INTRODUCTION

Glucose signaling in eukaryotic cells can be regarded as a process involving the detection of extracellular glucose levels, followed by the transduction to the nucleus of a glucose repression or induction signal. Hepatocytes and pancreatic β cells both stimulate gene transcription in response to high glucose concentrations (Chen et al., 1990; Girard et al., 1997; Towle and Kaytor, 1997; Vaulont and Kahn, 1994). In these cells, glucose transport is mediated by the GLUT2 glucose transporter (Thorens et al., 1988). A close correlation between GLUT2 levels and glucose-regulated gene expression is observed in hepatoma cells (Antoine et al., 1997), engineered β cells (Newgard et al., 1997) and GLUT2-null mice (Guillam et al., 1997), suggesting that in liver and pancreatic β cells, the presence of GLUT2 is required for the transcriptional effect of glucose.

The primary role of GLUT2 is to transport glucose inside the cell, where it is metabolized. The prevailing idea is that the glucose signal in eukaryotic cells originates from its own metabolism (Johnston, 1999). Extracellular glucose is detected by two nutrient sensors of low and high concentrations, Snf3p and Rgt2p, cloned in Saccharomyces cerevisiae (Özcan et al., 1996). These proteins are structurally similar to mammalian and yeast glucose transporters, except for a long cytoplasmic C terminus (Celenza et al., 1988; Özcan et al., 1996) that may serve as a signaling domain (Ko et al., 1993; Schmidt et al., 1999; Vagnoli et al., 1998). The nature of the glucose induction signal is unknown, but it is not generated by glucose metabolism, contrary to the glucose repression signal (Johnston, 1999).

The aim of this study was to test the hypothesis that, in addition to its transport activity, GLUT2 can function as a protein mediating transcriptional glucose signaling. To divert the putative interacting proteins from a glucose signaling pathway, two intracytoplasmic domains of GLUT2, the C terminus and the large loop located between transmembrane domains 6 and 7, were transfected into mhAT3F hepatoma cells. Glucose-induced accumulation of two hepatic gene mRNAs (GLUT2 and L-pyruvate kinase) was specifically inhibited in cells transfected with the GLUT2 loop and not with the GLUT2 C terminus. The dual effects of glucose were dissociated in cells expressing the GLUT2 loop; in fact a normal glucose metabolism into glycogen occurred concomitantly with the inhibition of the glucose-induced transcription. This inhibition by the GLUT2 loop could be due to competitive binding of a protein that normally interacts with endogenous GLUT2. In addition, the GLUT2 loop, tagged with green fluorescent protein (GFP), was located within the nucleus, whereas the GFP and GFP-GLUT2 C-terminal proteins remained in the cytoplasm. In living cells, a fraction (50%) of the expressed GFP-GLUT2 loop translocated rapidly from the cytoplasm to the nucleus in response to high glucose concentration and conversely in the absence of glucose. We conclude that, via protein interactions with its large loop, GLUT2 may transduce a glucose signal from the plasma membrane to the nucleus.

Key words: GLUT2, Glucose signaling, Living cell, Liver, Glucose metabolism, Glucose-regulated transcription

**SUMMARY**

The hypothesis that the glucose transporter GLUT2 can function as a protein mediating transcriptional glucose signaling was addressed. To divert the putative interacting proteins from a glucose signaling pathway, two intracytoplasmic domains of GLUT2, the C terminus and the large loop located between transmembrane domains 6 and 7, were transfected into mhAT3F hepatoma cells. Glucose-induced accumulation of two hepatic gene mRNAs (GLUT2 and L-pyruvate kinase) was specifically inhibited in cells transfected with the GLUT2 loop and not with the GLUT2 C terminus. The dual effects of glucose were dissociated in cells expressing the GLUT2 loop; in fact a normal glucose metabolism into glycogen occurred concomitantly with the inhibition of the glucose-induced transcription. This inhibition by the GLUT2 loop could be due to competitive binding of a protein that normally interacts with endogenous GLUT2. In addition, the GLUT2 loop, tagged with green fluorescent protein (GFP), was located within the nucleus, whereas the GFP and GFP-GLUT2 C-terminal proteins remained in the cytoplasm. In living cells, a fraction (50%) of the expressed GFP-GLUT2 loop translocated rapidly from the cytoplasm to the nucleus in response to high glucose concentration and conversely in the absence of glucose. We conclude that, via protein interactions with its large loop, GLUT2 may transduce a glucose signal from the plasma membrane to the nucleus.

