MOLECULAR AND CELLULAR BIOLOGY, Jan. 1992, p. 422–432
0270-7306/92/010422-11$02.00/0

Vol. 12, No. 1

# Glucose Transporter Isotypes Switch in T-Antigen-Transformed Pancreatic β Cells Growing in Culture and in Mice

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Received 23 April 1991/Accepted 25 September 1991

**High-level expression of the low-$K_m$ glucose transporter isoform GLUT-1 is characteristic of many cultured tumor and oncogene-transformed cells. In this study, we tested whether induction of GLUT-1 occurs in tumors in vivo. Normal mouse β islet cells express the high-$K_m$ (~20 mM) glucose transporter isoform GLUT-2 but not the low-$K_m$ (1 to 3 mM) GLUT-1. In contrast, a β cell line derived from an insulinoma arising in a transgenic mouse harboring an insulin-promoted simian virus 40 T-antigen oncogene (βTC3) expressed very low levels of GLUT-2 but high levels of GLUT-1. GLUT-1 protein was not detectable on the plasma membrane of islets or tumors of the transgenic mice but was induced in high amounts when the tumor-derived βTC3 cells were grown in tissue culture. GLUT-1 expression in secondary tumors formed after injection of βTC3 cells into mice was reduced. Thus, high-level expression of GLUT-1 in these tumor cells is characteristic of culture conditions and is not induced by the oncogenic transformation; indeed, overnight culture of normal pancreatic islets causes induction of GLUT-1. We also investigated the relationship between expression of the different glucose transporter isoforms by islet and tumor cells and induction of insulin secretion by glucose. Prehyperplastic transgenic islet cells that expressed normal levels of GLUT-2 and no detectable GLUT-1 exhibited an increased sensitivity to glucose, as evidenced by maximal insulin secretion at lower glucose concentrations, compared with that exhibited by normal islets. Further, hyperplastic islets and primary and secondary tumors expressed low levels of GLUT-2 and no detectable GLUT-1 on the plasma membrane; these cells exhibited high basal insulin secretion and responded poorly to an increase in extracellular glucose. Thus, abnormal glucose-induced secretion of insulin in prehyperplastic islets in mice was independent of changes in GLUT-2 expression and did not require induction of GLUT-1 expression.**

An increase in the rate of glucose uptake is one of the earliest biochemical events in cellular transformation and has a yet-unknown molecular basis (26, 36). GLUT-1 is a facilitated diffusion glucose transporter isoform with a low $K_m$, in the range of 1 to 2 mM, for glucose (37). Cultured fibroblasts infected with Rous sarcoma virus, Fujinami sarcoma virus, or simian virus 40 show an increased rate of uptake and oxidation of glucose. The increase in glucose uptake correlates with higher levels of GLUT-1 protein in Rous sarcoma virus (11, 14)-infected and Fujinami sarcoma virus (25)-infected cells, whereas in simian virus 40-infected cells, a redistribution of GLUT-1 protein from internal membranes to the plasma membrane is observed (17). More recently, the oncogenes *ras* and *src* were shown to increase the levels of GLUT-1 mRNA when transfected into fibroblasts, while transfection of the *myc* oncogene did not (8). Similarly, an increase in GLUT-1 mRNA in fibroblasts transfected with a temperature-sensitive mutant of Fujinami sarcoma virus and kept at the permissive temperature was observed (3). These results showed that the increased rate of glucose transport associated with oncogenic transformation correlated with increased expression of the high-affinity glucose transporter GLUT-1 and suggested that activated oncogenes may directly control the transcription of the GLUT-1 gene. However, these data were obtained with cell lines which already express a substantial level of GLUT-1. Therefore, they may reflect the regulation of glucose transporter expression in a cell culture environment which may be different from regulated expression of glucose transporters in tissues progressing toward tumor development in animals.

Indeed, recent experiments have shown that GLUT-1 expression is induced in tissue-cultured hepatocytes which normally express only GLUT-2 (28), a transporter isoform with a high $K_m$ (~20 mM) for glucose (13). This suggests that GLUT-1 expression may be the result of conditions of tissue culture and not directly of oncogenic transformation.

Insulin-secreting β islet cells normally express only GLUT-2 (23, 33) and correspondingly have a high $K_m$ (~20 mM) for glucose transport. The loss of glucose sensitivity for insulin secretion in β cells in diabetes and insulinomas correlates with, and might be caused by, reduced expression of GLUT-2 (4, 12, 24, 33, 34). In addition, low-level expression of GLUT-2 in cultured insulinoma cells is paralleled by high levels of GLUT-1 expression (33).

To determine the pattern of expression of GLUT-1 and GLUT-2 during oncogenic transformation of β cells and establishment of cell lines from tumor cells, we utilized a transgenic mouse model in which expression of the simian virus 40 large T antigen is controlled by the rat insulin II

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promoter (RIP Tag2 mice). These transgenic mice develop insulinomas within 12 to 20 weeks and die suddenly, probably from severe hypoglycemia (10). When transgenic mice are 4 weeks of age, all of their islets express the large T antigen but exhibit normal structure. At 9 weeks, some of their islets are of normal size but many islets show hyperplasia. About 1% of the ~400 islets in the pancreas develop into solid, highly vascularized β-cell tumors (10).

Here we show that GLUT-1 expression is not induced at any stage of tumor progression in mice. In contrast, in the cultured βTC3 cell line, which is derived from an end-stage tumor, GLUT-1 is the dominant glucose transporter; thus, high-level expression of GLUT-1 in these cells is induced by the cell culture conditions. Similarly, overnight culture of islets causes GLUT-1 induction in a majority of β cells. Also, we show that normal-size islets from 4- to 5-week-old transgenic mice express normal amounts of GLUT-2 but have an increased sensitivity to glucose for insulin secretion. A further reduction in GLUT-2 expression is observed during later stages of tumor progression in normal-size islets, hyperplastic islets, and tumors. These cells exhibit a very small increase in insulin secretion in response to increased glucose concentrations. Together, these results indicate that induction of GLUT-1 is not essential for abnormal glucose sensing.

## MATERIALS AND METHODS

**Northern (RNA) blots.** RNA was isolated by the lithium chloride method as described by Auffray and Rougeon (1), and Northern blots were prepared as described by Lehrach et al. (18). Briefly, the RNA was resolved on a 1% agarose-formaldehyde gel and transferred to a nylon membrane filter (ICN, Irvine, Calif.). Filters were hybridized with random-primed $^{32}$P-radiolabeled full-length cDNA probes of either rat GLUT-1 (a gift from M. Birnbaum) (2) or rat GLUT-2 (33). The hybridization solution contained 50% formamide, 5× SSC (1× SSC is 150 mM NaCl plus 75 mM sodium citrate [pH 7]), 5× Denhardt's solution (1× Denhardt's is 0.2 g each of polyvinylpyrrolidone, bovine serum albumin [BSA], and Ficoll 400 per liter), 5 mM EDTA, 1% sodium dodecyl sulfate (SDS), and 100 μg of poly(A) per ml. Hybridization was performed overnight at 42°C, and filters were washed twice in 2× SSC at 42°C and twice in 0.2× SSC at 65°C.

