and AdenoCre-treated islet (right)) under 1 mM glucose perifusion. Both islets show a low, but fairly uniform, signal level. B, same two islets after 5 min of 25 mM glucose in the perifusion medium. In the control islet, the NAD(P)H signal is greatly elevated in all cells, but in the AdenoCre-treated islet, many cells show a weak response.

**Fig. 3. Histogram of the NAD(P)H response ratio ((NAD(P)H signal at 1 mM glucose perifusion)/(NAD(P)H signal at 25 mM glucose perifusion)).** This histogram represents 220 nonperipheral β cells in 5 AdenoCre-treated islets from three different preparations. The normal distribution curve of islet cells from wild-type mice is overlaid. The bar graph histogram contains two populations of cells, the right-hand peak is consistent with what is observed in wild-type mice, although untreated $g^k_{\text{lox/lox}}$ islets show a ~5% reduction in NAD(P)H response ratio from wild-type mice. About one-third of the β cells analyzed fall in the left-hand peak that shows considerably less than normal NAD(P)H response, which is consistent with a knockout of GK activity by Cre infection.
their response below 5 mM, which is consistent with the loss of GK from the impaired cells (2, 25–28). While the evidence for GK as the β cell glucose sensor is compelling, it has been difficult to functionally distinguish the role GK from other hexokinases in β cells. Mannohexitol, a competitive inhibitor of hexokinases, has been used to inhibit glucose phosphorylation in β cells (e.g. Ref. 27), but it is not specific for GK. In fact, application of mannohexitol to AdenoCre-treated islets caused a uniform decrease in autofluorescence from all β cells (not shown). Thus, to distinguish the specific role of GK apart from other hexokinase gene family members with lower Km values at ~5 mM glucose. The kinetics of response in these cells is consistent with glucose usage being determined by one or more other hexokinase gene family members with lower Km values and not by GK.

**DISCUSSION**

Many previous studies have pointed to a rate-determining role for GK in glucose-stimulated responses of β cells (2, 25–28). While the evidence for GK as the β cell glucose sensor is compelling, it has been difficult to functionally distinguish the role GK from other hexokinases in β cells. Mannohexitol, a competitive inhibitor of hexokinases, has been used to inhibit glucose phosphorylation in β cells (e.g. Ref. 27), but it is not specific for GK. In fact, application of mannohexitol to AdenoCre-treated islets caused a uniform decrease in autofluorescence from all β cells (not shown). Thus, to distinguish the specific role of GK apart from other hexokinase isoforms, we have made use of the highly specific Cre/loxP system to inducibly eliminate GK within β cells in intact islets. This approach allows GK gene expression to remain normal in the adult animal prior to islet isolation, and avoids the lethality caused by either a global or β cell-specific deficiency in GK (3–6). By using a recombinant adenoaviruses to express Cre, conversion of the gklox allele to gkd allele was delayed until after islets are isolated from the adult animal. While an infection efficiency of only ~30% was achieved using this approach, it offered the advantage in this instance of creating heterogeneities of glucose sensing within the islet. These heterogeneities allowed us to examine the role of cell-cell communication during glucose-stimulated metabolic activity, in addition to determining the specific functional role of GK.

The results from these studies are important in at least two regards. First, they provide strong additional evidence that GK does indeed function as the glucose sensor in β cells. TPEM analysis showed that approximately one-third of the cells in AdenoCre-treated islets lacked a normal metabolic response to glucose (Figs. 2 and 3). This percentage was closely correlated to the amount of Cre-mediated recombination determined by Southern blot. Because AdenoCre uses the potent immediate early cytomegalovirus promoter to drive Cre expression, infection of a cell probably results in efficient Cre expression and thus recombination of both copies of the gklox allele into the gkd allele. Because of this, intermediate levels of GK gene expression within infected (i.e. recombination of only a single allele) are unlikely, and the amount of recombination probably reflects the percentage of cells without GK. Similar percentages were observed in Western blots for GK. Unfortunately, it was not possible to examine the AdenoCre-treated islets by immunofluorescence after NAD(P)H imaging because, unlike normal islets (13), they did not remain mobilized on the Cell-Tak during fixation (despite over 20 attempts). We have found this to be a limitation regardless of the recombinant adenoaviruses used. Consequently, we were unable to correlate NAD(P)H responses with either Cre expression or GK expression on a cell-by-cell basis in the islets. Nonetheless, a few AdenoCre-treated islets that did survive the immunostaining process in parallel preparations showed increased heterogeneity and an overall reduction in GK immunofluorescence compared with untreated gklox/lox islets. Furthermore, cells that were presumed to lack GK showed a muted NAD(P)H response to glucose that saturated at a lower glucose concentration than the more highly responding cells, consistent with glucose phosphorylation by other hexokinases.