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**INTRODUCTION**

Glucose signaling in eukaryotic cells can be regarded as a process involving the detection of extracellular glucose levels, followed by the transduction to the nucleus of a glucose repression or induction signal. Hepatocytes and pancreatic β cells both stimulate gene transcription in response to high glucose concentrations (Chen et al., 1990; Girard et al., 1997; Towle and Kaytor, 1997; Vaulont and Kahn, 1994). In these cells, glucose transport is mediated by the GLUT2 glucose transporter (Thorens et al., 1988). A close correlation between GLUT2 levels and glucose-regulated gene expression is observed in hepatoma cells (Antoine et al., 1997), engineered β cells (Newgard et al., 1997) and GLUT2-null mice (Guillam et al., 1997), suggesting that in liver and pancreatic β cells, the presence of GLUT2 is required for the transcriptional effect of glucose.

The primary role of GLUT2 is to transport glucose inside the cell, where it is metabolized. The prevailing idea is that the glucose signal in eukaryotic cells originates from its own metabolism (Girard et al., 1997; Towle and Kaytor, 1997; Vaulont and Kahn, 1994). A recent report, however, provides evidence that the glucose-signaling pathway can be triggered by other means. Originating from a plasma membrane sensor, this pathway, described in yeast, is mediated by protein-protein interactions (Johnston, 1999). Extracellular glucose is detected by two nutrient sensors of low and high concentrations, Snf3p and Rgt2p, cloned in Saccharomyces cerevisiae (Özcan et al., 1996). These proteins are structurally similar to mammalian and yeast glucose transporters, except for a long cytoplasmic C terminus (Celenza et al., 1988; Özcan et al., 1996) that may serve as a signaling domain (Ko et al., 1993; Schmidt et al., 1999; Vagnoli et al., 1998). The nature of the glucose induction signal is unknown, but it is not generated by glucose metabolism, contrary to the glucose repression signal (Johnston, 1999).

The aim of this study was to test the hypothesis that, in addition to its transport activity, GLUT2 can function as a glucose sensor, triggering glucose-regulated gene expression. GLUT2 is a member of a family of proteins (GLUTs) whose predicted structure consists of 12 transmembrane gene domains, and N- and C-terminal cytoplasmic domains. A large loop between transmembrane domains 6 and 7 is predicted to reside in the cytoplasm. The specificity of GLUT isoforms is determined by the intracytoplasmic domains, which are not conserved in terms of their amino acid sequences (Bell et al., 1990). Signal transduction from a transmembrane receptor protein requires the activation of its intracytoplasmic domain. If GLUT2 transmits a glucose signal, one of its intracytoplasmic domains must be part of the signaling pathway. In order to identify the
GLUT2 domain that affects glucose sensing in the cell, the C terminus and large intracellular loop of GLUT2 were expressed in a hepatoma cell line. In large amounts, the intracytoplasmic domains would appear to compete with endogenous GLUT2 for binding to proteins involved in the glucose signaling pathway and thereby modify glucose-regulated gene expression. We assessed the impact of expression of the two domains on both a metabolic and a transcriptional effect of glucose.

MATERIALS AND METHODS

Cell culture

The mhAT3F hepatoma cell line was derived from transgenic mice synthesizing the SV40 large T and small t antigens under the control of the antithrombin III promoter (Antoine et al., 1992). mhAT3F cells were grown in Dulbecco’s modified Eagle’s medium/Ham-F12, Glutamax (Life Technologies) supplemented with 100 UI/ml penicillin, streptomycin, 0.1 μM insulin, 1 μM dexamethasone, 1 μM triiodothyronine and 5% fetal calf serum. The mhAT3F cell line was chosen because GLUT2 is abundantly expressed and the stimulation of gene transcription by glucose is preserved (Antoine et al., 1997).

The cells were cultured in 17 mM glucose unless otherwise stated. Cells were also cultured in glucose-free medium for 24 hours (it should be noted that some glucose was present in the fetal calf serum). When mhAT3F cells were cultured in the absence of glucose, the residual glucose concentration in the medium after 24 hours was 0.03±0.01 mM. When mhAT3F cells were cultured with an initial glucose concentration of 17 mM, the residual glucose concentration after 24 hours was 8.65±0.10 mM.

