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Cloning and functional expression of a human pancreatic islet glucose-transporter cDNA

(human liver/pancreatic islet glucose transporter/human islet cDNA library/functional expression)

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ABSTRACT **Previous studies have suggested that pancreatic islet glucose transport is mediated by a high-K_m, low-affinity facilitated transporter similar to that expressed in liver. To determine the relationship between islet and liver glucose transporters, liver-type glucose-transporter cDNA clones were isolated from a human liver cDNA library. The liver-type glucose-transporter cDNA clone hybridized to mRNA transcripts of the same size in human liver and pancreatic islet RNA. A cDNA library was prepared from purified human pancreatic islet tissue and screened with human liver-type glucose-transporter cDNA. We isolated two overlapping cDNA clones encompassing 2600 base pairs, which encode a pancreatic islet protein identical in sequence to that of the putative liver-type glucose-transporter protein. *Xenopus* oocytes injected with synthetic mRNA transcribed from a full-length cDNA construct exhibited increased uptake of 2-deoxyglucose, confirming the functional identity of the clone. These cDNA clones can now be used to study regulation of expression of the gene and to assess the role of inherited defects in this gene as a candidate for inherited susceptibility to non-insulin-dependent diabetes mellitus.**

Defining the molecular mechanisms involved in the synthesis and secretion of insulin may help clarify the nature of the deficiency that characterizes diabetes. Glucose appears to be the major regulator of insulin synthesis and secretion, as it modulates the effects of other nutrients, hormones, and neurotransmitters (1). Both first-phase and second-phase secretion appear tightly linked to glucose metabolism (2, 3). With pancreatic islet isolation and quantitative histochemical techniques, the glucose concentration in islet cells was found to equal that in blood within 60 sec of glucose-load injection into rats (4). Saturable stereospecific uptake of D-glucose by pancreatic islet cells had been shown in isolated mouse islets (5). These studies suggested that islet glucose transport is mediated by a high-K_m, low-affinity transporter as in liver (6) and that transport is not rate limiting for glucose metabolism. In three different transformed islet cell lines, however, in which glucose-stimulated insulin secretion was impaired, the uptake of 3-0-methylglucose was slow (6, 7). Thus, while glucose transport in normal islets is rapid, impaired glucose transport may, in certain circumstances, be the rate-limiting step for glucose control of insulin secretion.

Because islets represent only ≈1% of total pancreatic tissue and membrane proteins represent only a minor component of total cell protein, the islet glucose transporter has not been isolated. Recombinant DNA techniques have been used to identify glucose transporters in other tissues, however. The human erythrocyte glucose transporter was isolated from a human hepatoma (HepG2) cDNA library (8). This 2800-base-pair (bp) cDNA encoded a 492-amino acid residue polypeptide with an estimated size of the translated deglycosylated protein of 46 kDa. The HepG2 cDNA has been functionally expressed in *Escherichia coli* (9) and *Xenopus* oocytes (K.K. and M.M., unpublished work). This transporter appears to be the major transporter of brain, whereas it is expressed at various levels in other tissues (10–12). The HepG2/erythrocyte glucose transporter cDNA has been used to screen adipocyte, muscle, and heart cDNA libraries. cDNA clones were isolated that encode a protein with 65% sequence identity to the human HepG2 glucose transporter (13–16). This cDNA hybridizes to an mRNA present only in skeletal muscle, heart, and adipose tissue and appears to be the translocatable glucose transporter expressed in insulin-responsive tissues.

Because the HepG2/erythrocyte glucose-transporter cDNA was expressed at very low levels in fetal and adult liver, an adult human liver cDNA library was screened at low stringency, and cDNA clones were isolated that encode a 524-amino acid liver protein with 55% identity with the HepG2/erythrocyte glucose transporter (17). A similar strategy was used to isolate a cDNA clone encoding a rat liver glucose-transporter mRNA shown to be expressed in liver, intestine, kidney, and pancreatic islets (18). An antibody directed towards a synthetic peptide encoded by the cDNA was used to show by immunocytochemistry that an immunologically related protein is expressed in the plasma membrane of rat pancreatic islet beta cells (18). Because the HepG2/erythrocyte glucose-transporter mRNA was not detected in islets, these studies suggested that a protein either identical or very similar to the liver-type glucose transporter was the major transporter in pancreatic islets of rats. Neither rat nor human protein has been isolated, however. In the current report, we describe the cloning and functional expression of a glucose-transporter cDNA isolated from human pancreatic islets. DNA sequence analysis indicates that the islet transporter is identical to the human liver-type glucose-transporter polypeptide.

