Role of the GLUT 2 Glucose Transporter in the Response of the L-type Pyruvate Kinase Gene to Glucose in Liver-derived Cells* (Received for publication, July 8, 1996, and in revised form, April 1, 1997)

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Twenty-six different hepatoma cell lines established from cancer-prone transgenic mice exhibited a close correlation between expression of the GLUT 2 glucose transporter and activation of the L-type pyruvate kinase (L-PK) gene by glucose, as judged by Northern blot analyses and transient transfection assays. The L-PK gene and a transfected L-PK construct were silent in GLUT 2(+) cells and active in GLUT 2(−) cells cultured in glucose-free medium. Transfection of GLUT 2(−) cells with a GLUT 2 expression vector restored the inducibility of the L-PK promoter by glucose, mainly by suppressing the glucose-independent activity of this promoter. Culture of GLUT 2(−) cells, in which the L-PK gene is constitutively expressed, in a culture medium using fructose as fuel selected GLUT 2(+) clones in which the L-PK gene responded to glucose.

The expression of the L-PK gene in GLUT 2(−) cells cultured in the absence of glucose was correlated with a high intracellular glucose 6-phosphate (Glu-6-P) concentration while under similar culture conditions Glu-6-P concentration was very low in GLUT 2(+) cells. Consequently, a role of GLUT 2 in the glucose responsiveness of glucose-sensitive genes in cultured hepatoma cells could be to allow for Glu-6-P depletion under gluconeogenic culture conditions. In the absence of GLUT 2, glucose endogenously produced might be unable to be exported from the cells and would be phosphorylated again to Glu-6-P by constitutively expressed hexokinase isoforms, continuously generating the glycolytic intermediates active on the L-PK gene transcription.

Glucose is an important regulator of gene transcription in most prokaryotic and eukaryotic species. In vertebrates, it modulates the expression of genes for enzymes involved in metabolic regulation in the liver and adipose tissue and of insulin in β cells of the endocrine pancreas (1). In these three glucose-sensitive tissues, glucose transport and phosphorylation are performed by various isoforms of glucose transporters and hexokinases, respectively. Glucose phosphorylation to glucose 6-phosphate, required for glucose action on the transcriptional machinery (2–6), is mainly mediated by hexokinase (HK)1 II in adipocytes and by hexokinase IV (or glucokinase) in the liver and β cells; expression of the glucokinase gene is constitutive in the pancreas and insulin-dependent in the liver due to the existence of two different tissue-specific alternative promoters (7). However, glucokinase is replaced by other insulin-independent hexokinase isoforms (mainly HK I) in cultured hepatoma cell lines (2, 8, 9). Tissues whose function is regulated by glucose also possess particular glucose transporters, GLUT 4 in adipocytes and GLUT 2 in tissues secreting glucose into the blood (liver, small intestine, and proximal tubular cells of the kidney) as well as GLUT 2 in the β cells of the islets of Langerhans (10, 11). In pancreas β cells, it has been suggested that GLUT 2 and glucokinase are essential components of the “glucose sensor” (12, 13). However, the mechanisms of the GLUT 2-specific role in the regulation of insulin secretion by glucose remain unclear (13, 14).

In most hepatoma cell lines in culture, glucose responsiveness of glucose-dependent genes is lost (15) even when these cells remain well differentiated (2). In fact, expression of glucose-responsive genes is generally constitutive in these cells in which glucokinase is replaced by HK I and GLUT 2 is replaced by GLUT 1, another glucose transporter isoform expressed in most cells not specialized in metabolism regulation, e.g. in cancerous cells (8, 16).

However, we recently succeeded in isolating various well differentiated cell lines that have conserved sensitivity of their transcriptional machinery to glucose (2). Here, we show that glucose responsiveness of the L-PK gene in these cells requires synthesis of GLUT 2, and we suggest that GLUT 2, but not GLUT 1, allows for efflux of glucose endogenously produced by the gluconeogenic pathway when cells are cultured without glucose. Therefore, in the absence of GLUT 2, glucose-responsive genes would be permanently stimulated by glycolytic intermediates of endogenously produced glucose.

