Glucose-6-phosphatase (G6Pase) catalyzes the release of glucose from glucose 6-phosphate. This enzyme was mainly studied in the liver, but while detected in the small intestine little is known about the regulation of its intestinal expression. This study describes the mechanisms of the glucose-dependent regulation of G6Pase expression in intestinal cells. Results obtained in vivo and in Caco-2/TC7 enterocytes showed that glucose increases the G6Pase mRNA level. In Caco-2/TC7 cells, glucose stabilized G6Pase mRNA and activated the transcription of the gene, meaning that glucose-dependent G6Pase expression involved both transcriptional and post-transcriptional mechanisms. Reporter-gene studies showed that, although the −299/+57 region of the human G6Pase promoter was sufficient to trigger the glucose response in the hepatoma cell line HepG2, the −1157/−1133 fragment was required for maximal activation of glucose-6-phosphatase gene transcription in Caco-2/TC7 cells. This fragment binds the aryl receptor nuclear translocator (ARNT), CAMP-responsive element-binding protein, and upstream stimulatory factor transcription factors. The DNA binding activity of these transcription factors was increased in nuclear extracts of differentiated cells from the intestinal villus of mice fed sugar-rich diets as compared with mice fed a no-sugar diet. A direct implication of ARNT in the activation of G6Pase gene transcription by glucose has been observed in Caco-2/TC7 cells using RNA interference experiments. These results support a physiological role for G6Pase in the control of nutrient absorption in the small intestine.

The intestinal absorption of glucose, allowing its transfer from the lumen to the blood, occurs through enterocytes. The uptake of glucose is mediated at the apical membrane of the absorptive cells by the sodium-glucose co-transporter SGLT1 and by the rapid and transient sugar-dependent recruitment of the glucose transporter GLUT2 to the brush-border membrane (1, 2). Glucose is then released into the blood by GLUT2 in the basolateral membrane. Glucose is also metabolized by enterocytes via several metabolic pathways such as glycolysis and triglyceride synthesis. Moreover, it has been shown recently that, in fasted or diabetic animals, the small intestine is able to release endogenous glucose in the blood, thus significantly contributing to the total endogenous production of glucose (3). The last step of gluconeogenesis, which is the hydrolysis of glucose-6-phosphate into glucose and inorganic phosphate, is catalyzed by the glucose-6-phosphatase (G6Pase)1 (EC 3.1.3.9) complex.

The regulation of G6Pase expression has been extensively studied in the liver, a major site of G6Pase expression (for review, see Ref. 4). In this tissue G6Pase is induced by fasting and repressed by refeeding and insulin. Carbohydrates and lipids also modulate the expression of G6Pase in the liver. Glucose (5–7) and sucrose (8) were reported to increase the expression of G6Pase in rat liver and in hepatocyte models. The induction of G6Pase expression by carbohydrates occurs at both transcriptional and post-transcriptional levels (7) and involves the glycolytic/gluconeogenic pathway (5). Dietary lipids have opposite effects on G6Pase expression in the liver, depending on their chain length and degree of saturation. Short-chain (9) and long-chain fatty acids (10) increase the expression of G6Pase, whereas polyunsaturated fatty acids inhibit the activity of the rat G6Pase promoter (11). However, most of the mechanisms, which modulate G6Pase expression, still need to be elucidated.

G6Pase is also expressed in human and rat small intestine (4, 12, 13) as well as in the human enterocyte cell line Caco-2 (12, 14), which displays the morphological differentiation and most of the functional properties of mature small intestinal enterocytes (15, 16). However, little is known about the regulation of G6Pase expression in the intestine. In rat small intestine, fasting and diabetes increase G6Pase expression (12, 17). An increase of G6Pase activity has also been observed in fructose-fed animals (18, 19). The molecular mechanisms involved in the regulation of G6Pase expression by nutrients in the intestine remain unknown. The purpose of this study was to determine whether simple carbohydrates, such as glucose, regulate the expression of G6Pase in enterocytes and to investigate the molecular mechanisms involved in this process.