MATERIALS AND METHODS

Synthesis of the cDNA Library. Double-stranded cDNA was synthesized with a kit, as described by the supplier (Bethesda Research Laboratories). First-strand synthesis used 10 μg of human islet poly(A)$^+$ RNA [twice-purified on oligo(dT)-cellulose] and Moloney murine leukemia virus reverse transcriptase, followed by second-strand synthesis with *E. coli* DNA polymerase I and ribonuclease H. A total of 2.4 μg of double-stranded cDNA was synthesized. The

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first *Eco*RI sites were methylated with *Eco*RI methylase (New England Biolabs), the termini were repaired with T4 DNA polymerase (Bethesda Research Laboratories), and phosphatased *Eco*RI linkers (New England Biolabs) were ligated to the cDNA. The cDNA was then digested with *Eco*RI, and unligated linkers were separated by Sephadex chromatography and preparative gel electrophoresis, as described (19). The cDNA larger than 600 bp was eluted from the gel with Geneclean (Bio 101, La Jolla, CA), ligated to *Eco*RI-digested λgt11 (Promega Biotec), packaged with an *in vitro* packaging extract (Gigapack Gold, Stratagene), and grown on *E. coli* Y1090 cells. A total of 10^6 clones were obtained with ≈90% recombinants. Average size of the clones obtained was 1.1 ± 0.4 kilobases (kb) (mean ± SD). The cDNA library was amplified in Y1090 cells and stored over 0.3% chloroform in aliquots at 4°C. A fraction of the library was frozen after adding 7% (vol/vol) dimethyl sulfoxide in 1.5-ml aliquots in Cryotube tubes at −80°C.

cDNA Library Screening. For screening of the liver and islet λgt11 cDNA libraries, phage were plated on a lawn of *E. coli* Y1090 cells at a density of 5×10^4 plaque-forming units per 150-mm Petri dish (Falcon 1058), as described (20). Plaque lifts were performed with nylon membranes (MSI, Fisher), as described by Maniatis *et al.* (20). After baking for 1 hr at 80°C, the filters were prehybridized 2 hr at 42°C in 5× SSPE (1 × SSPE is 0.18 M NaCl, 1 mM EDTA, 10 mM NaH_2PO_4, pH 7.4)/0.2% SDS/0.1% each of bovine serum albumin, polyvinylpyrrolidone, and Ficoll/denatured sheared salmon sperm DNA (200 μg/ml). The filters were then hybridized with nick-translated or random primer-labeled probes at 10^6–10^7 cpm/ml. After being washed in 0.1× SSPE at 50°C, the blots were exposed to Kodak XAR-5 film at −70°C for various periods by using a DuPont Cronex LightningPlus intensifying screen.

Human Tissues. Pancreatic islets were isolated from cadaver donors as described (21). All human tissues were obtained with institutional approval and informed consent. Human heart, skeletal muscle, jejunum, liver, and kidney were obtained under a rapid autopsy protocol under the supervision of Marian Peters of the Pathology Department.