Plasmid constructs

The coding regions of the intracellular loop between transmembrane domains 6 and 7 of the rat GLUT2 glucose transporter (amino acids 237-301) and the C terminus GLUT2 domain (amino acids 481-521) were amplified by means of PCR using a plasmid containing the full-length GLUT2 cDNA (Thorens et al., 1988) as template. The fragments were then inserted, in-frame, into the pEGFPc vector (Clontech), in fusion with green fluorescent protein (GFP).

Transfection experiments

mhAT3F cells were plated and transfected by using the calcium phosphate-DNA precipitation method (Chen and Okayama, 1988). The GLUT2 loop and GLUT2 C terminus in pEGFP vectors, and the vector alone, were transfected and harvested after 2 days for transient expression measurements. Transfected cells were selected with geneticin sulphate G-418 (0.4 mg/ml) (Gibco-BRL) for 3 weeks. Stable clones of mhAT3F cells were grown on Permanox four-chamber slides (Lab-Tek, Nunc) with 17 mM glucose. Cells were also cultured in glucose-free medium for 24 hours (it should be noted that some glucose was present in the fetal calf serum). Cells were washed twice with ice-cold 0.9% NaCl and scraped free in 30% KOH lysis buffer. Glycogen was precipitated with ethanol and radioactivity was counted. Results are expressed as nmole glucose incorporated into glycogen/106 cells.

Western blot analysis

Proteins were separated by SDS-PAGE (10% acrylamide) and electrotransferred to a nitrocellulose membrane. After overnight incubation in 5% (w/v) non-fat milk in Tris-buffered saline (TBS) (20 mM Tris-HCl, pH 7.5, 150 mM NaCl), the blots were washed in TBS containing 0.1% (v/v) Triton X-100 and incubated with a monoclonal anti-GFP (Clontech) or anti-GLUT2 C terminus antibody (East-Acres). Immune complexes were detected by ECL (Amersham).

Northern blot analysis

Total RNA was purified from confluent mhAT3F cells after 24 hours of culture in the various experimental conditions, and northern analysis was performed as previously described (Postic et al., 1993). The northern blots were hybridized with radiolabeled liver-pyruvate kinase and GLUT2 cDNAs and mRNAs were quantified by means of computer-based scan analysis. An 18S rRNA probe was used to visualize RNA loading (Postic et al., 1993).

Determination of radiolabeled glucose incorporation into glycogen

[14C]glucose (2 μCi, Amersham Pharmacia) incorporation into glycogen was measured in confluent cells cultured in 30 mm dishes, after 24 hours in the different experimental conditions, as previously described (Kasus-Jacobi et al., 1997). Cells were washed twice with ice-cold 0.9% NaCl and scraped free in 30% KOH lysis buffer. Glycogen was precipitated with ethanol and radioactivity was counted. Results are expressed as nmole glucose incorporated into glycogen/106 cells.

Fluorescence analysis

Stable clones of mhAT3F cells were grown on Permanox four-chamber slides (Lab-Tek, Nunc) with 17 mM glucose. Cells were washed three times with PBS (137 mM NaCl, 1.3 mM KCl, 16.1 mM Na2HPO4, 1.5 mM KH2PO4), fixed for 30 minutes in 4% paraformaldehyde in PBS, and quenched for 10 minutes in 50 mM NH4Cl. The nuclei were stained bright blue using Hoechst 33258 (0.5 μg/ml) for 5 minutes. After extensive washes in PBS the coverslips were mounted in Mowiol (Hoechst). Fluorescence microscopy was performed using an Olympus IMT2 inverted microscope, a
fluorescence immersion lens (40×) and an FITC filter. Photographs (Kodak Panther P1600 film) were taken with an Olympus MO-4Ti camera adapted to the microscope. The location of the fusion proteins was quantified by using the Imagetool Software.