**Immunoblots.** Tumor tissues and livers were excised, and cells were disrupted in lysis buffer (5% SDS, 80 mM Tris [pH 6.8], 5 mM EDTA, 5% β-mercaptoethanol, 1 mM phenylmethylsulfonyl fluoride) by sonication for 1 min (Heat Systems; Ultrasonics, Inc., New York, N.Y.) at maximum speed. Equal amounts of protein (30 μg) were resolved on an SDS–10% polyacrylamide gel and transferred to a nitrocellulose membrane filter (35). Immunoblotting was performed as described by Thorens et al. (33). The antibody used for detection of GLUT-1 was a 1:100 dilution of a polyclonal serum raised against a peptide corresponding to the COOH terminus of the protein (amino acids 477 through 492) (32).

**Immunofluorescence.** Pancreas and tumor tissue fragments were fixed by immersion in paraformaldehyde-lysine-periodate fixative (20) overnight at 4°C and then kept at 4°C in phosphate-buffered saline (PBS) until use. For preparing 3-μm-thick frozen sections, tissue fragments were equilibrated for several hours at 4°C in PBS containing 0.6 M sucrose and 0.02% sodium azide, subsequently embedded in O.C.T. compound (Miles Inc., Elkhardt, Ind.), frozen in 2-methylbutane in liquid nitrogen for 10 s, and kept at −70°C until sectioning in a Reichert Frigocut cryostat. Sections were placed on polylysine-coated glass slides, air dried, and kept at −70°C until staining. Tissue sections were incubated for 10 min in 1% BSA in PBS. Sections were then incubated for 40 min with one of the following antisera (diluted in 1% BSA-PBS): affinity-purified sera raised against the COOH terminus of GLUT-1 (32), affinity-purified sera raised against a peptide corresponding to the COOH terminus (amino acids 512 through 523) of mouse GLUT-2 (29), or a guinea pig antiserum for insulin diluted 1:200 (Accurate Chemical & Scientific Corp., New York, N.Y.). Thereafter, sections were washed three times for 5 min each with PBS. The sections were then incubated with fluoresceinated goat or swine anti-rabbit immunoglobulin G (Calbiochem, San Diego, Calif.) diluted 1:100 in 1% BSA-PBS. The sections were washed as described above and mounted in 60% glycerol–2% *n*-propyl gallate–0.2 M Tris-HCl (pH 8.1). Sections were observed and photographed with Kodak TMAX or Ektachrome film (ISO 400) on a Zeiss Photomicroscope III. For double staining, the coverslips were removed after photography and the sections were washed for 5 min in PBS. The sections were then incubated for 1 h with an affinity-purified goat anti-rabbit immunoglobulin G (Cappel, Malvern, Pa.) (0.1 mg/ml) in 1% BSA-PBS. Thereafter, the sections were washed four times for 15 min each with PBS. The second antibody (anti-insulin) was then applied as described for the initial staining, and detection was with rhodamine-conjugated goat anti-rabbit immunoglobulin G (Cappel) diluted 1:100 in 1% BSA-PBS.

**Generation of secondary tumors.** A total of $10^6$ βTC3 cells in PBS were injected intraperitoneally into syngeneic male B6D2/$F_1$ mice. The development of solid tumors 6 to 10 weeks later was detected by monitoring reduced blood glucose levels.

**Insulin secretion assays.** Islets were isolated from 4- to 5-week-old RIP Tag2 and control C57BL/6 mice by collagenase infusion of the pancreas through the bile duct and manual picking under a dissecting microscope (9). Tumors were excised from the mice and mechanically disrupted in Hanks' balanced salt solution (6). Groups of 10 islets and aliquots of tumor cell suspensions were preincubated in Hanks' balanced salt solution (supplemented with 10 mM *N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid [HEPES] [pH 7.2] and containing 5 mM glucose and 0.1% BSA and then bubbled with 95% $O_2$–5% $CO_2$) in a 37°C 5% $CO_2$ humidified incubator for 2 h. The medium was then replaced with fresh medium containing various concentrations of glucose (each assayed in triplicate) and 0.5 mM 1-isobutyl-3-methylxanthine, and the cells were incubated for 2 h at 37°C. The incubation medium and the cell extracts were assayed by insulin radioimmunoassay in quadruplicate as described previously (5).

**Immunofluorescence of primary islet β cells in tissue culture.** Pancreatic islets were isolated from C57B mice by collagenase infusion of the pancreas through the bile duct followed by a Ficoll gradient (19). Fresh islets or islets cultured for 24 h in Dulbecco's minimal essential medium (containing 4.5 g of glucose per liter and supplemented with 10% fetal bovine serum, 0.2 mM L-glutamine, 100 U of penicillin per ml, and 100 U of streptomycin solution per ml [both from JRH Biosciences]) were washed in PBS and fixed overnight with paraformaldehyde-lysine-periodate at 4°C (see above). Thereafter, islets were incubated overnight in PBS at 4°C, warmed to 37°C, and embedded in 100 μl of 2% agarose-PBS at 42°C following brief centrifugation in a microcentrifuge. Agarose-embedded islets were equilibrated with PBS containing 0.6 M sucrose and 0.02% sodium azide

by rotation overnight at 4°C and subsequently embedded in O.C.T. compound, frozen, and sectioned. Glucose transporter isoform GLUT-1 was detected by the indirect immunofluorescence staining method as described above.

## RESULTS

**Pancreatic islet β cells of RIP Tag2 mice express only GLUT-2.** In RIP Tag2 mice, expression of the large T antigen initiates neoplastic transformation of a few islets according to a predictable pattern. The islets of the transgenic mice are normal in size (50 to 250 μm in diameter) up to about 4 to 5 weeks. Beginning at 6 to 8 weeks, most of the islets become hyperplastic; by 12 weeks, a few of these multifocal hyperplastic nodules progress into solid tumors which grow to up to 10 to 20 mm in diameter. We analyzed expression of GLUT-1 and GLUT-2 during this period of tumorigenesis to determine whether the neoplastic transformation affected the normal pattern of expression. Pancreas sections from RIP Tag2 mice prepared at different stages of tumorigenesis were stained with sera against GLUT-1, GLUT-2, and insulin (see Materials and Methods). Figure 1 shows islets from a normal mouse (Fig. 1A through C) and islets of a comparable size from RIP Tag2 mice at different stages of oncogenic progression (Fig. 1D through L). In normal islets, GLUT-2 was expressed in all the β cells (Fig. 1A); no islet cells expressed GLUT-1 (Fig. 1B), and all the β cells expressed insulin (Fig. 1C). In RIP Tag2 mice at 4.5 weeks, all β cells showed the same intense staining for GLUT-2 as did islets from control mice (Fig. 1D), while the intensity of GLUT-2 staining was reduced in islet β cells from an 8.5-week-old mouse (Fig. 1G). In an islet from a 12.5-week-old tumor-bearing mouse, a mosaic staining for GLUT-2 was observed; some β cells showed a distinct, albeit reduced, membrane staining, while other β cells were not stained (Fig. 1J). No changes occurred in the expression of insulin. No GLUT-1 staining of β cells was observed at any stage of tumor progression in RIP Tag2 mice (Fig. 1B, E, H, and K).