RNA Preparation and Northern (RNA) Blots. RNA was prepared by the guanidinium thiocyanate/cesium chloride extraction method of Chirgwin *et al.* (22). Poly(A)$^+$ RNA was selected by two passages over oligo(dT)-cellulose (type 3, Collaborative Research). RNA was electrophoresed on 1.2% agarose-formaldehyde gels, blotted to MSI nylon membranes, and hybridized to probes labeled either by random priming, by nick-translation (Amersham), or by cRNA synthesis using T7 RNA polymerase or T3 RNA polymerase (Promega) according to directions of the manufacturer. Prehybridization and hybridization was done under high-stringency conditions, as described for the library screening, or for variable stringency, as described below. For preparation of the HepG2/erythrocyte glucose-transporter cRNA probe, the *Eco*RI 2.4-kb fragment of clone pGT25L (8) was subcloned into the bacterial plasmid Bluescript (Stratagene) and linearized before transcription. For preparation of the liver glucose-transporter cRNA probes, the *Eco*RI inserts from λ–liver glucose-transporter clones (see Fig. 3 λLGT) were subcloned into the *Eco*RI site of Bluescript and linearized with enzymes in the polylinker, depending on their orientation relative to the T3 and T7 promoters. Hybridization with cRNA probes was under the same conditions as described for cDNA library screening but at the temperatures indicated. The blots were washed in 0.1× SSC (1× SSC is 0.15 M sodium chloride/0.015 M sodium citrate, pH 7)/0.1% SDS at the temperatures indicated.

DNA Sequencing. The *Eco*RI inserts from the clones λA1–A3 were subcloned into the *Eco*RI sites of Bluescript. These plasmids were grown in either strains JM105 or XL1-Blue and harvested; inserts were subcloned into the *Eco*RI site of M13mp18 and -mp19 vectors (Bethesda Research Laboratories). Sequencing of the fragments subcloned in M13 vector was by the dideoxy chain-termination method of Sanger *et al.* (23) by using Sequenase (United States Biochemical) and ^{35}S-labeled dATP (Amersham) with either the universal M13 primer or synthetic oligonucleotides, as described by the manufacturer. Reaction mixtures were separated on buffer gradient gels, as described (24).

Expression in Oocytes. *Xenopus* oocytes were isolated from excised ovaries and then injected with glucose-transporter mRNAs, as described elsewhere (K.K. and M.M., unpublished work). 2-Deoxy[^{3}H] glucose (25 μM, 1 μCi/ml; 1 Ci = 37 GBq) uptake measurements were conducted on groups of five to seven oocytes in 0.5 ml of modified Barths' saline at 22°C. After 30 min the oocytes were washed three times in 3 ml of ice-cold modified Barths' saline and then dissolved in 1 ml of 1% SDS. Radioactivity was then quantitated in a liquid-scintillation spectrophotometer.

RESULTS

Northern Blot Analysis of Islet RNA. Pancreatic islets obtained from cadaver donors maintain viability even after prolonged cold time of ischemia and the isolation process (25); condition of the RNA that could be obtained from these islets was not known, however. Furthermore, the donors had suffered from hours to weeks of either shock or chronic disease or both before death. RNA was, therefore, isolated from a number of different human pancreatic islet preparations, electrophoresed on denaturing gels, and subjected to Northern blot analysis for hybridization with a ^{32}P-labeled insulin probe (Fig. 1) Proinsulin mRNA was clearly abundant in these islet RNA preparations even after 3-day culture; yet the amount of proinsulin mRNA varied markedly. Dot-blot analysis with synthetic human proinsulin mRNA as standard revealed that insulin mRNA concentration varied over a 50-fold range, from 0.3–15 ng/μg of total islet RNA.

The HepG2/erythrocyte glucose transporter is encoded by a 2.8-kb mRNA expressed in placenta, brain, and, to a lesser degree, in heart, kidney, and skeletal muscle (10, 11). Although this mRNA is expressed at low levels in liver, it is