**EXPERIMENTAL PROCEDURES**

Caco-2/TC7 Cell Culture—Caco-2/TC7 cells (20) were seeded at 6 × 10^5 cells/cm² on 6-well semipermeable filters (3 μm high pore density, BD Biosciences, Meylan, France). Cells were cultured in Dulbecco's modified Eagle's minimum essential medium (DMEM) supplemented

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1 The abbreviations used are: G6Pase, glucose-6-phosphatase; ARNT, aryl receptor nuclear translocator; CREB, CAMP-responsive element-binding protein; USF, upstream stimulatory factor; CHREBP, carbohydrate responsive element-binding protein; DEMEM, Dulbecco's modified Eagle's medium; siRNA, small interfering RNA; EMSA, electrophoretic mobility shift assay; PEPCK, phosphoenolpyruvate carboxykinase; HNF4α, hepatic nuclear factor 4α.
with 1% nonessential amino acids (Invitrogen) and 20% heat-inactivated (30 min, 56°C) fetal bovine serum (AbCys, France). From the confluence, only the basal compartment contained 20% heat-inactivated fetal bovine serum. The medium was changed 48 h after seeding and daily thereafter. To ensure the same growth rate, cells were cultured with the same glucose concentration (i.e. no glucose or 25 mM glucose in both compartments) until confluence. From the confluence (i.e. before differentiation), and for 6 days (i.e. during the process of differentiation), cells were switched to the appropriate glucose concentration or to mannitol containing medium. The final glucose concentration was obtained by addition of 2.5 mM glucose to glucose-free DMEM, and supplied in the upper and/or lower compartments, as required. Fetal bovine serum (20%) provided 1% glucose in the lower compartment.

**Plasmids**—The luciferase reporter-gene constructs containing the −397/0 to −1227/−57, and −299+/−57 regions of the human G6Pase promoter (phG6Pase-luc) were previously described (21).

The −1227/−57 phG6Pase-luc fragment was generated from the −1227/−57 phG6Pase-luc plasmid using the QuickChange site-directed mutagenesis kit (Stratagene) with the following oligonucleotides: 5′-CCCCATCTCCTACAAAGTGAAATTTAGAATCGTTTT CGCACGTAC-3′ and 5′-GTACGTGGGACACATGGTCAAATTGGTCAT TCTTTTGGTAGAGGGG-3′. The 1227/−57 fragment was then sequenced and subcloned into the −1227/−57 fragment of the wild-type −1227/−57 fragment.

**RNA Interference**—Double-stranded RNAs (small interfering RNA (siRNA)) were designed and synthesized by Dharmacon Research (LaFayette, CO) according to the “custom smart pool” option. Caco-2/TC7 cells, stably transfected with the −1227/−57 phG6Pase-luc construct, were seeded in six-well tissue culture plates. One day before the confluence, the cells were transfected with 50 pmol of ARNT siRNA using Oligofectamine (Invitrogen) in Optimem (Invitrogen). After transfection, cells were cultured in DMEM supplemented or not with 25 mM glucose. Post-confluent cells were harvested after 72 h after the transfection for luciferase assays and Western blot analysis.

**Western Blot Analysis**—Homogenates (100 μg) from Caco-2/TC7 cells transfected with or without the −1227/−57 phG6Pase-luc construct and cultured in the presence of ARNT siRNA, as described under “RNA Interference,” were separated by 7.5% SDS-PAGE in reducing conditions and transferred onto nitrocellulose membrane. The blots were incubated in 5% low fat milk powder (w/v) in phosphate-buffered saline-T (phosphate-buffered saline containing 0.1% Tween 20). They were then probed with a rabbit antibody against human ARNT (dilution: 1/4000, Santa Cruz Biotechnology Inc., Santa Cruz, CA) or goat anti-rabbit antibody (dilution: 1/5000, Santa Cruz, CA) was used as secondary antibody. The blots were then developed with enhanced chemiluminescence (ECL) reagents according to the manufacturer’s instructions (Amersham Pharmacia Biotech). Densitometry analyses were performed using Image J software.