The pancreas from a 12.5-week-old RIP Tag2 mouse contains β cells in a few normal-size islets, a large number of hyperplastic islets, and several large vascularized tumors. In the hyperplastic islets (Fig. 2A through C), β cells showed a mosaic staining for GLUT-2 (Fig. 2A) and no staining for GLUT-1 (Fig. 2B). Importantly, there were groups of β cells that stained for insulin (Fig. 2C) but not for GLUT-2 (Fig. 2A). Note that some background cytoplasmic GLUT-1 staining was observed with exocrine cells surrounding the islet (Fig. 2B). In the tumor, staining for GLUT-2 also appeared mosaic in insulin-containing cells (Fig. 2D), while there was no plasma membrane staining for GLUT-1 (Fig. 2E) and most cells stained for insulin (Fig. 2F); some weak intracellular staining for GLUT-1 was observed. These results are representative of seven RIP Tag2 mice with tumors.

Figure 3 demonstrates that some insulin-positive β cells in a hyperplastic islet and a tumor from a 12-week-old RIP Tag2 mouse do not express GLUT-2. The same sections were double-stained for GLUT-2 (Fig. 3A, C, and E) and for insulin (Fig. 3B, D, and F). In a normal islet, every cell that stained for insulin (Fig. 3B) also stained for GLUT-2 (Fig. 3A). In a hyperplastic islet (Fig. 3C and D) and in a tumor (Fig. 3E and F), many GLUT-2-negative cells contained insulin (Fig. 3, arrows).

**GLUT-1 and GLUT-2 expression is regulated in tumors and in βTC3 cells.** Cultured βTC3 cells, which are derived from T antigen-expressing β-cell tumors, were immunostained for glucose transporters and insulin (Fig. 2G through I). Only a few cells showed plasma membrane staining for GLUT-2 (Fig. 2G), while many cells stained for GLUT-1 (Fig. 2H) and most stained for insulin (Fig. 2I). βTC3 cells are capable of forming tumors when injected into syngeneic mice. These secondary tumors showed staining patterns for GLUT-1 and GLUT-2 which were similar to that of the primary tumor: a heterogeneous expression of GLUT-2 (Fig. 2J), no GLUT-1 expression (Fig. 2K), and general staining for insulin (Fig. 2L). To confirm the immunofluorescence results, Western blotting (immunoblotting) analysis was performed with equal amounts of protein from βTC3 cells and the primary and secondary tumors. The βTC3 cells contained at least seven- to eightfold more GLUT-1 protein than did normal islets or primary or secondary tumors (Fig. 4).

The differences in GLUT-1 and GLUT-2 expression between the βTC3 cells in culture and the tumors in vivo were also observed at the level of mRNA accumulation. The levels of GLUT-1 and GLUT-2 mRNAs in primary tumors, βTC3 cells at different passages in culture, and secondary tumors were analyzed. Northern blots were first probed with GLUT-1 cDNA. Following autoradiography, the probe was removed and the filter was rehybridized with a cDNA probe for GLUT-2 (Fig. 5). The primary tumor (Fig. 5A, lane T-RIP Tag2) showed very low-level expression of both glucose transporters; GLUT-2 expression was ~20% that of normal islets. The GLUT-1 mRNA level in βTC3 cells progressively increased during passage in tissue culture; compare the intensity at passage 14 (lane βTC3-p14) with that at passage 31 (lane βTC3-p31). In contrast, the levels of GLUT-2 mRNA in βTC3 cells were much lower than those in normal islets and decreased slightly during propagation in culture (compare lane βTC3-p14 with βTC3-p31). The two secondary tumors analyzed (Fig. 5B, lanes $T^21$-RIP Tag2 and $T^22$-RIP Tag2) had much lower levels of GLUT-1 mRNA than βTC3 cells. In contrast, the level of GLUT-2 mRNA, which was low in the primary tumor and the βTC3 cells, increased in one of the secondary tumors (Fig. 5A, lane $T^21$-RIP Tag2) and remained unchanged in the other (Fig. 5A, lane $T^22$-RIP Tag2) but was still lower than the level in normal islets. Importantly, in normal rat islets that were kept overnight in culture, GLUT-1 mRNA was also detected. This mRNA was induced during tissue culture, since RNA prepared from fresh rat islets did not contain detectable levels of GLUT-1 (30a). Thus, we determined whether GLUT-1 protein is induced during in vitro culture by immunofluorescence microscopy of freshly isolated mouse pancreatic islets and islets after 24 h in tissue culture (see Materials and Methods). Figure 6 shows that GLUT-1 protein is detected in the plasma membrane of islet cells only after tissue culture; GLUT-1 is not detected in freshly isolated islets. Northern blotting of mRNA from primary mouse islets confirms that GLUT-1 mRNA is undetectable in fresh islets and is induced after 24 h in tissue culture; GLUT-2, which is expressed at high levels in fresh islets, is barely seen after 24 h in tissue culture (Fig. 7). Hence, induction of GLUT-1 mRNA and protein in RIP Tag2 insulinoma cells is a tissue culture phenomenon and is not a result of transformation per se.

Table 1 summarizes the relative expression of the two glucose transporter isoforms in islets and tumors. GLUT-1 is not detected by immunofluorescence in the plasma membranes of normal islets, plasma membranes of islets from RIP Tag2 mice, or membranes from primary or secondary tumors. In contrast, high levels of GLUT-1 mRNA and protein are detected in βTC3 cells and also in normal islets kept in culture overnight. GLUT-2 expression is reduced