**MATERIALS AND METHODS**

**Cell Lines and Culture**—mhAT and mhPKT hepatoma cell lines were derived from transgenic mice synthesizing the SV40 large T and small t antigens in the liver under the direction of either the antithrombin III gene or L-PK gene regulatory sequences (17, 18). Hepatocytes were obtained from transgenic animals of different ages with either normal, fetal (mhPKT35), or tumoral (mhPKT, mhAT1, and mhAT5) livers and established in culture as described previously (19–21). About 30 mhAT1- and mhAT3-derived subclones were isolated using different selective media, particularly containing fructose as the only carbohydrate source (19, 20). Lines mhPKT35F1 and F2 were subcloned from

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1 The abbreviations used are: HK, hexokinase; Glu-6-P, glucose 6-phosphate; L-PK, L-type pyruvate kinase; PABP, poly(A)-binding protein; CAT, chloramphenicol acetyltransferase; RSV, Rous sarcoma virus; CMV, cytomegalovirus; GIRE, glucose response element.
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mhPKT3 cells by using such a fructose medium. All hepatoma cell lines were usually grown in Ham’s F12/Dulbecco’s modified Eagle’s medium (v/v), Glutamax medium (Life Technologies, Inc., Chagrin Falls, OH) supplemented with penicillin, streptomycin, 0.1 μM insulin, 1 μM dexamethasone, 1 μM triiodothyronine, and 5% (v/v) fetal calf serum.

Induction Studies—For the glucose induction studies, cells were cultured in a serum-free medium supplemented with 10 μM transferrin and 100 μg/ml albumin. In general, cells were cultured for 24 h before induction in a glucose-free, serum-free medium supplemented with 1 μM triiodothyronine, 1 μM dexamethasone, and 10 mM lactate (“lactate” conditions). Induction was then performed by replacing lactate with 17 mM glucose for 24 h (or less when mentioned).

Induction of the L-PK and GLUT 2 mRNA levels was also analyzed in the presence of various concentrations of glucose for 24 h or in the presence of 17 mM glucose and protein inhibitors, such as 10 mM cycloheximide or 25 mM anisomycin, for the times given in the legend to Fig. 5.

Northern Blot Analysis—Total RNAs were isolated from cell lines by lysis in 7 M guanidinium HCl followed by phenol extraction (22). RNAs were denatured with methylmercury (II) hydroxide, were electro- phoretically separated on formaldehyde, 1.2% agarose gels, and then were transferred and UV cross-linked to nylon membranes (Hybond N, Amersham Corp.). Prehybridization (1 h with 100 μg/ml salmon sperm DNA) and hybridization were carried out as described previously (19) except that the temperature was 55°C when rat or human probes were used. Quantitation of the radioactive bands on the nylon membranes was performed using a PhosphorImager (Molecular Dynamics).

The probes for rat L-PK and human poly(A)-binding protein (PABP) sequences have been described previously (19). The specific 341-base pair probe for mouse L-PK cDNA was obtained by reverse transcriptase-polymerase chain reaction of mouse mRNA in the laboratory.2 The probes for rat GLUT 2 (10) and human GLUT 1 (23) sequences were obtained from corresponding expression vectors kindly given by Bernard Thorens (Lausanne, Switzerland). The probe for rat hexokinase I sequence was from the laboratory of D. Granner (24).

Transfection Experiments—The LAL3–119 L-PK/CAT and -119L-PK/CAT constructs have been described previously (25). Transfection was performed by lipofection using N-[1-(2,3-dioleoyloxy)propyl]-N,N,N,N-tetramethylammonium methyl sulfate (DOTAP, Boehringer-Mannheim, Mannheim, Germany) according to the manufacturer instructions in cells cultured in glucose-free, serum-free medium for 3–4 h. Five μg of CAT constructs and 2 μg of pBSV-luciferase (or 1 μg of pCMV-GLUT) were cotransfected in 60-mm plastic dishes (Falcon, Oxnard, CA) when cells were 60–80% confluent. After 16 h, the medium containing the lipoliposome/DNA complex was removed and replaced with a 17 mM glucose or a 10 mM lactate medium for 24 h. CAT and luciferase activity assays were performed as described (25). CAT activity was calculated as the percentage of diacetylated form versus nonmetabolized chloramphenicol. Results were expressed as a ratio of CAT activity to luciferase activity to correct for the variable transfectability.

When transfections were performed with pCMV expression vectors, pBSV-luciferase was omitted because of the risk of titration of ubiquitous transcription factors. In such a case, CAT activities were measured in the entire extract of each dish to compensate for the differential growth of the cells (i.e. dilution of the CAT activity) when cultured in glucose versus lactate medium for induction studies. The GIRE-mediated glucose responsiveness of the L-PK promoter was assessed by standardizing the activities obtained with the LAL3–119L-PK/CAT construct according to those obtained with the control -119L-PK/CAT construct.