**Transfection Experiments and Luciferase Assay**—For stable transfection experiments, Caco-2/TC7 cells were seeded at 4 × 10⁴ cells/cm² in Petri dishes (Corning Glassworks, Corning, NY). The cells were transfected 48 h after seeding using Lipofectamine (Invitrogen) according to the manufacturer’s protocol, with phG6Pase-luc constructs and pPUR vector (Clontech), which contains the puromycin-resistance gene. Stable transfectants were selected with 10 μg/ml puromycin (Sigma). Total puromycin-resistant cellular populations were analyzed. After selection, all experiments were performed using the appropriate culture medium without puromycin. For stable transfection experiments, HepG2 cells were seeded at 3.75 × 10⁵ cells/cm² in six-well tissue culture plates (BD Biosciences) were cultured for 48 h in low glucose (1 mM glucose) or high glucose (25 mM glucose) medium in the presence of 1% fetal bovine serum.

For transient transfection, HepG2 cells were seeded at 4.2 × 10⁴ cells/cm² in six-well tissue culture plates and cultured in DMEM (5 mM glucose) supplemented with 10% fetal bovine serum. The cells were transfected 24 h after seeding by the calcium phosphate precipitation method with 4 μg of the phG6Pase-luc constructs and 1 μg of pCMV-β-galactosidase plasmid. After transfection, HepG2 cells were cultured for 48 h in low glucose (1 mM) or high glucose (25 mM) medium in the presence of 1% fetal bovine serum.

Luciferase activities were assayed as previously described (22) and were equalized for the transient transfection efficiencies by β-galactosidase assays using standard protocol.

**Northern Blot Analysis**—Total RNA was isolated using RNAplus reagent (qiBiogene, Ilkirch, France) according to the manufacturer’s protocol. The reverse transcription experiments were performed as previously described (23) with 2 μg of total RNA in a total volume of 40 μl. cDNA was quantified using the Light-Cycler System according to the manufacturer’s procedures (Roche Molecular Biochemicals). PCR were performed with a 1:400 final dilution of the reverse transcription product in the SYBR Green I master mixture with specific primers (0.4 μM each of primer) for the cDNA of interest. PCR conditions were one step of denaturation (8 min at 95°C) followed by 40 cycles (each cycle consisted of 10 s at 95°C, 10 s at 65°C, and 10 s at 72°C). The following primers were used:

- 5′-TCATCTTGGTGTCCGTGATCG-3′
- 5′-TTTACCAAGGCGACG GAATGG-3′
- 5′-TACCTTCCTGGTGCTCGATCG-3′
- 5′-TTTATCAGGGGCACG GAAGTG-3′
- 5′-GAGCATATGGAGGCTTCTAG-3′
- 5′-GAGGCTGTGGTCGTTAGTGTCG-3′
- 5′-AGACCATGATGAGGGTGAGA-3′
- 5′-CACACACGACGAGCTG TGC-3′
- 5′-AGTCTTGCTTCGGCTTCTAGA-3′
- 5′-AGACCATGACCGCACATTACA-3′

The mRNA level of L19 was unaffected by the presence or absence of sugars in the culture medium. Results were expressed as the ratio between the levels of G6Pase or L-Pyruvate kinase mRNA and L19 mRNA. The same results were obtained if specific primers for the 18 S rRNA (using a 1:2000 dilution of reverse transcriptase) were used, instead of L19 primers, as internal control.

**Northern Blot Analysis**—Total RNA was extracted by the guanidium isothiocyanate method as previously described (23). After glycolic denaturation, 20 μg were electrophoresed on 1% agarose and transferred onto nylon membranes (Applicene, France) with 20× SSC. Prehybridization and hybridization were performed as described previously (23).

G6Pase mRNA was detected with the cDNA human G6Pase prepared from phG6Pase plasmid obtained from G. Mithieux (12). The mRNA level of G6Pase was determined as described under “Reverse Transcription and Real-time PCR Analysis.”