**Fluorescence imaging of GFP-GLUT2 loop**

A stable clone of GFP-GLUT2 loop was grown on glass coverslips Labtek (Nunc), with or without glucose added in the culture medium for 24 hours. The experiments were performed at 25°C in circulating PBS with or without glucose (17 mM). The microscopy was conducted using a confocal Zeiss LSM 510 microscope and a 63× objective lens. One 1 μm slice located in the nucleus of the cell was observed, and images were captured every 30 seconds during the periods of the experiments. The PBS was circulating constantly; the total replacement of PBS in the culture chamber took 1 minute. Captured images were then analysed using the provider software. Quantification of green fluorescence was established on a scale of grey intensity (256 grey levels). The mean grey intensities were measured in a circle (diameter of the region of interest: 5 μm) arbitrarily positioned inside the nucleus and in the cytoplasm of the cell. The results, expressed as mean grey intensities, were recorded in each captured image during the experimental period. We verified that the replacement of the circulating medium by a medium of the same composition did not affect fluorescence location (results not shown).

**Statistical analysis**

Results are expressed as means ± s.e.m. Statistical analysis was performed by using Student’s *t*-test for unpaired data.

**RESULTS**

**Expression of GLUT2 intracytoplasmic domains in mhAT3F cells**

The production of fusion proteins in clonal stable mhAT3F
transfectants was analyzed by using an antibody directed against green fluorescent protein. The fusion proteins recognized by the anti-GFP antibody were of the expected sizes, i.e. 29 kDa for GFP alone, 32.5 kDa for GFP-GLUT2 C terminus and 34.8 kDa for GFP-GLUT2 loop (Fig. 1). The expression levels of the three fusion proteins were similar. As necessary for this study, the expression of the fusion proteins was not affected by the presence of glucose in the culture medium.

Expression of the GFP-GLUT2 C terminus and loop was 3-4 times higher than that of endogenous GLUT2 in untransfected cells, as estimated by northern blotting (not shown). All mRNA signals were detected using the radiolabeled full-length GLUT2 cDNA as probe. The probe hybridized with about 10% of the mRNAs for GFP-GLUT2 C terminus and loop compared with 100% of GLUT2 mRNA, leading to a probable underestimation of the transgene amounts.

Accumulation of GLUT2 mRNA in mhAT3F cells

The transcriptional effect of glucose on two glucose-dependent liver genes, encoding GLUT2 and L-pyruvate kinase, was assayed in clonal stable mhAT3F transfectants (Fig. 2). The cells were cultured in the absence or presence of 17 mM sugar (n=4). GLUT2 mRNA amounts in mhAT3F cells expressing the GFP-GLUT2 loop were always statistically lower than GLUT2 mRNA amounts in wild-type cells (P<0.01; n=4).

GLUT2 mRNA in mhAT3F

Arbitrary Units

![Graph showing GLUT2 mRNA levels in wild type and GFP-GLUT2 loop expressing cells](image)

**Fig. 3.** Effect of glucose, fructose and xylose on GLUT2 mRNA accumulation in wild-type mhAT3F cells and mhAT3F cells stably transfected with the GFP-GLUT2 loop. Northern blots were hybridized with GLUT2 cDNA and quantified by scanning densitometry. *Significant increase (P<0.05) compared cells cultured for 24 hours in the absence (no) or presence of 17 mM sugar (n=4). GLUT2 mRNA amounts in mhAT3F cells expressing the GFP-GLUT2 loop were always statistically lower than GLUT2 mRNA amounts in wild-type cells (P<0.01; n=4).

**Fig. 4.** Effect of the glucose concentration on glycogen synthesis in mhAT3F cells stably transfected with GFP or GFP fusions of intracytoplasmic domains of GLUT2. Results are expressed in nmol [U-14C]glucose/10^6 cell / 24h

4 times higher than that of endogenous GLUT2 in untransfected cells, as estimated by northern blotting (not shown). All mRNA signals were detected using the radiolabeled full-length GLUT2 cDNA as probe. The probe hybridized with about 10% of the mRNAs for GFP-GLUT2 C terminus and loop compared with 100% of GLUT2 mRNA, leading to a probable underestimation of the transgene amounts.