Immunofluorescence Studies—mhPKT3 cells were attached to coverslips and treated as described previously by Hughes et al. (26) except that permeabilization was done with 0.2% Triton X-100 for 5’. Immunofluorescent staining of GLUT 1 and GLUT 2 was carried out using a 1:100 dilution of antisera kindly provided by B. Thorens (Lausanne, Switzerland) that recognized GLUT 1 from human, rat, and mouse and GLUT 2 from rat. Staining of recombinant or endogenous GLUT 2 was also confirmed with other antisera kindly provided by S. W. Cushman (Bethesda, MD) and Luc Pénaud (Toulouse, France) (data not shown).

Determination of Glucose 6-Phosphate Concentration—Glucose 6-phosphate in cultured hepatoma cells was assayed enzymatically according to Slein (27) as described previously (9).

Statistics—Results are given as means + S.D. Statistical significance

RESULTS

Correlation between GLUT 2 mRNA Level and Glucose Responsiveness of the L-PK Gene in 26 Different Hepatoma Cell Lines—Different lines of hepatoma cells were derived from transgenic mice synthesizing the SV40 large T and small t antigens in the liver under the direction of either antithrombin III gene or L-PK gene regulatory sequences (17, 18). Hepatocytes were obtained from fetal or adult transgenic animals with either normal (mhPKT3) or tumoral livers (mhPKT, mhAT1, and mhAT3 cell lines) and established in culture as described previously (19, 20). Each line was derived from a single clone. Fig. 1A shows that induction of L-PK mRNA accumulation by 17 mM glucose in 26 independent clones was proportional (r = 0.92 by linear regression analysis) to their GLUT 2 mRNA content at the same time. In addition, all these clones also contain more or less GLUT 1 mRNA roughly in reverse proportion to GLUT 2 mRNA (not shown). The results obtained for three clones, which synthesized different amounts of GLUT 2 mRNA when cultured in the presence of 17 mM glucose, are presented in Fig. 1B. In cells with low GLUT 2 mRNA levels, L-PK mRNA accumulation was practically constitutive, independent of glucose concentration in the medium. In contrast, in GLUT 2-expressing cells, the amplitude of the glucose responsiveness of the L-PK mRNA expression was correlated with the GLUT 2 mRNA level. It should be observed that GLUT 2 mRNA accumulation is itself dependent on glucose, as previously demonstrated in hepatocytes and mhAT3F cells (3, 28, 29), whereas abundance of GLUT 1 mRNA was independent of glucose (Fig. 5).

To confirm whether the positive correlation between GLUT 2 expression and response of the L-PK gene to glucose was me-
mediated by the L-PK glucose response element (GlRE), we chose 8 clones among the 26 presented in Fig. 1 in which we tested glucose responsiveness of the L4L3–119L-PK/CAT construct (25). It was previously demonstrated that the responsiveness of this construct to glucose was due to the GlRE located from 2168 to 2144 base pairs with respect to the cap site (25). Fig. 2A shows that induction of the CAT activity in transiently transfected cells was also dependent on GLUT 2 mRNA level. Activity of the transfected L-PK promoter was almost constitutive in GLUT 2(−) cells (mhAT1G8) and inducible in GLUT 2(+) cells (mhAT3F) (Fig. 2B). It is noteworthy that 2 mM fructose was also able to activate the L-PK promoter in GLUT 2(+) cells, in agreement with the known efficiency of GLUT 2 on fructose transport (30).

**Forced Expression of GLUT 2 but Not of GLUT 1 Glucose Transporter in Cells Lacking GLUT 2 Restores Some Glucose Responsiveness to the L-PK Promoter**—To study the effect of GLUT2 (or GLUT1) on the glucose responsiveness of the L-PK promoter, two hepatoma cell lines lacking GLUT 2, i.e. mhPKT3 previously established3 and HepG2, were transiently transfected with L-PK CAT constructs and CMV-expression vectors for GLUT 1 or GLUT 2.

Immunofluorescence studies were performed to provide evidence of the GLUT 1 and GLUT 2 expression induced by transient transfection of the relevant vectors (Fig. 3A).

In the mhPKT3 cell line, GLUT 1 immunofluorescence was easily detectable at the plasma membrane of the cells while no

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3 A. Vandewalle, unpublished results.