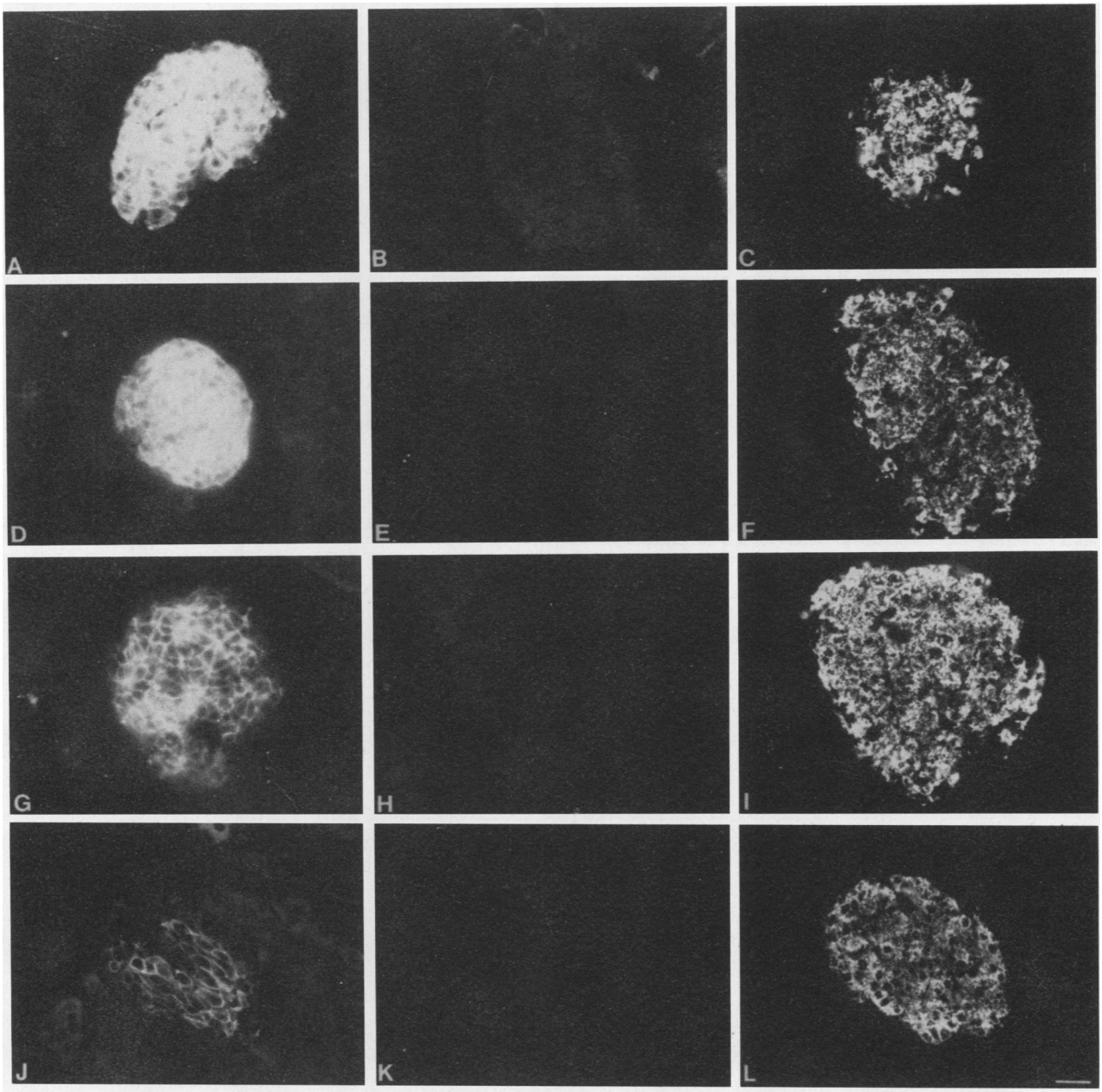


FIG. 1. Immunohistochemical localization of GLUT-2, insulin, and GLUT-1 in pancreatic islets from RIP Tag2 mice. (A to C) Pancreatic islets from a normal mouse; (D to L) islets from RIP Tag2 mice at the following ages (in weeks): 4.5 (D to F), 8.5 (G to I), and 12.5 (J to L). Sections were stained for GLUT-2 (A, D, G, and J), GLUT-1 (B, E, H, and K), and insulin (C, F, I, and L). Note that the photographs in panels A and D were exposed twice as long as those in panels G and J (therefore, panels A and D appear brighter than they really are). The GLUT-2 protein is localized to the plasma membrane of the β cells (A, D, G, and J), and its level is reduced gradually during tumor progression. Normal-size islets from tumor-bearing mice show a mosaic staining for GLUT-2 (J). No staining is observed for GLUT-1 in β cells at any stage (B, E, H, and K). Bar, 30 μm.

gradually during neoplastic transformation, while insulin is expressed during all stages. Thus, in βTC3 cells in culture, GLUT-1 is the predominant glucose transporter isoform, while GLUT-2 is the dominant isoform expressed in normal islets and transgenic islets and tumors in vivo.

**Altered glucose-stimulated insulin secretion in transformed β cells from RIP Tag2 mice.** βTC3 cells in culture secrete insulin in response to very low levels of glucose (5, 6); the stimulation obtained with 1 mM glucose is approximately 75% of the maximal stimulation achieved by the further elevation of glucose concentration. This is in contrast to results with isolated normal islets, in which insulin secretion occurs at a low basal rate in the presence of 5 mM glucose and reaches its maximum rate at 16 to 20 mM. To determine whether this increased sensitivity to glucose correlates with altered expression of GLUT-1 or GLUT-2, we assayed glucose-induced insulin release from β cells in RIP Tag2 islets and tumors, which express only GLUT-2. As shown in