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The transcriptional effect of glucose on two glucose-dependent liver genes, encoding GLUT2 and L-pyruvate kinase, was assayed in clonal stable mhAT3F transfectants (Fig. 2). The cells were cultured in the absence or presence of 17 mM glucose for 24 hours. In mhAT3F cells expressing GFP alone, the level of GLUT2 and L-pyruvate kinase mRNAs increased three- to fourfold in response to glucose, a level similar to that observed in wild-type cells. Expression of the GFP-GLUT2 C terminus did not affect the accumulation of GLUT2 and L-pyruvate kinase mRNA in response to glucose. In contrast, expression of the GFP-GLUT2 loop specifically abolished both GLUT2 and L-pyruvate kinase mRNA accumulation in response to glucose. After hybridization with L-PK cDNA, two bands were detected: a 2.8-kb signal corresponding to liver pyruvate kinase (L-PK) and a 2.4-kb signal corresponding to

**Fig. 5.** Location of fluorescent proteins in cells stably transfected with GFP (A,B), GFP-GLUT2 C terminus (C,D) and GFP-GLUT2 loop (E,F). (B,D,F) The nuclei were stained with Hoechst dye. (A,C,E) The green fluorescent fusion proteins are visualized by epifluorescence with a light microscope.
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Fig. 6. Intracellular movements of GFP-GLUT2 loop in one living mhAT3F cell. These results were obtained by confocal microscopy. Bar, 10 μm. In the same cell, images of the GFP-GLUT2 loop were recorded in the absence (A) and presence (B) of 17 mM glucose. The fluorescence was quantified (see Materials and Methods), in the nucleus (black squares) and the cytoplasm (open squares) as mean grey intensity in an area of 5 μm diameter (right part). (A,B) Two captured images from the same cell at the times indicated on the right. This experiment was representative of three different experiments.

muscle pyruvate kinase (M-PK). m-PK is constitutively expressed in mhAT3F cells and served as a control (Fig. 2).

Inhibition of the glucose effect was also observed in a pool of stable mhAT3F cells constitutively expressing GFP-GLUT2 loop (not shown), indicating that the inhibition was due solely to the fusion protein, and not to a peculiar insertion of the GFP-GLUT2 loop construct in the mhAT3F cell genome.

The intracellular loop of GLUT2 was thus identified as the protein domain that interferes with the transcriptional signaling triggered by glucose.

Effect of glucose, fructose and xylose on GLUT2 mRNA accumulation

To determine the nature of the triggers that stimulate the glucose signaling pathway, several sugars were tested. In wild-type mhAT3F cells, 17 mM fructose, a hexose transported by GLUT2 (although less efficiently than glucose; Gould et al., 1991), mimicked the effect of glucose on GLUT2 mRNA accumulation. With 17 mM xylose, a pentose poorly transported by GLUT2 (Gould et al., 1991), no significant stimulation of GLUT2 mRNA accumulation was detected in wild-type mhAT3F cells (Fig. 3). A correlation between the amount of transported sugar and GLUT2 mRNA accumulation was thus observed. No stimulation in GLUT2 mRNA accumulation was observed in mhAT3F cells expressing GFP-GLUT2 loop with any type of sugar (Fig. 3).

Incorporation of radioactive glucose into glycogen in mhAT3F cells

To assess the impact of GLUT2 domains upon a metabolic pathway, glycogen synthesis was studied using glucose as a substrate. When mhAT3F cells, transfected with GFP, GFP-GLUT2 C terminus or GFP-GLUT2 loop, were cultured for 24 hours with increasing glucose concentrations (0, 5 and 17 mM), incorporation of radioactive glucose into glycogen was dependent upon the concentration of glucose (Fig. 4). When compared to GFP-transfected cells, the incorporation of radioactive glucose into glycogen did not vary in the presence

Fig. 7. Intracellular movements of GFP-GLUT2 loop in six nuclei from living transfected mhAT3F cells. These results were obtained by confocal microscopy. Images were captured in the GFP-GLUT2 loop cells incubated for 7 minutes in the presence of 17 mM glucose and then for 7 minutes in the absence of glucose. GFP-GLUT2 loop fluorescence in the nuclei was quantified as mean grey intensity (see Materials and Methods), the dark line represents the mean values of six different cells (pale lines). This experiment was reproduced three times.
of GFP-GLUT2 loop or GFP-GLUT2 C terminus in transfected cells, thus demonstrating that the metabolic pathway was not altered (Fig. 4). In contrast, a slight increase in glucose incorporation into glycogen was observed in cells transfected with GFP-GLUT2 C terminus when compared to GFP-GLUT2 loop (Fig. 4). The reason for this difference remained unexplained but could be due to the intrinsic property of GLUT2 C terminus, which is responsible for the high $K_m$ for glucose of GLUT2 protein (Katagiri et al., 1992).