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**Fig. 2.** Correlation between transcriptional response of the L-PK gene to glucose and GLUT 2 mRNA abundance in hepatoma cell lines. Cell lines were cultured for 24 h in a glucose-free medium before induction by 17 mM glucose. A. A plot of the stimulation by glucose of the L4L3–119L-PK/CAT construct versus the simultaneous GLUT 2 mRNA accumulation in eight hepatoma cell lines. GLUT 2 mRNA levels were quantitated from Northern blots as in Fig. 1 and normalized with PABP mRNA used as unvariant standard. CAT activities of the L4L3–119L-PK/CAT construct transfected in each cell line were determined by the percentage of chloramphenicol conversion to its acetylated forms and expressed after standardization by the luciferase activity to correct for variable transfectability. Fold increase represents percent of conversion under 17 mM glucose induction/percent conversion under lactate conditions. Results are the mean of at least two experiments run separately. B, representative chromatograms of CAT experiments performed on extracts of mhAT1G8 and mhAT3F cells transfected with 5 μg of L4L3–119L-PK/CAT and 2 μg of pRSV-luciferase in a glucose-free medium and then cultured for 30 h in 10 mM lactate, 2 mM fructose, or 17 mM glucose. Slots were loaded with extract dilutions representative of constant luciferase activity.

**Fig. 3.** Effect of transient expression of GLUT 2 and GLUT 1 in cells lacking GLUT 2 on glucose responsiveness of the L-PK promoter. A, immunofluorescence studies in mhPKT3 cell line before and after transfection with GLUT expression vectors. mhPKT3 cells expressed mainly GLUT 2 (top left) as shown by the immunofluorescence staining at the surface membrane of the cells and no detectable level of GLUT 2 (bottom left). When transfected with GLUT expression vector, 4–5% of cells exhibited a very high fluorescence located around the plasma membrane of the cells. The fluorescence intensity of these transfected cells was so high that the fluorescence of non-transfected cells could no longer be detected, not even the GLUT 1-specific fluorescence.

B, 5 μg of either L4L3–119L-PK/CAT or -119L-PK/CAT constructs were co-transfected with 1 μg of CMV, CMV-GLUT 2, or CMV-GLUT 1 expression vectors in two cell lines lacking GLUT 2, mhPKT3, and HepG2. Cells were maintained for 8 h in a glucose-free medium, transfected for 16 h in the same medium, and then cultured for another 24 h either in lactate (LO), 17 mM glucose (G17), or 17 mM fructose (F17). CAT activities were measured as described under “Materials and Methods.” The glucose responsiveness of the L-PK promoter was expressed as the ratio of the L4L3–119L-PK/CAT activity to the -119L-PK/CAT activity. Results are the mean ± S.E. of four separate experiments. Statistical significance between LO and G17 culture conditions: *, p < 0.01; ***, p < 0.001. Statistical significance between CMV-vector versus GLUT 2-vector transfected cells in the same medium: ○, p < 0.05; ◯, p < 0.02.
membrane-associated GLUT 2 immunofluorescence was observed. When mhPKT3 cells were transfected with CMV-GLUT 1 or CMV-GLUT 2 expression vectors, 4–5% of the cells exhibited greatest immunofluorescence at the plasma membrane level, confirming the correct localization of the forced expressed transporters.

Response of the L4L3-L-PK promoter to glucose was calculated by dividing the CAT activities with the glucose-sensitive L4L3–119L-PK/CAT construct by those of with glucose insensitive -119L-PK/CAT construct (Fig. 3 B). Glucose responsiveness was nil in mhPKT3 cells and very weak (×1.58) in HepG2 cells. Co-transfection with the GLUT 2 expression vector resulted in a clear and significant L-PK glucose responsiveness in both cell lines (3.0- and 3.8-fold increase between lactate (Fig. 3B, LO) and glucose (Fig. 3B, G17) medium in mhPKT3 and HepG2 cells, respectively). In HepG2 cells, 17 mM fructose stimulated the L-PK promoter almost as efficiently as glucose, which confirms that GLUT 2 synthesized by transfected cells was active on fructose entry into the cells (30).

Interestingly, the response to glucose in HepG2 cells resulted in both decreased activity of the L-PK promoter under lactate conditions (×0.70) and increased activity under glucose condition (×1.70). In contrast, forced expression of GLUT 1 in these cells, which already synthesize GLUT 1 (Fig. 3A), did not significantly change glucose responsiveness of the L-PK promoter.

Induction of GLUT2 Expression by Selection of Clones in a Fructose Medium Parallels the Appearance of Glucose Responsiveness of the L-PK Gene—We observed that most of the clones isolated in our laboratory that exhibit a high GLUT 2 expression and, thus, a good glucose responsiveness had been selected for “liver-specific functions” by culture in a medium in which glucose was replaced by 2 mM fructose (2). Initially, in designing these conditions, we wanted to select cells able to use fructose efficiently through their endogenous fructokinase and aldolase, B, two enzymes specific to liver, kidney, and small intestine (31). In fact, we also selected for the expression of GLUT 2, a transporter permissive for fructose transport (30). Accordingly, we subjected mhPKT3 cells, which had never been selected by culture with fructose before, to fructose selection.