It is well established that other hexokinases are expressed within β cells and that glucose responsiveness requires a high GK/hexokinase ratio (29). Indeed, transformed β cells maintained in culture for extended times demonstrate a lower GK/hexokinase ratio and exhibit diminished glucose-stimulated responses (30). Thus, the significant NAD(P)H response observed at the lower glucose levels in the AdenoCre-infected islet cells is consistent with the activity of hexokinases other than GK. The absence of GK may cause an up-regulation of these other hexokinases, all of which exhibit a more pronounced inhibition by glucose 6-phosphate than does GK (29). Because of this differential inhibition, however, it is difficult to assess precisely the contribution of other hexokinases to glucose sensing in a normal β cell. To rigorously determine the role of each hexokinase isoform, mice with conditional knockouts of each gene would have to be created and examined.

Second, these studies demonstrate that individual β cells within intact pancreatic islets show independent metabolic responses. Because gradients in NAD(P)H levels between adjacent β cells persist even after 30 min, there does not appear to be any mechanism within the islet to generate metabolic uniformity among heterogeneous cells. Thus, we conclude that intercellular communication is not involved in glucose-stimulated metabolic flux and that each β cell senses glucose independently at the metabolic level. Exactly how cell-cell communication, which clearly plays a significant role in increasing insulin secretion, is involved in more distal signaling events (e.g. membrane depolarization, Ca2+ influx, and insulin exocytosis) remains to be determined.

The detection of artificial heterogeneities among single β cells within AdenoCre-treated islets helps to further validate the use of TPEM for high-resolution measurements of cellular metabolism in thick samples. Because the optical section in TPEM is ~1 μm thick, information from single cells should be uncontaminated by background fluorescence that arises from other cells. In fact, TPEM has proved useful for measuring activity of single synapses in brain slices using exogenous fluorescent probes (31). Here we have shown that TPEM measurements of NAD(P)H accurately report the metabolic flux in individual cells. Since TPEM offers submicron resolution, it also opens the possibility to perform subcellular metabolic measurements, such as differentiating the NAD(P)H signals between the cytoplasm and mitochondria.

It may be important to note that islets from gklox/lox mice are slightly less responsive than islets from wild-type mice (~5% difference between the line and the right-hand peak of the histogram in Fig. 3). This was not unexpected because the...
blood glucose levels in the gklox/lox mice are slightly elevated (<10%) from those in wild-type mice (6). Both of these findings indicate that the gklox allele may express slightly less GK than the wild-type gene Gk gene. This slight difference in responsiveness is not likely to be a limitation of the present study, since the difference in NAD(P)H response is less than we have observed between different mouse strains.2

The use of in vitro gene knockouts offers great potential for investigations of cellular signal transduction. Because the Cre/loxP approach allows normal gene expression to continue while the whole animal are avoided. Another advantage of targeted gene knockouts is that, unlike pharmacological approaches, the cells. Although we were able to take advantage of the heterogeneous introduced by the adenovirus infection in this study, complete in vitro gene knockouts could often be advantageous. To obtain 100% efficient recombination of the loxP sites, methods other than adenovirus-mediated transfections could be used for the introduction of Cre. One such alternative is to use mice that contain inducible Cre transgenes (32).

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REFERENCES
1. Neuberg, C. B., and McGarry, J. D. (1995) Annu. Rev. Biochem. 64, 689–719
2. Matechinsky, F. M., Gisler, B., and Magnuson, M. A. (1998) Diabetes 47, 367–315
3. Grupe, A., Hultgren, B., Ryan, A., Ma, Y. H., Bauer, M., and Stewart, T. A. (1995) Cell 83, 69–78
4. Ishihara, H., Tashiro, F., Ikuta, K., Asano, T., Katagiri, H., Inukai, K., Kikuchi, M., Yazaki, Y., Oka, Y., and Miyazaki, J. (1995) FEBBS Lett. 371, 329–332
5. Bali, D., Svetlanov, A., Lee, H.-W., Fusco-DeMane, D., Leiser, M., Li, B., Barzili, N., Surana, M., Hou, H., Fleischer, N., DePinho, R., Rossetti, L., and Efrat, S. (1995) J. Biol. Chem. 270, 21484–21467