**Location of the overexpressed proteins in mhAT3F cells**

The subcellular location of the fusion proteins was studied using the green fluorescence of GFP (Fig. 5). GFP and GFP-GLUT2 C terminus were distributed uniformly in mhAT3F cells. In contrast, the GFP-GLUT2 loop was concentrated in the nuclei, as visualized by superimposition of the green (GFP) and bright blue (nuclear) fluorescence. This specific location was observed regardless of the position of the tag, i.e. GFP-GLUT2 loop (Fig. 5) or GLUT2 loop -GFP (result not shown).

The fluorescence of the fusion proteins was carefully quantified. The ratio of mean grey intensity of identical areas in the nucleus and cytoplasm showed that the GFP alone and GFP-C terminus proteins had the same cellular location. Their mean grey ratios were, respectively, 1.51±0.05, $n=10$ and 1.51±0.10, $n=10$. In contrast, the mean grey ratio of GFP-GLUT2 loop in AT3F cell was 2.15±0.11, $n=12$, showing a nuclear accumulation of the fusion protein. This specific location was observed with the light microscope (Fig. 5) and further confirmed by confocal (Fig. 6) microscopic observations.

**Intracellular movements of GFP-GLUT2 loop in living cells in response to glucose**

Using confocal microscopy in living cells, intracellular movements of the GFP-GLUT2 loop were detected despite the high level of transgene expression. The translocations of GFP-GLUT2 loop in mhAT3F were followed in response to switches of glucose concentrations in the culture medium. In cells equilibrated at 25°C in PBS, the GFP-GLUT2 loop was located in the nucleus and to a lesser extent in the cytoplasm (Fig. 6). Upon a switch from PBS to PBS containing 17 mM glucose, the fluorescence from GFP-GLUT2 loop decreased in the cytoplasm and increased in the nucleus during the 14 minutes experimental period (Fig. 6). When the cells equilibrated in PBS containing 17 mM glucose were switched to PBS, the fluorescence from GFP-GLUT2 loop decreased by twofold in the nuclei of six recorded cells, during the experimental period (Fig. 7). A fraction (50%) of the GFP-GLUT2 loop translocated within a few minutes from the cytoplasm to the nucleus in response to high glucose, and conversely during glucose deprivation.

**DISCUSSION**

The presence of the glucose transporter isoform GLUT2 is required for appropriate responses to glucose in both pancreatic $\beta$ cells and hepatocytes. We studied the role of two GLUT2 protein domains in the transduction of the glucose signal in hepatic cells. Specific functions have been attributed to domains of glucose transporters. For example, subcellular targeting of GLUTs has been attributed to the N- and C-terminal domains, whereas the substrate binding site and its specificity are mainly due to transmembrane domains (Saravolac and Holman, 1997). Expression of intracellular domains of GLUT4, the insulin-sensitive glucose transporter, demonstrated that the C terminus of GLUT4 transfected into adipocytes could cause insulin-like stimulation of glucose transport (Lee and Jung, 1997).

The potential signaling functions of intracytoplasmic domains of GLUT2 were studied in an mhAT3F cell line. The effect of glucose on the accumulation of mRNA encoding two glucose-sensitive hepatic genes, GLUT2 and L-pyruvate kinase, was abolished in cells expressing the large loop but not the C terminus of GLUT2. The GLUT2 loop was thus identified as a GLUT2 domain mediating the transcriptional glucose signal. Overexpression of the GLUT2 loop relative to endogenous GLUT2 was sufficient to divert the glucose signal from its usual pathway, suggesting that the GFP-GLUT2 loop competitively binds a protein that normally binds to endogenous GLUT2. We therefore created a dominant-negative mutant mammalian cell, allowing the investigation of the effects of glucose on gene transcription.

The effect of glucose on GLUT2 mRNA accumulation was mimicked by sugars that are efficiently transported by GLUT2 in wild-type cells. Therefore, sugar transport by GLUT2 appears to be necessary to initiate the pathway and control its amplitude. In contrast, in cells expressing the GFP-GLUT2 loop, the entry of glucose and fructose mediated by endogenous GLUT2 was not sufficient to trigger the transcription signal.