Fig. 4, A and B, shows that the amount of endogenous L-PK mRNA in mhPKT3 GLUT 2(−) cells was rather independent of glucose (×1.8) before selection and became significantly glucose-responsive in fructose-selected mhPKT3F1 (×3, 7) and mhPKT3F2 (×11) clones, concomitantly with appearance of GLUT 2 expression. Fig. 4C confirms that the recovery of GLUT 2 expression in mhPKT3F1 clone is parallel with restoration of the L-PK promoter activation by glucose (×2.5) or by fructose (×2.4).

In GLUT 2(+) cells, L-PK and GLUT 2 Genes Are Similarly Regulated by Glucose—The GLUT 2 gene is known to be regulated by glucose (3, 28, 29). We raised the question of whether the kinetics of glucose responsiveness of the GLUT 2 and L-PK genes were similar or different in mhAT3F cells. Fig. 5 shows that time course (Fig. 5B) and concentration dependence of the response of the endogenous GLUT 2 and L-PK genes to glucose (Fig. 5C) were practically similar, both requiring active protein synthesis and, therefore, being blocked by translation inhibitors such as cycloheximide and anisomycin (Fig. 5A). However, these results did not preclude that a major cause of the delay of L-PK gene response to glucose (and perhaps of its dependence on active protein synthesis) could be the need for active GLUT 2 protein synthesis. In fact, the same requirement could exert itself on GLUT 2 mRNA accumulation, with GLUT 2 gene induction by glucose being subjected, in such a case, to a positive auto-regulatory loop. Fig. 5A also shows that mhAT3F cells expressed the GLUT 1 and HK 1 genes regardless of the presence of glucose in the medium and with a low sensitivity to translation inhibitors. In contrast, these cells, like all the lines used in this study or reported in the literature (2, 8, 9), were devoid of glucokinase gene expression (not shown).

GLUT 2 Production from an Expression Vector Neutralizes the Latency of L-PK Gene Transcription by Glucose in mhAT3F Cells—To further test the hypothesis that the need for previous GLUT 2 synthesis could be involved in the delayed response of the L-PK gene to glucose in cells deprived of glucose for more than 30 h before induction, we forced the expression of GLUT 2 in mhAT3F cells cultured in lactate for 64 h (endogenous GLUT 2 mRNA being absent) and then analyzed the time course of the L4L3–119L-PK/CAT construct activation by glucose (Fig. 6B). As recalled in Fig. 6A, when mhAT3F cells were deprived of glucose for more than 30 h, activation of the L-PK promoter was very low until the 14th h (2) while induction was more rapid after glucose deprivation of only 15 h. In cells that accu-
mM glucose and 10 nM insulin for the indicated times. The same blot was sequentially hybridized with the different probes. A, time course of L-PK and GLUT 2 mRNA induction by 17 mM glucose and a quantitative representation of the Northern blot as shown in panel A. C, GLUT 2 mRNAs by culture in 10 mM lactate for 40 h (time 0), cells were grown for 24 h in a medium containing from 2.5 to 17 mM glucose. The mRNAs were cultured and GLUT 2 mRNAs in mhAT3F cells.

Fig. 5. Parallelism between glucose responsiveness of GLUT 2 and L-PK mRNAs in mhAT3F cells. Time-course and glucose dose-response of L-PK and GLUT 2 endogenous gene expression in mhAT3F cells. A, analysis by Northern blot. After decreasing the levels of L-PK and GLUT 2 mRNAs by culture in 10 mM lactate for 40 h (time 0), cells were cultured with 17 mM glucose, or 17 mM glucose and protein synthesis inhibitors (25 mM anisomycin or 10 mM cycloheximide), or 17 mM glucose and 10 nM insulin for the indicated times. The same blot was sequentially hybridized with the different probes. B, time course of L-PK and GLUT 2 mRNA induction by 17 mM glucose and a quantitative representation of the Northern blot as shown in panel A. C, glucose dose-response of L-PK and GLUT 2 mRNA synthesis. After decreasing their levels by culture for 24 h in 10 mM lactate, cells were grown for 24 h in a medium containing from 2.5 to 17 mM glucose. The mRNAs were analyzed by Northern blot and quantitated versus PABP gene expression by PhosphorImaging. Results are the mean of at least three separate experiments.