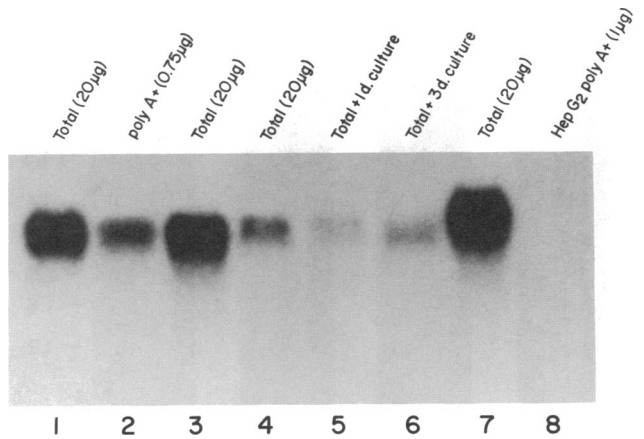

FIG. 1. Northern blot analysis of RNA from several human pancreatic islet preparations. Total islet RNA (20 μg) (lanes 1, 3–7), islet poly(A)$^+$ RNA (0.75 μg) (lane 2), and HepG2 polyA$^+$ RNA (1 μg) (lane 8) were denatured with formaldehyde, electrophoresed on 1.2% agarose-formaldehyde gels, and blotted to a nylon membrane as described. RNA in lanes 5 and 6 was extracted from islets previously cultured for 1 (1d) and 3 (3d) days, respectively (21, 25). A ^{32}P-labeled human insulin 1.6-kb genomic DNA probe was hybridized, the blot was washed, and autoradiographs were obtained as described. Size of the transcripts was estimated by coelectrophoresis of a 1-kb DNA ladder and an RNA ladder (both from BRL).

expressed at high levels in transformed cells, such as the human hepatoma cell-line HepG2 (26). When human liver, HepG2 cell, and pancreatic islet poly(A)$^+$ RNA were examined by Northern blotting and hybridization with the HepG2/erythrocyte glucose-transporter cRNA at high stringency, a 2.8-kb mRNA was seen only in HepG2 cells (Fig. 2), even after long exposure (5 days). After hybridization of the HepG2 cRNA probe at reduced stringency (57°C, 50°C, or 42°C), a 2.8-kb mRNA could be detected in both liver and islet mRNA, as well as less abundant mRNAs. From these observations we speculated that a HepG2/erythrocyte-related glucose transporter was common to both liver and islet tissue.

Synthesis of an Islet cDNA Library and Characterization of Glucose-Transporter Clones. To isolate the putative HepG2/erythrocyte-related islet glucose-transporter cDNA, a human islet cDNA library was synthesized from poly(A)$^+$ RNA in the expression vector λgt11. Recombinant phage (1×10^6) were screened under conditions of both high- and low-stringency, from the reasoning that HepG2-identical clones might be obtained at high stringency and HepG2/erythrocyte glucose-transporter-related glucose-transporter clones at lower stringency. Screening of 1×10^6 recombinants yielded no positive clones.

Subsequent to the above studies, human liver-type glucose-transporter cDNA clones were isolated from liver and kidney cDNA libraries by Fukumoto *et al.* (17). From the published sequence of this liver-type glucose-transporter cDNA, oligonucleotides were synthesized and used to screen a human liver cDNA library. A total of 1.5×10^6 recombinants were screened, yielding 3.2 kb of overlapping cDNA clones (Fig. 3). Restriction endonuclease mapping of these clones indicated that they were identical to the liver glucose-transporter cDNA isolated by Fukumoto *et al.* (17). The 5′ end of λ–liver glucose-transporter-2 clone (λHLGT-2) was sequenced (250 bp) and, with the exception of being shorter by 10 bp, was identical to the published sequence of the liver-type glucose-transporter clone.

To compare islet and liver glucose-transporter mRNA, restriction fragments of the liver-type glucose-transporter cDNAs were subcloned in the bacterial plasmid Bluescript

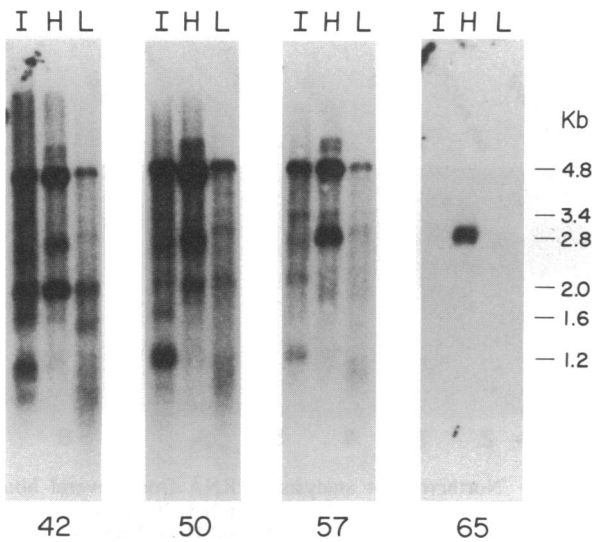

FIG. 2. Northern blot analysis with variable-stringency hybridization of human RNA to ^{32}P-labeled human HepG2/erythrocyte cRNA. Poly(A)$^+$ RNA (1 μg) from islets (I), HepG2 cells (H), or liver (L) was applied to each of four lanes. RNA was electrophoresed and blotted, and the filter was cut into four strips and hybridized under the described conditions but at hybridization and wash temperatures in °C indicated beneath the lanes.