In bacteria, yeast and plants, complex signal transduction networks are activated in response to glucose, and the transcriptional effects of glucose appear to be dissociated from the metabolic effects. In mammalian cells, the transcriptional and metabolic processes have always been closely related in glucose signaling. By expressing the intracytoplasmic domain of GLUT2, we discriminated between the dual effects of glucose. Indeed, increasing extracellular glucose concentrations modulated glucose incorporation into glycogen in both wild-type and transfected cells. This showed that glucose metabolism via glucose-6-phosphate was preserved when the loop domain was transfected. Taken together, these results show that the transfected intracytoplasmic loop of GLUT2 inhibits the transcriptional effect of glucose on glucose-sensitive genes independently of glucose storage into glycogen and suggests the existence of a parallel pathway in mammalian cells, as in other cells.

The intracellular location of the fusion protein might help to clarify its inhibitory role on glucose-induced transcription. Indeed, we showed that the GFP-GLUT2 loop specifically accumulated in the nucleus of mhAT3F cells, whereas the C terminus remained in the cytoplasm. It is known that a globular protein under 50 kDa (about 9 nm) can freely cross the nuclear pore. The nuclear location of the GFP-GLUT2 loop was probably not size-dependent, as the GFP and GFP-GLUT2 C terminus are smaller than the GFP-GLUT2 loop. Using the PSORT II program, the GLUT2 loop (not the C terminus) was shown to contain 20% basic amino acids, which are a target for nuclear import machinery. The location of the fusion protein might be affected by extracellular glucose concentrations. In response to a high glucose concentration and...
within a few minutes, a fraction of the expressed GFP-GLUT2 loop translocated from the cytoplasm to the nucleus in living mhAT3F cells. These regulated movements favor the hypothesis that GFP-GLUT2 loop translocates via a complex mechanism involving other proteins. In these cells, the ratio of GFP-GLUT2 loop versus endogenous GLUT2 favored the former in the competition for protein binding. The proteins that would normally interact with the wild-type GLUT2 protein in the plasma membrane would now bind cytoplasmic GFP-GLUT2 loop. The higher nuclear location of the GFP-GLUT2 loop in response to glucose accompanied inactivation of transcription of glucose-sensitive genes in transfected cells. This suggests that, in wild-type cells, the protein that binds to endogenous GLUT2 has to be released from its anchoring site on the plasma membrane to activate the transcription of glucose-sensitive genes inside the nucleus.

There are several mechanisms by which the large intracellular loop of GLUT2 may transduce a signal from the plasma membrane to the nucleus. A recent discovery in the Notch-1 pathway shows that growth factors stimulate intracellular cleavage of a transmembrane receptor, and the fragment peptide released mediates the Notch-1 signaling pathway (Schroeter et al., 1998). Cleavage of the intracellular loop of the endogenous glucose transporter by the action of an external signal is unlikely, since the glucose transporter GLUT2 is an integral plasma membrane protein with 12 transmembrane domains, and has never been found in the cytoplasm or nucleus of hepatocytes or pancreatic β cells. Alternatively, endogenous GLUT2 could sequester a cytoplasmic protein, on receipt of a sugar signal; the protein would then be released from the plasma membrane, unmasking a site for mediation of the transcriptional effect of glucose. The external signal that provokes glucose signaling from glucose transporter GLUT2 might be the sugar itself. Indeed, glucose crosses the cell membrane by means of structural changes (inward- or outward-facing substrate binding sites) of its transporters (Gould and Seatter, 1997). In this study, however, we cannot rule out a role for one of the early glucose metabolites.

In conclusion, we provide evidence that GLUT2, through its large intracytoplasmic loop, mediates a glucose signaling pathway from the plasma membrane to the nucleus of hepatoma cells.

We thank Edith Brot-Laroche and Anne-Françoise Burnol for helpful discussions. We also thank Dominique Perdereau for DNA sequencing, and Françoise Vialat (CNRS UPR 9068, Nogent) and Christophe Klein (IFR 58, Paris) for the illustrations. GLUT2 cDNA was a kind gift from B. Thorens (Institute of Pharmacology and Toxicology, University of Lausanne, Switzerland) and the liver pyruvate kinase cDNA was from A. Kahn (INSERM U129, Paris, France). mhAT3F cells were established and generously provided by B. Antoine (INSERM U129, Paris, France). G.G. is the recipient of a grant from Ministère de la Recherche et de la Technologie (France); I.G. is supported by Fondation pour la Recherche Médicale; A.L. is supported by Association pour la Recherche sur le Cancer, Grant no. 9303.

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