Fig. 6. Effect of forced GLUT 2 expression in mhAT3F cells on induction of the L-PK gene by glucose. mhAT3F cells were co-transfected with 5 µg of L43-119L-PK/CAT and 1 µg of pCMV-, or pCMV-GLUT 2, or pCMV-GLUT 1 in glucose-free medium and then induced with 17 mM glucose according to different protocols. A, the glucose activation of L-PK gene expression is delayed in mhAT3F cells when cells are deprived of glucose for >30 h before induction by glucose (■ ■). (Results are drawn similar to those presented in Fig. 3 of Ref. 2.) However, activation was more rapid when glucose deprivation was short (15 h) (○-○). B and C, effect of forced expression of GLUT 2 or GLUT 1 on the time-course of L-PK gene activation by glucose. Cells were cultured for 24 h before transfection in a lactate medium containing 4% (w/v) diazoylated fetal calf serum, transfected for 16 h in a serum-free lactate medium, and then kept for a further 24 h in 4% diazoylated fetal calf serum lactate medium before glucose induction for 2, 4, or 6 h. Cells were harvested 48 h after the beginning of the transfection. Results of panels B and C are given as mean ± S.E. of the same four separate experiments except that, in panel B, results are expressed in □° of glucose stimulation and, in panel C, in percent acetylation. Statistical significance compared with time 0 of the same experiment: *, p < 0.001; statistical significance compared with time 0 of the experiment using the void CMV expression vector: ○, p < 0.001.

DISCUSSION

Hepatocytes and β cells were the only cells to express the couple “GLUT 2-glucokinase” for transport and phosphorylation of glucose from the blood. In the hepatoma cell lines de-
scribed to date, neither GLUT 2 nor glucokinase were ever reported to be significantly expressed, being replaced by GLUT 1 and insulin-independent hexokinase isoforms, mainly HK1 and HK2 (8, 16). We have previously established and characterized various new hepatoma cell lines, some of which express GLUT 2 but not glucokinase and yet are responsive to glucose (2, 19, 20). Hepatoma cells that express mostly GLUT 1 exhibit a glucose-independent L-PK gene expression, whereas GLUT 2(−) cell lines express the L-PK gene poorly under glucose-free conditions and strongly under glucose conditions. Induction of GLUT 2 synthesis in GLUT 2(−) cells, either by selection for ability to grow in a fructose medium or by transient transfection of a GLUT 2 expression vector, allows for concomitant occurrence of glucose responsiveness of the L-PK promoter.

Thus, the main question is what is the mechanism of GLUT 2 action on the response of glucose-sensitive genes to glucose in liver cells? In other words, what does GLUT 2 do that GLUT 1 is unable to do? GLUT 1 and GLUT 2 have similar Vmax, but affinity of GLUT 1 is 2–3-fold higher than that of GLUT 2. In addition, GLUT 1 is highly asymmetrical, much more efficient for glucose influx than efflux, whereas GLUT 2 is symmetrical, which explains its specific role in organs whose function is to secrete glucose (11). Finally, GLUT 2 but not GLUT 1 is capable to transport fructose (30).

In endocrine pancreas β cells, the predominant glucose-sensor has been definitively identified as glucokinase by knock out of the gene in mice (32, 33), and the significance of the specific GLUT 2 expression in rodent β cells is still disputed, especially as the amount of the GLUT 2 isoform seems to be very low in human β cells (34, 35). German (36) has shown that β cells overexpressing GLUT 1 do not lose their ability to sense changes in glucose concentration within the physiological range and to respond by an appropriate stimulation of the insulin gene promoter. In contrast, Newgard and co-workers (12, 26) hypothesized that GLUT 2, perhaps by specific interaction with glucokinase, could generate an intracellular signal needed for the response of insulin secretion to glucose. This view is consistent with the report by Valera et al. that, in mice, an antisense GLUT 2 mRNA impaired the normal activation of insulin secretion by glucose (37). Therefore, GLUT 2 could be needed to allow normal regulation for insulin secretion by glucose in β cells, at least in rodents, but be non-essential for glucose-dependent regulation of insulin gene transcription.

A glucose sensor system is also essential in the liver to provide a correct metabolic response to dietary modifications, either glucose utilization and glycogen storage or glucose production. This sensor seems to be composed, as in β cells, of GLUT 2 and glucokinase. However, glucokinase could be replaced by other insulin-independent hexokinases, in vivo and ex vivo. In vivo, knockout mice specifically deficient in glucokinase in liver have an almost normal glucose regulation (32), whereas deficiency in pancreas glucokinase leads to neonatal death from severe insulin-dependent diabetes mellitus (33). Ex vivo, we have shown that hepatoma cells with hexokinase 1 (2, and this paper) respond to glucose in modulating transcription of glucose-responsive genes, e.g. the L-PK gene. Thus, in contrast to the essential role of glucokinase in glucose-dependent insulin secretion by β cells, glucokinase seems to be needed in the liver mainly for phosphorylating glucose to Glu-6-P, a role that can be performed by other hexokinase isoforms as well. However, GLUT 2 seems to be essential for the liver glucose sensor function, as suggested by its requirement for the normal response of the L-PK gene to glucose in liver-derived cells.