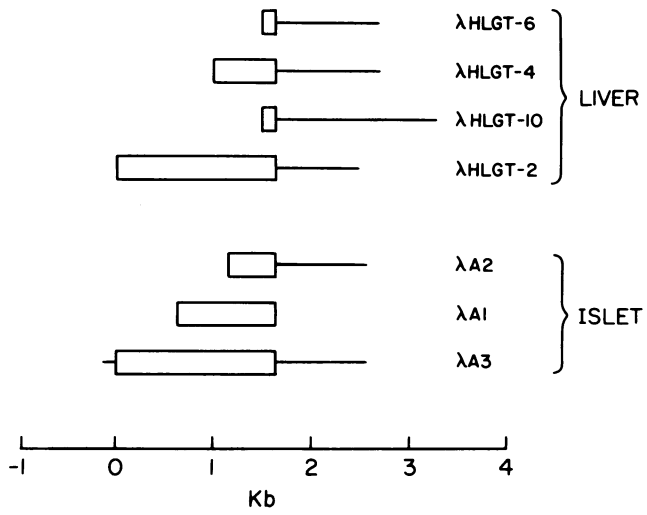

FIG. 3. A schematic diagram of the liver and pancreatic islet glucose-transporter cDNA clones isolated. The liver cDNA clones (λLGT-2, -4, -6, and -10) were isolated from a human liver cDNA library in λgt11 by ^{32}P-labeled synthesized oligonucleotides based on the published sequence. The islet cDNA clones (λA1–3) were isolated from human islet cDNA libraries in λgt11 as described in text. Boxes represent translated portions of the cDNA.

and then ^{32}P-labeled and hybridized to Northern blots of human tissue RNA (Fig. 4). RNA transcripts of 6.4, 3.4, and 2.8 kb were identified in human liver, jejunum, heart, and at low abundance in some human islet preparations (see, for example, HI556, Fig. 4*A*). The abundance of liver-type glucose transporter mRNA in islet tissue was estimated to represent only 10% or less that in liver and jejunum. Although no homologous RNA was observed in psoas muscle, mRNAs comparable in size to liver and jejunum were seen in heart muscle.

From these observations, the human liver-type glucose-transporter cDNA was used to screen the islet cDNA library. A total of 1.5×10^6 recombinant λgt11 phage were screened, and nine clones were plaque-purified. Crosshybridization of these clones revealed two positive clones, λA1 and λA2 (Fig. 3). Restriction mapping indicated that the 5′ end was missing. Another human islet cDNA library (supplied by Dan Rabin, Molecular Diagnostics, West Haven, CT) was subsequently screened, which yielded a 2.5-kb clone, λA3. The cDNA inserts of these clones were subcloned in M13mp18 and M13mp19 vectors and sequenced (Fig. 5). The sequence of the cDNAs for the putative islet glucose-transporter encompassed 2586 bp and was identical to the reported sequence of the human liver glucose-transporter-like cDNA, except for the presence of an additional 12 bp of 5′ noncoding sequence.

Functional Expression of the Human Liver/Pancreatic Islet Glucose Transporter. The human liver/pancreatic islet glucose-transporter-like cDNA had not, to our knowledge, been characterized by functional expression. Synthetic mRNA was produced from a full coding length cDNA construct and injected into *Xenopus* oocytes. mRNA-injected oocytes exhibited 7-fold higher rates of 2-deoxyglucose uptake than did water-injected controls (Fig. 6), thus confirming the functional identity of the encoded protein. For comparison, uptake into oocytes injected with the human HepG2-type and the rat insulin-regulatable glucose-transporter mRNAs is also shown.