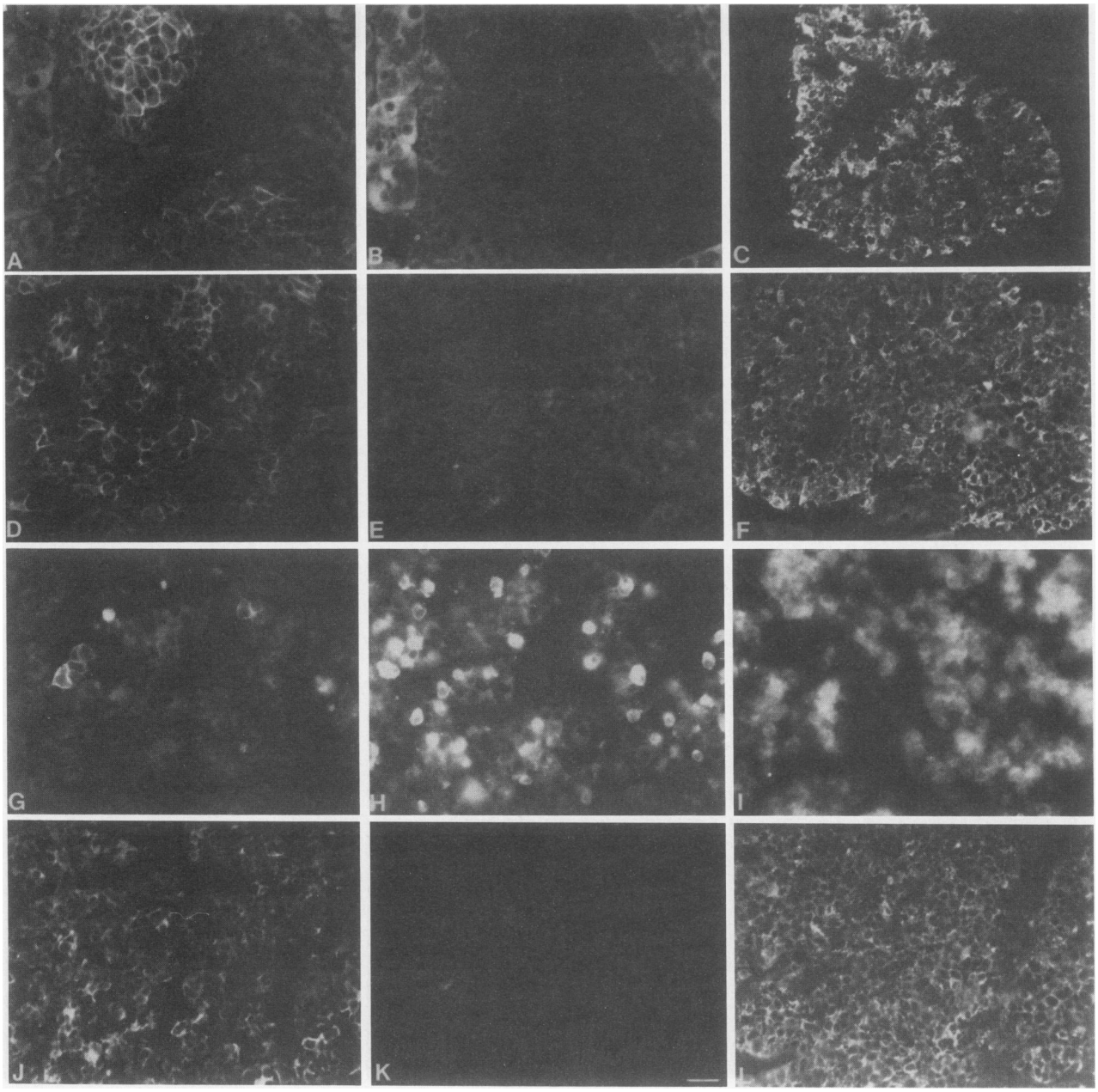


FIG. 2. Immunofluorescence detection of GLUT-2, insulin, and GLUT-1 in primary and secondary tumors and βTC3 cells. (A to C) Consecutive sections from a single hyperplastic islet from a 12.5-week-old RIP Tag2 mouse; (D to F) sections from a primary tumor from the same mouse; (G to I) sections from the βTC3 cell line fixed in situ; (J to L) sections from a secondary tumor which developed in a syngeneic mouse 10 weeks after an intraperitoneal injection of βTC3 cells. Sections in panels A, D, G, and J are stained for GLUT-2; those in panels B, E, H, and K are stained for GLUT-1; and those in panels C, F, I, and L are stained for insulin. In the hyperplastic islet (A to C), all three consecutive sections are similarly orientated and are characterized by the triangular shape of this islet. The hyperplastic islet (A) shows a mosaic staining for GLUT-2; there are some islet β cells that do not stain for GLUT-2 (A) but do stain for insulin (C). No staining of β cells with antibodies for GLUT-1 is observed (B); some background staining of the cytoplasm of exocrine cells is observed (B). The tumor shows a mosaic staining for GLUT-2 (D) but no staining for GLUT-1 (E); most cells stain for insulin (F). The pattern of glucose transporter expression in the βTC3 cell line (passage 33) is opposite that of the tumor; a few cells stain weakly for GLUT-2 (G), while most of the cells stain for GLUT-1 (H) and insulin (I). In contrast, the staining pattern for the secondary tumor (J to L) is similar to that for the primary tumor; there is a low, mosaic staining for GLUT-2 (J) and no staining for GLUT-1 (K), and all cells stain for insulin (L). Bar, 30 μm.

Table 2, insulin release from normal islets increased progressively as glucose concentrations increased from 5 to 22 mM, with maximal secretion at 22 mM. Islets from 4- to 5-week-old RIP Tag2 mice also released increasing amounts of insulin in response to increasing glucose concentrations. However, the maximal insulin release from the transgenic islets occurred at 10 mM glucose, a concentration lower than that for normal islets. Insulin release from cell suspensions obtained from both primary and secondary tumors was also stimulated by glucose. However, the basal (constitutive)