We have found that the main effect of GLUT 2 does not seem to be to generate a positive signal needed for glucose action, but rather to allow for L-PK gene extinction in cells cultured in the absence of glucose. All the hepatoma cells used in this work are more or less capable of gluconeogenesis and, accordingly, several of them have been selected for persistence of liver-specific functions by prolonged culture in glucose-free media (18, 19). Therefore, hepatoma cells cultured under glucose-free conditions are expected to synthesize Glu-6-P and glucose, and to secrete glucose provided that Glu-6-Pase and the transporter specialized in glucose export, i.e. GLUT 2, are present.

In the absence of GLUT 2, glucose efflux from the cells might be limited. As a consequence, the sequestered intracellular glucose could be phosphorylated back to Glu-6-P in a sort of futile cycle by the constitutive hexokinase isoforms replacing glucokinase, resulting in a high Glu-6-P concentration. This hypothesis is in complete agreement with our results that hepatoma cells cultured for 30 or 40 h in lactate medium had a high Glu-6-P concentration when they are GLUT 2(−) and an undetectable or low Glu-6-P concentration when they are GLUT 2(+). Intracellular Glu-6-P concentration in GLUT 2(+) cells seems to increase between hour 30 and 40 of culture in glucose-free medium (Table I). In fact, this result was not surprising because expression of the GLUT 2 gene is itself glucose-dependent (3). Consequently, prolonged culture in lactate medium should result in the progressive disappearance of the GLUT 2 transporter. Glu-6-P is the first glucose metabolite needed for the transcriptional response of glucose-responsive genes to glucose (4–6). Accumulation of this compound (and derived glycolytic intermediates) in GLUT 2(−) cells cultured without glucose could therefore activate L-PK gene transcription. In contrast, GLUT 1 expression could contribute to limited glucose export or promote rapid reentry of glucose secreted in the extracellular medium.

In conclusion, our results provide a new insight into the correlation between expression of the GLUT 2 transporter and the physiological response to glucose observed in gluconeogenic cells. In hepatoma cell lines, the usually expressed “couple” for transport and phosphorylation of glucose is GLUT 1-HK1, whereas it is GLUT 2-glucokinase in the liver. We show in this paper that hepatoma cell lines that express the couple GLUT 2-HK1 conserve the sensitivity of metabolic genes to glucose concentration and suggest that GLUT 2 acts in this process more as a component of the gluconeogenic pathway than as an essential element for glucose influx. In vivo, GLUT 2 is present in the liver under both gluconeogenic and glycolytic conditions. Under the former condition, GLUT 2 activity would be essential for both glucose secretion and keeping the intracellular Glu-6-P concentration low and thus avoid permanent activation of glycolytic and lipogenic genes, which otherwise would result in a futile metabolic cycle and inappropriate energy dissipation.