2 S. M. Knobel, J. S. Salmon, and D. W. Piston, unpublished observations.

6. Postic, C., Shiota, M., Niwender, K. D., Jetton, T. L., Chen, Y., Moates, J. M., Shelton, K. D., Lindner, J., Cherrington, A. D., and Magnuson, M. A. (1999) J. Biol. Chem. 274, 305–315
7. Lennmark, Å. (1974) Diabetologia 10, 431–438
8. Meda, P., Atwater, I., Goncalves, A., Bangham, A., Oeri, L., and Rojas, E. (1984) Q. J. Exp. Physiol. 69, 719–735
9. Gyfie, E., Grupenziesser, E., and Helledam, B. (1991) Cell Calcium 12, 229–240
10. Meda, P., Bosc, D., Chanson, M., Giordano, E., Vallar, L., Willecke, K., el Anamari, A., Gros, D., and Beyer, E. C. (1991) Exp. Cell Res. 192, 469–480
11. Smolen, P., Rinzel, J., and Shermman, A. (1993) Biophys. J. 64, 1668–1680
12. Bennett, B. D., Jetton, T. L., Ying, G., Magnuson, M. A., and Piston, D. W. (1996) J. Biol. Chem. 271, 3647–3651
13. Noma, Y., Bonner-Weir, S., Latimer, J. B., Davalli, A. M., and Weir, G. C. (1996) Endocrinology 137, 1485–1491
14. Pipeleers, D. G., Kiekens, R., Ling, Z., Wilikens, A., and Schuit, F. (1994) Diabetes 37, S57–S64
15. Gu, H., Marth, J. D., Orban, P. C., Mossmann, H., and Rajewsky, K. (1994) Science 265, 103–106
16. Anton, M., and Graham, F. L. (1995) J. Virol. 69, 4600–4606
17. Becker, T. C., NoellandlRio, H., Noel, R. J., Johnson, J. H., and Newgard, C. B. (1994) J. Biol. Chem. 269, 21224–21238
18. Denk, W., Strickler, J. H., and Webb, W. W. (1999) Science 284, 73–76
19. Piston, D. W., Bennett, B. D., Ying, G. (1995) J. Microbiol. Soc. Am. 1, 25–34
20. Graham, F. L., Smiley, J., Russel, W. C., and Naira, R. (1997) J. Gen. Virol. 36, 59–72
21. Becker, T. C., Noel, R. J., Coats, W. S., Gomez-Foix, A. M., Alam, T., Gerard, R. D., and Newgard, C. B. (1994) Methods Cell Biol. 43, 161–189
22. Sharp, D. W., Kemp, C. B., Knight, M. J., Ballinger, W. F., and Lacy, P. F. (1973) Transplantation 16, 686–689
23. Stefan, Y., Meda, P., Neufeld, M., and Orci, L. (1987) J. Clin. Invest. 80, 175–183
24. Liang, Y., Najafi, H., Smith, R. M., Zimmerman, E. C., Magnuson, M. A., Tal, M., and Matschinsky, F. M. (1992) Diabetes 41, 792–806
25. Becker, T. C., Noel, R. J., Johnson, J. H., Lynch, R. M., Hirose, H., Tokuyama, Y., Bell, G. I., and Najafi, H. (1990) J. Gen. Virol. 71, 372–378
26. Denk, W., Strickler, J. H., and Webb, W. W. (1994) J. Biol. Chem. 269, 469–480
27. Sweet, I. R., Li, G., Najafi, H., Berner, D., and Matschinsky, F. M. (1996) Endocrinology 137, 371–378
28. Wang, H. L., and Iynedjian, P. B. (1997) Proc. Natl. Acad. Sci. U. S. A. 94, 4572–4577
29. Geman, M. S. (1990) Proc. Natl. Acad. Sci. U. S. A. 90, 1781–1785
30. Piechaczek, U., Blondell, B., Wollheim, C. B., Höppner, W., Seitz, H. J., and Iynedjian, P. B. (1997) Biochem. J. 314, 249–255
31. Denk, W., Yuste, R., Svoboda, K., and Tank, D. W. (1996) Curr. Opin. Neurobiol. 6, 372–378
32. Kühn, R., Schwenk, F., Aguet, M., and Rajewsky, K. (1995) Science 269, 1427–1429