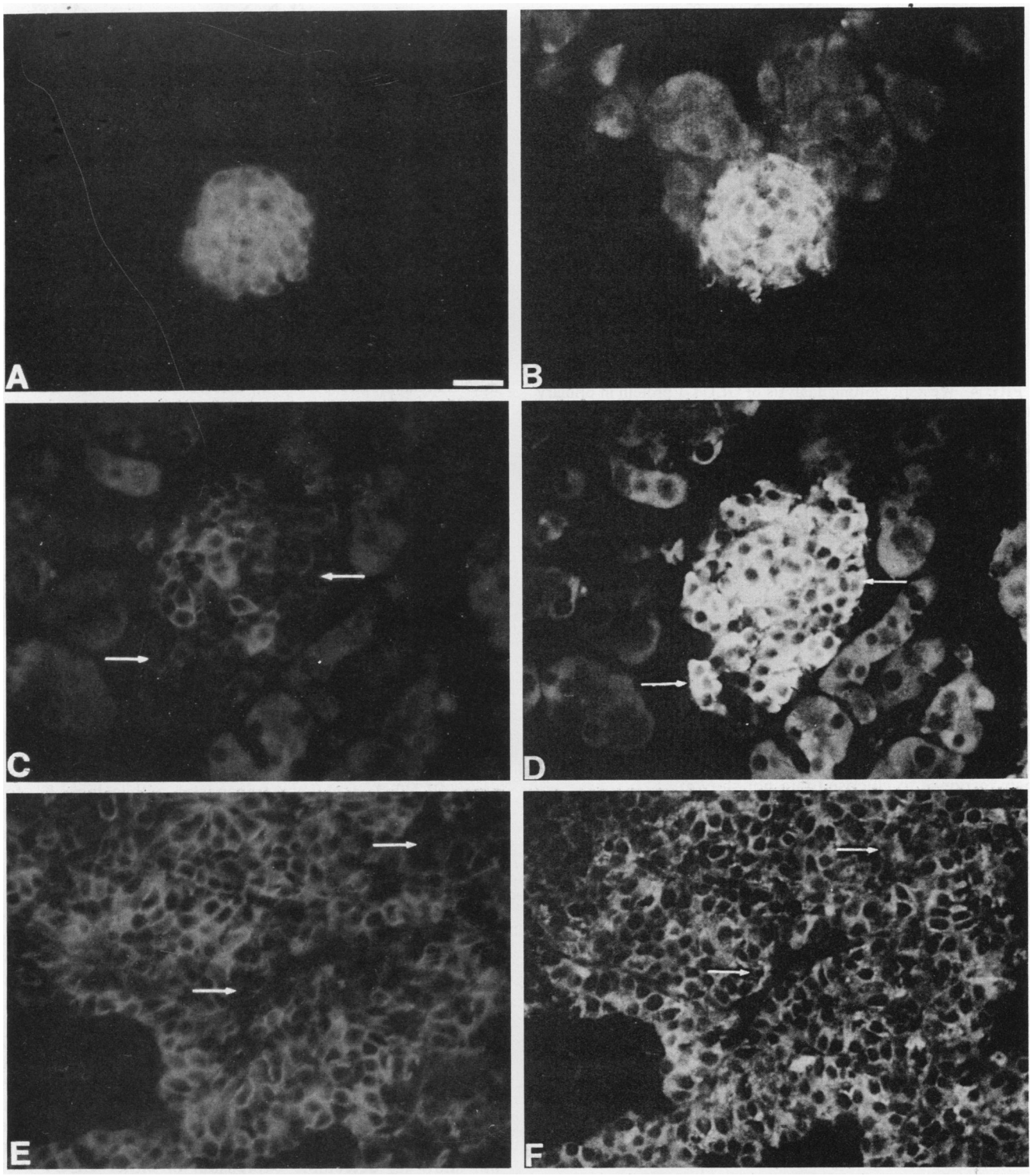


FIG. 3. Double-immunofluorescence detection of GLUT-2 and insulin in pancreatic islet β cells from RIP Tag2 mice. (A and B) Islet from a normal mouse; (C to F) islets from a 12.5-week-old RIP Tag2 mouse; (C and D) hyperplastic islet; (E and F) primary tumor. Sections were stained for GLUT-2 (fluorescein) (A, C, and E) and then stained for insulin (rhodamine) (B, D, and F). Every β cell from the normal mouse expresses both GLUT-2 and insulin (A and B). In the hyperplastic islet and the tumor, many β cells that stain for insulin do not express GLUT-2 (arrows). Bar, 30 μm.

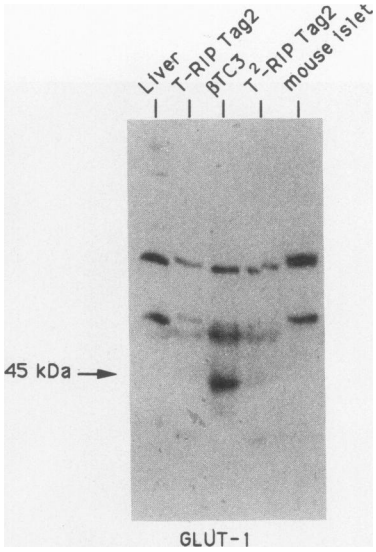


FIG. 4. Western blotting analysis of GLUT-1 protein in primary and secondary insulinomas from RIP Tag2 transgenic mice and βTC3 cells. Cell lysates (30 μg of protein) from mouse liver and islets, a primary tumor (lane T-RIP Tag2) of a RIP Tag2 mouse, a secondary tumor (lane $T^2$-RIP Tag2) from mice injected with βTC3 cells, and cultured βTC3 cells were resolved by SDS-PAGE (7.5% acrylamide) and transferred to a nitrocellulose filter. The filter was incubated for 1 h with a polyclonal serum raised against a peptide corresponding to the COOH-terminal amino acids of GLUT-1 (32), and bound antibody was detected with $^{125}$I-protein A. Exposure time with Kodak XAR-5 film at −70°C with an intensifying screen was 3 days. The arrow on the left indicates the expected position of the GLUT-1 polypeptide. The two upper bands are nonspecific and unrelated to GLUT-1.

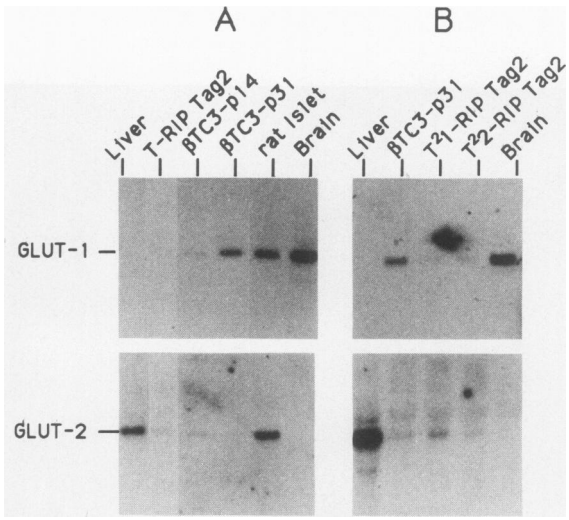


FIG. 5. Northern blotting analysis of GLUT-1 and GLUT-2 mRNA in tumors and in a cell line derived from RIP Tag2 mice. Ten micrograms of total RNA was resolved by electrophoresis on a 1% agarose-formaldehyde gel, and was transferred to nylon filters. The filters were hybridized under high-stringency conditions with a GLUT-1 cDNA probe (yield, 2.8-kb message). Following autoradiography, the probe was removed and the filter was rehybridized with the GLUT-2 cDNA probe (yield, 2.5-kb message). (A) RNA was extracted from rat liver, a primary tumor from a RIP Tag2 mouse (lane T-RIP Tag2), βTC3 cells at passages 14 and 31 (lanes βTC3-p14 and βTC3-p31), rat pancreatic islets that were kept overnight in tissue culture, and rat brain. (B) RNA was extracted from two different secondary tumors (lanes $T^2$1-RIP Tag2 and $T^2$2-RIP Tag2) and the control tissues used for panel A. Exposure times with Kodak XAR-5 film at −70°C using an intensifying screen were 4 days for GLUT-1 and 6 days for GLUT-2.

release at 5 mM glucose was high compared with release from both normal islets and islets from transgenic mice, as well as βTC3 cell lines (6). Hyperplastic islets in tumor-bearing transgenic mice resembled the primary tumor cells in their response to glucose (data not shown).

It therefore appears that the progression of β cells through tumorigenesis is accompanied by a gradual loss of the normal response to glucose, manifested by maximal insulin secretion at lower-than-normal glucose concentrations. Changes in the response to glucose are observed with mice at early stages of tumorigenesis that appear by both immunofluorescence (Fig. 1A and D) and Western blotting (data not shown) to have normal GLUT-2 expression in their islets. Later stages of neoplastic progression show a gradual loss of GLUT-2 expression that correlates with a loss of glucose-dependent insulin secretion. In contrast, insulin secretion in cultured βTC3 cells is stimulated by glucose concentrations below 1 mM (5), which may result from induction of the low-$K_m$ GLUT-1 transporter during propagation in culture.

## DISCUSSION

**Regulation of GLUT-1 and GLUT-2 expression in β cells from RIP Tag mice.** The facilitated diffusion glucose transporter GLUT-1 has a low $K_m$ for glucose (1 to 3 mM) (37), well below the level of glucose in normal blood (5 mM) or tissue culture medium. It is expressed normally in many cell types (2, 7) but is not detectable in normal islets (27, 33). However, its expression is very high in all transformed cell lines studied, including insulinomas (33). Increased expression of GLUT-1 has been suggested to be the direct result of oncogenic transformation (3, 8). Islet β cells normally express GLUT-2, a glucose transporter with a high $K_m$ for glucose (17 mM) (13). A transgenic mouse model in which the development of pancreatic islet β-cell tumors is heritable (10) was used to determine the regulated expression of GLUT-1 and GLUT-2 during oncogenic transformation of islet β cells. Our principal conclusion is that in this model, GLUT-1 is not induced by transformation per se but appears only in cells propagated in culture, an event which is reversed in secondary tumors formed after injection of cultured cells into syngeneic mice. Indeed, GLUT-1 expression is induced in normal islets upon culture overnight (Fig. 6 and 7).