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REFERENCES
1. Vaulont, S., and Kahn, A. (1993) *FASEB J.* 8, 28–35
2. Lefrançois-Martinez, A. M., Diaz-Guerra, M. J. M., Vallet, V., Kahn, A., and Antoine, B. (1993) *FASEB J.* 8, 89–96
3. Rencurel, F., Waeber, G., Antoine, B., Rocchiccioli, F., Maulard, P., Girard, J., and Leturque, A. (1996) *Biochem. J.* 314, 903–909
4. Foufelle, F., Gouhot, B., Pégorier, J.-P., Perdereau, D., Girard, J., and Ferré, P. (1992) *J. Biol. Chem.* 267, 20543–20546
5. Marie, S., Diaz-Guerra, M.-J., Miquerol, L., Kahn, A., and Iynedjian, P. B. (1993) *J. Biol. Chem.* 268, 23881–23890
6. Foufelle, F., Gouhot, B., Pégorier, J.-P., Perdereau, D., Girard, J., and Ferre´, P. (1992) *J. Biol. Chem.* 267, 20543–20546
7. Iynedjian, P. B. (1993) *Biochem. J.* 293, 1–13
8. Arora, K. K., and Pedersen, P. L. (1988) *J. Biol. Chem.* 263, 17422–17428
9. Doiron, B., Cuif, M.-H., Kahn, A., and Diaz-Guerra, M.-J. M. (1994) *J. Biol. Chem.* 269, 10213–10216
10. Thorens, B., Sarkar, H. K., Kaback, H. R., and Lodish, H. F. (1988) *Cell* 55, 281–290
11. Gould, G. W., and Holman, G. D. (1993) *Biochem. J.* 295, 329–341
12. Hughes, S. D., Quaade, C., Johnson, J. H., Ferber, S., and Newgard, C. B. (1993) *J. Biol. Chem.* 268, 15205–15212
13. Newgard, C. B. (1994) *Diabetes* 43, 513–515
14. Lachaal, M., Spangler, R. A., and Jung, C. Y. (1993) *Am. J. Physiol.* 268, 51–55
15. Meinenhofer, M. C., De Medicis, E., Cognet, M., and Kahn, A. (1987) *Eur. J. Biochem.* 169, 237–244
16. Flier, J. S., Mueckler, M. M., Usher, P., and Lodish, H. F. (1987) *Science* 235, 1492–1497
17. Dubois, N., Bennoun M., Allemand, I., Molina, T., Grimmer, G., Daude-Monsac, M., Abelant, R., and Briand, P. (1991) *J. Hepatol.* 13, 237–239
18. Cartier, N., Miquerol, L., Talie, M., Lepetit, N., Levrat, F., Grimmer, G., Briand, P., and Kahn, A. (1992) *Oncogene* 7, 1413–1422
19. Antoine, B., Levrat, F., Vallet, V., Berbar, T., Cartier, N., Dubois, N., Briand, P., and Kahn, A. (1992) *Exp. Cell Res.* 200, 175–185
20. Levrat, F., Vallet, V., Berbar, T., Miquerol, L., Kahn, A., and Antoine, B. (1993) *Exp. Cell Res.* 206, 307–316
21. Antoine, B., Courjault-Gautier, F., Bens, M., Vallet, V., Cluzeaud, H., Toutain, H., Pringault, E., Kahn, A., and Vandewalle, A. (1997) *Exp. Cell Res.* in press
22. Chomzynski, P., and Sacchi, N. (1987) *Anal. Biochem.* 162, 156–159
23. Mueckler, M., and Lodish, H. F. (1986) *Cell* 44, 629–637
24. Printz, R. L., Koch, S., Potter, L. R., O'Doherty, R. M., Tiesinga, J. J., Moritz, S., and Granner, D. K. (1995) *J. Biol. Chem.* 268, 5209–5215
25. Bergot, M. O., Diaz-Guerra, M. J. M., Puzenat, N., Raymondjean, M., and Kahn, A. (1992) *Nucleic Acids Res.* 20, 1781–1878
26. Hughes, S. D., Johnson, J. H., Quaade, C., and Newgard, C. B. (1992) *Proc. Natl. Acad. Sci. U. S. A.* 89, 688–692
27. Stein, M. W. (1963) in *Methods of Enzymatic Analysis* (Bergmeyer, H. U., ed) pp. 1238–1242, Academic Press, New York
28. Asano, T., Katagiri, H., Tsukuda, K., Lin, J. J., Inihara, H., Yasaki, Y., and Oka, Y. (1992) *Diabetes* 41, 22–25
29. Postic, C., Burelreci, R., Rencurel, F., Pégorier, J. P., Girard, J., and Leturque, A. (1993) *Biochem. J.* 293, 119–124
30. Gould, G. W., Thomas, H. M., Jess, T. J., and Bell, G. I. (1991) *Biochemistry* 30, 5139–5145
31. Shapira, F., Shapira, G., and Dreyfus, J. C. (1961) *Enzymol. Biol. Clin.* 7, 170–175
32. Groupe, A., Hultgren, B., Ryann, A., Hui Ma, Y., Bauer, M., and Stewart, T. A. (1995) *Cell* 83, 69–78
33. Terunishi, Y., Sakurai, H., Yasuda, K., Iwamoto, K., Takahashi, N., Ita, K., Kasai, H., Suzuki, H., Ueda, O., Kamada, H., Jishage, K., Komeda, K., Noda, M., Kanazawa, Y., Taniguchi, S., Miwa, I., Akuma, Y., Komeda, T., Yasaki, Y., and Kadewaki, T. (1995) *J. Biol. Chem.* 270, 30253–30256
34. De Vos, A., Himberg, H., Quattet, E., Bouwens, L., Pipeleers, D., and Schutt, F. (1995) *J. Clin. Invest.* 96, 2489–2495
35. Ferrer, J., Benito, C., and Gomis, R. (1995) *Diabetes* 44, 1369–1374
36. German, M. S. (1993) *Proc. Natl. Acad. Sci. U. S. A.* 90, 1741–1745
37. Valera, A., Solanes, G., Fernandez-Alvarez, J., Pujol, A., Ferrer, J., Asins, G., Gomis, R., and Bosch, F. (1994) *J. Biol. Chem.* 269, 28543–28546