DISCUSSION

From previous observations of the similarities of glucose metabolism in pancreatic islets and liver (4), that islet glucose

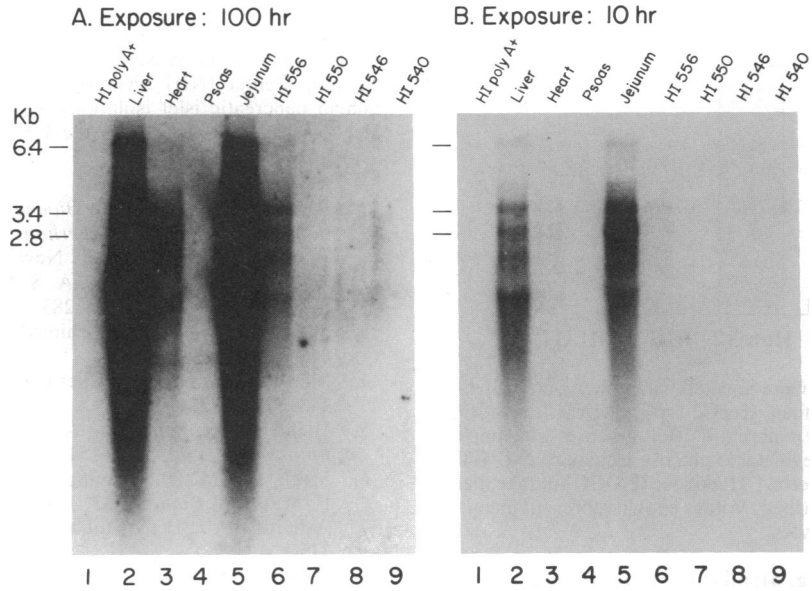

FIG. 4. RNA was extracted from human autopsy tissues and from five different human pancreatic islet preparations as indicated. In each lane, 40 μg of total RNA or 1 μg of human islet (HI) poly(A)$^+$ RNA was electrophoresed, blotted onto nylon membranes, and hybridized with ^{32}P-labeled liver glucose-transporter cDNA isolated from clone λLGT-2. The filter was washed and exposed to film for the times indicated in *A* and *B*.

transport would be mediated by a high K_m, low-affinity transport protein similar to that in liver was predictable. The studies of Thorens *et al.* (18) indicated that a rat liver glucose-transporter cDNA shared nucleotide homology and the protein shared immunoreactivity with a pancreatic islet glucose transporter; yet, isolation and positive identification of an islet cDNA clone has not been reported. The islet cDNA clones described in this report encode a protein identical to that encoded by the human liver cDNA. This information together with the functional-expression data confirms the identity of the glucose transporter in these two tissues. Further evidence that the major pancreatic islet glucose transporter is the liver-type glucose transporter was the failure to identify HepG2/erythrocyte glucose-transporter mRNA, or insulin-regulatable glucose-transporter mRNA in Northern blots of islet RNA hybridized under high-stringency conditions. Because beta cells make up the majority of islet cells and because Thorens *et al.* (18) demonstrated specific immunofluorescence in pancreatic beta cells, the liver-type glucose-transporter cDNA that we isolated from islet RNA is most likely the major transporter of human pancreatic islet beta cells. We note that we were unable to identify either HepG2, liver-type, or insulin-regulatable glucose-transporter mRNA in exocrine pancreas, which suggests that another glucose-transporter protein may mediate transport in this tissue.

The explanation for differences in expression of the three types of glucose-transporter mRNA in various human and rat tissues is currently unresolved. In some tissues, such as liver, pancreatic islets, or brain, one transporter appears to be predominantly expressed (Fig. 4; refs. 11, 17, and 18). In the kidney, both the liver-type and the HepG2-type mRNAs are expressed in ≈10:1 ratio of the former to the latter (L.K. and M.A.P., unpublished data). In human skeletal muscle, both insulin-regulatable and HepG2/erythrocyte glucose transporters are expressed, whereas we detected no liver-type glucose-transporter mRNA. We were surprised to find expression of the liver-type glucose-transporter mRNA in