In young transgenic mice (4 to 5 weeks old), GLUT-2 expression in islets is similar to that in normal mice. During tumorigenesis, we detected reduced and heterogeneous expression of GLUT-2 protein but no GLUT-1 protein on the plasma membranes of islet β cells. In particular, there are islet β cells and insulinomas in situ that express insulin but show no detectable expression of either GLUT-1 or GLUT-2 (Fig. 2 and 3). Our results demonstrate that transformation of β cells in vivo results in a gradual reduction in expression of GLUT-2 but that the decrease in GLUT-2 is not accompanied by detectable expression of GLUT-1 on plasma membranes, as judged by immunofluorescence. However, a low level of expression of GLUT-1 can be detected in primary tumors by Northern and Western blot

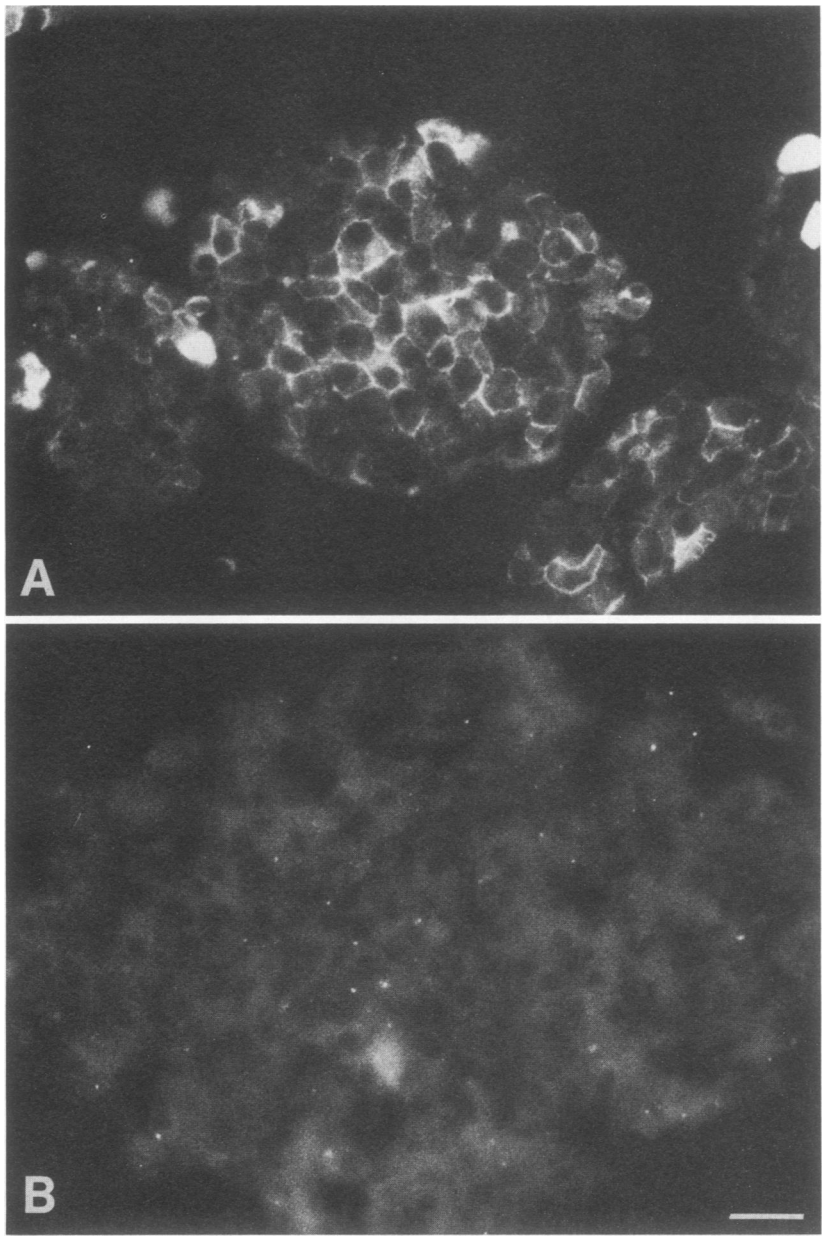


FIG. 6. Immunofluorescence detection of GLUT-1 in primary cultured pancreatic islet cells. Sections (5 μm thick) were cut from normal mouse islets that were cultured for 24 h (A) and from a freshly isolated islet (B). Both sections were stained for GLUT-1 (see Materials and Methods) and showed expression of plasma membrane GLUT-1 protein only in cultured islets. The photograph of the section in panel B was exposed 1.3 times longer than the one in panel A to show absolutely no staining for GLUT-1 in freshly isolated islet cells. Bar, 30 μm.

analyses (Fig. 4 and 5). The reduced level of GLUT-2 in hyperplastic islets and tumors could be caused by either the elevation in blood insulin or the reduction in blood glucose generated by the insulinomas; Chen et al. (4) showed that maintenance of rat blood glucose at 50 mg/dl (3 mM) for several days causes a reduction in islet GLUT-2 expression.

In contrast, GLUT-1 expression is modulated in βTC3 cells by continuous passage in tissue culture and by introduction into animals (Fig. 5 and Table 1). Thus, GLUT-1 expression by βTC3 cells is induced during propagation in culture and is decreased when the cells are returned to animals, in which secondary tumors form. In contrast, the GLUT-2 level is very low in cultured cells. It is induced in βTC3 cells grown in animals and reached a level similar to that in the primary RIP Tag tumor. The difference in GLUT-1 expression observed in βTC3 cells in culture and in animals might be due to exposure to different growth factors in culture, the lack of an extracellular matrix, or a selection for certain cell properties in vitro. Indeed, expression of GLUT-1 mRNA is induced in normal islets cultured for 24 h, whereas normal islets in vivo do not express detectable levels of GLUT-1 (Fig. 5 through 7). Clearly GLUT-1 expression is not essential for tumorigenesis in vivo. In this context, Miyazaki et al. (22) reported the establishment of two cell lines from transgenic mice similar to RIP Tag2 mice. Both cell lines expressed high levels of GLUT-2, and one of these lines, MIN7, expressed high levels of GLUT-1 and had no sensitivity to glucose. The second cell line, MIN6,

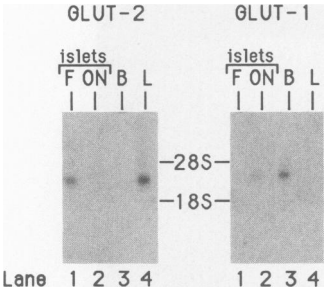


FIG. 7. Analysis of GLUT-1 and GLUT-2 mRNA in primary cultured mouse pancreatic islets. RNA was extracted from freshly isolated C57BL/6 mouse islets (lane 1), mouse islets that were cultured for 24 h in Dulbecco's minimal essential medium containing 5 mM glucose, 15% horse serum, and 2.5% fetal bovine serum (lane 2), rat brain (lane 3), and rat liver (lane 4). One microgram of total RNA was resolved by electrophoresis on each of two identical 1% agarose-formaldehyde gels and transferred to nylon filters. The filters were hybridized under high-stringency conditions with GLUT-2 cDNA probe (yield, 2.5-kb message) or GLUT-1 cDNA probe (yield, 2.8-kb message). Exposure time with Kodak XAR-5 film at −70°C with an intensifying screen was 2 weeks.