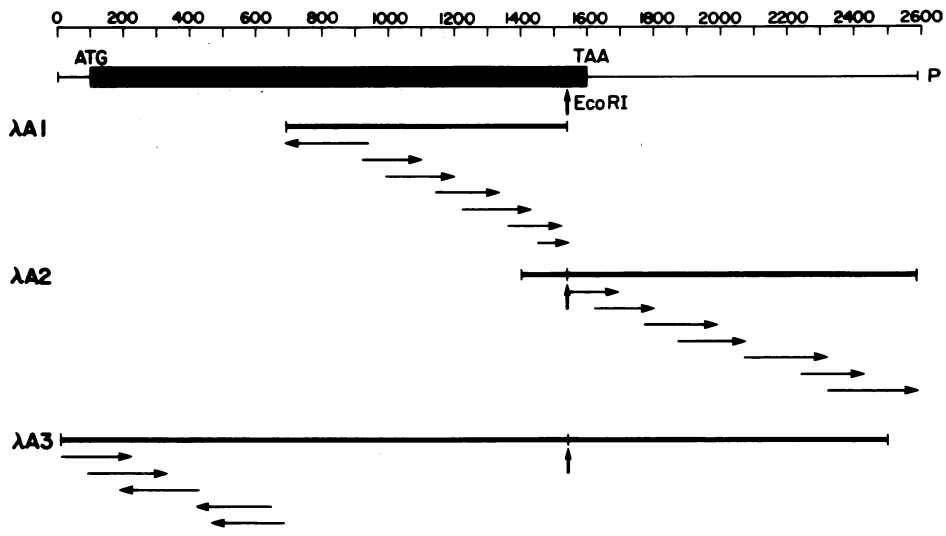

FIG. 5. Sequencing strategy using M13 and oligonucleotide primers for pancreatic islet glucose-transporter clones λA1–3. The beginning and end of the putative translated region is indicated, as well as the *Eco*RI site (vertical arrows) at position 1545.

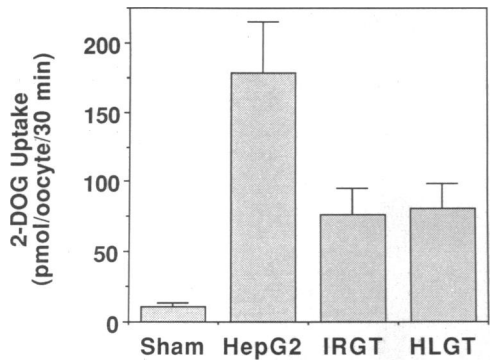

FIG. 6. *Xenopus* oocytes were injected with either water (Sham) or mRNA encoding the human HepG2-type glucose transporter (HepG2), the human liver/pancreatic islet glucose transporter (HLGT), or the rat insulin-regulatable glucose transporter (IRGT). Three days after injection 2-deoxy[^{3}H]glucose (2-DOG)-uptake measurements were done as described. Values shown represent mean ± SD for uptake into 5–10 oocytes.

human heart tissue (see Fig. 4); all three types of mRNAs are expressed in this organ. Although the relationship between expression of the various glucose-transporter genes and glucose metabolism has yet to be established, the observation that both liver and islet tissues express the same glucose transporter provides additional evidence for the similarity in the glucose metabolic process in these tissues.

Impaired glucose utilization by tissues is a characteristic feature of non-insulin-dependent diabetes mellitus (27). Whether the defect in glucose utilization is due to insulin lack or a primary defect in the biochemical steps involved in glucose uptake and use has yet to be determined. Now that some major molecules involved in glucose uptake have been isolated, these processes can be evaluated in diabetics at the level of molecular genetics. Some prominent defects in non-insulin-dependent diabetes mellitus include impaired hepatic glucose use as well as impaired glucose-stimulated insulin secretion (27). These pathophysiological processes could be mediated by a defect of the liver/pancreatic islet glucose transporter, either by diminished regulation at the level of gene expression or by a primary defect in the gene. A single gene encodes the liver and pancreatic islet glucose transporter, and thus cDNA and genomic DNA probes can now be used by Southern blot analysis of peripheral leukocyte DNA to assess the role of inherited defects in this gene. Such defective genes would be candidates for the cause of inherited susceptibility to non-insulin-dependent diabetes mellitus.

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