TABLE 2. Glucose-regulated insulin release from islet and tumor cells derived from RIP Tag2 mice[a]

| Glucose concn (mM) | Insulin secretion (ng/100 ng of content)[b] | | | |
|---|---|---|---|---|
| | Islets | | Tumors | |
| | Normal | RIP Tag2 | Primary | Secondary |
| 5 | 0.85 ± 0.68 | 1.66 ± 0.40 | 4.52 ± 1.61 | 6.86 ± 0.99 |
| 6 | 1.45 ± 0.85[c] | 3.13 ± 1.16[c] | 5.73 ± 1.87 | 8.09 ± 1.58 |
| 10 | 3.89 ± 1.44[c] | 11.24 ± 3.04[d] | 7.52 ± 2.25[d] | 8.01 ± 1.30 |
| 22 | 15.78 ± 6.05[c] | 10.57 ± 3.27[e] | 10.47 ± 5.12[e] | 9.77 ± 2.07[e] |

[a] Islets from normal mice and 4- to 5-week-old RIP Tag2 mice, primary tumor cells, and secondary tumor cells were incubated for 2 h in medium containing 5 mM glucose. The medium was replaced with fresh media containing various glucose concentrations and 0.5 mM 1-isobutyl-3-methylxanthine. After 2 h of incubation at 37°C, the medium and cell extract were assayed for insulin by radioimmunoassay.

[b] Values are the means ± standard error of the mean for three independent experiments. Statistical analysis was done by *t* test.

[c] $P < 0.05$, compared with value for 5 mM glucose and the adjacent glucose concentration.

[d] $P < 0.05$, compared with value for 5 mM glucose and the preceding concentration.

[e] $P < 0.005$, compared with value for 5 mM glucose.

expressed very low levels of GLUT-1 and exhibited glucose-inducible insulin secretion at normal glucose levels. Thus, expression of GLUT-1 is induced in all cultured β cells, and a high level of GLUT-2 expression is necessary, though not sufficient, for normal glucose sensing (see below).

It is noteworthy that most normal liver cells express only GLUT-2, while some of them, the perivenous hepatocytes, also express GLUT-1 (31). Starvation or diabetes of rats (30) or introduction of hepatocytes into cell culture (28) causes an induction of GLUT-1 expression in otherwise GLUT-1-negative cells. Thus, conditions of cell culture or other metabolic changes can trigger GLUT-1 induction in several types of cells.

**GLUT-2 expression and glucose-regulated insulin secretion in β cells from RIP Tag2 mice.** Glucose efficiently and rapidly enters islet cells, and the intracellular glucose concentration approximates that of blood glucose (21). Inside the β cell, glucose is metabolized through the glycolytic pathway and one of the metabolic products, probably ATP, activates insulin secretion. There are several examples of diabetic rats and mice in which glucose-insensitive insulin secretion is correlated with a large (≥60%) reduction in GLUT-2 expression in β cells. Some cells lose GLUT-2 expression altogether. This includes both pharmacologic and genetic models of diabetes (4, 12, 24, 34). The same is true for tumors and hyperplastic islets in the RIP Tag2 mice studied here, which exhibit a poor response to elevations in extracellular glucose (Tables 1 and 2). In contrast, in islets from 4- to 5-week-old RIP Tag2 mice, insulin secretion responds to glucose but maximal secretion occurs at lower glucose concentrations than in normal islets. This is in spite of the fact that all β cells express apparently normal amounts of GLUT-2, as judged by immunostaining (Fig. 1A and D). Although immunostaining is not quantitative, it is capable of detecting variations in staining intensity greater than 25%. This increased sensitivity to glucose may be caused by inhibition of GLUT-2 transport functions or an alteration in another component of glucose sensing, such as glucokinase, which has a high $K_m$ for glucose and which catalyzes a rate-limiting step in glycolysis (21). In particular, induction of hexokinase, an enzyme with a low $K_m$ for glucose, could abrogate normal glucose sensing.

TABLE 1. Relative plasma-membrane expression of glucose transporters during oncogenic transformation in RIP Tag2 mice and βTC3 cells

| Transporter | Level of expression in[a]: | | | | | |
|---|---|---|---|---|---|---|
| | Normal islets | | RIP Tag2 mice (wk) | | | |
| | | | | | 12 | |
| | In situ | 24-h culture | 4 | 8 | Normal-size islet | Hyperplastic islet |
| GLUT-2 | ++++ | ++[b] | ++++ | ++ | + | + |
| GLUT-1 | − | +++ | − | − | − | − |
| Insulin | +++ | +++[b] | +++ | +++ | +++ | +++ |

[a] −, no expression; + through ++++, increasing levels of expression. Data were confirmed by Western or Northern blotting or both.

[b] Data are from reference 30a.

The mosaic staining for GLUT-2 in tumors in situ (Fig. 1 through 3) and the fact that some tumor cells appear to express neither GLUT-1 nor GLUT-2 suggest that another glucose transporter isoform (known or unknown) may be expressed in islet β cells or tumors of RIP Tag2 mice. We assessed the repertoire of glucose transporters in RIP Tag2 insulinomas by performing polymerase chain reaction amplification of glucose transporters from a cDNA library of RIP Tag2 tumor cells and by using degenerate oligonucleotides which are complementary to the mRNA sequence of all known facilitated diffusion glucose transporters. This analysis detected, in addition to GLUT-1 and GLUT-2, a few clones encoding the mouse homologs of GLUT-3 (16) and GLUT-5 (15, 30b). The complete cloning and studies of the role of these glucose transporters in insulinomas are in progress, and we do not know yet whether the glucose transporters are expressed in transgenic β cells. Both GLUT-2 and GLUT-1 are expressed in perivenous hepatocytes (31), so there is a precedent for expression of two glucose transporter isoforms in the plasma membrane of the same cell. It is possible that a second glucose transporter (e.g., GLUT-3 or GLUT-5) is expressed together with GLUT-2 in some β cells and that a balanced expression of low- and high-$K_m$ glucose transporters might be a part of the

mechanism of glucose sensing. Alterations in the number or activity of glucose transporters on the plasma membrane could cause glucose insensitivity in pathologic conditions such as insulinomas and diabetes.

## ACKNOWLEDGMENTS

We thank M. J. Charron, G. Christofori, H. Y. Lin, and H. Youssoufian for comments on the manuscript; O. A. Emran and D. Fusco-Demane for excellent technical assistance; and M. L. Stuppard for helping with manuscript typing.

This work was supported by NIH grants GM 40916 and HL 41484 to H.F.L. and DK 20541 to the Albert Einstein College of Medicine Diabetes Research and Training Center. M.T. was supported by a fellowship from the European Molecular Biology Organization, B.T. was supported by the Swiss National Science Foundation, and S.E. was supported by the Juvenile Diabetes Foundation.

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