**Reverse Transcription and Real-time PCR Analysis**—Total RNA was isolated using RNAplus reagent (qiBiogene, Ilkirch, France) according to the manufacturer’s protocol. The reverse transcription experiments were performed as previously described (23) with 2 μg of total RNA in a total volume of 40 μl. cDNA was quantified using the Light-Cycler System according to the manufacturer’s procedures (Roche Molecular Biochemicals). PCR were performed with a 1:400 final dilution of the reverse transcription product in the SYBR Green I master mixture with specific primers (0.4 μM of each of primer) for the cDNA of interest. PCR conditions were one step of denaturation (8 min at 95°C) followed by 40 cycles (each cycle consisted of 10 s at 95°C, 10 s at 65°C, and 10 s at 72°C). The following primers were used:

- 5′-TCATCTTGGTGTCCGTGATCG-3′
- 5′-TTTACCAAGGCGACG GAATGG-3′
- 5′-TACCTTCCTGGTGCTCGATCG-3′
- 5′-TTTATCAGGGGCACG GAAGTG-3′
- 5′-GAGCATATGGAGGCTTCTAGA-3′
- 5′-AGACCATGATGAGGGTGAGA-3′
- 5′-CACACACGACGAGCTG TGC-3′
- 5′-AGTCTTGCTTCGGCTTCTAGA-3′
- 5′-AGACCATGACCGCACATTACA-3′

The mRNA level of L19 was unaffected by the absence of sugars in the culture medium. Results were expressed as the ratio between the levels of G6Pase or L-Pyruvate kinase mRNA and L19 mRNA. The same results were obtained if specific primers for the 18 S rRNA (using a 1:2000 dilution of reverse transcriptase) were used, instead of L19 primers, as internal control.
Intestinal Expression of Glucose-6-phosphatase and Glucose

**FIG. 1.** Effect of glucose on the G6Pase mRNA level in intestinal cells. A, in vivo increase of G6Pase mRNA level by glucose. C57Bl/6J mice were fed no-sugar (NS) or glucose-rich (Glc) diets for 3 days. Total RNA was extracted from the jejunum, and mRNA levels of G6Pase (left panel) and L-pyruvate kinase (right panel) were analyzed by the real-time PCR method. Results are expressed as arbitrary units of the ratio between the mRNA levels of G6Pase or L-pyruvate kinase and L19, and represent mean ± S.E. (n = 5). **,** p < 0.01 as compared with the no-sugar diet. B, Northern blot analysis of G6Pase and PEPCK mRNAs in Caco-2/TC7 cells under various glucose supply conditions. Post-confluent Caco-2/TC7 cells were cultured on semipermeable filters for 6 days from the confluence in the presence of various glucose concentrations in the apical and/or basal compartments, as indicated. rRNA 18S probe was used as loading control. C, relative variations of G6Pase mRNA levels determined by real-time PCR analysis in post-confluent Caco-2/TC7 cells. Cells were cultured on semipermeable filters for 6 days from the confluence in the presence of increasing concentrations of glucose or in the presence of mannitol (used as osmotic control) in both compartments. Results are expressed as in A and represent mean ± S.E. from four independent experiments. *, p < 0.05, and **, p < 0.01 as compared with low glucose (0 mM apical/1 mM basal) condition. D, time course analysis of the glucose-dependent increase of G6Pase mRNA. **,** p < 0.01 as compared with low glucose (0 mM apical/1 mM basal) condition, until confluence (T0), and then maintained in low glucose medium (0 mM apical/1 mM basal) or switched to high glucose medium (25 mM apical/25 mM basal). NS, no sugar; Glc, glucose; Olt, mannitol.

**Statistical Analyses—**Statistical analyses were performed using Student’s t test for unpaired data.

**RESULTS**

Glucose Increases the Expression of G6Pase in Intestinal Cells—The effect of glucose on the expression of G6Pase in intestinal cells was first analyzed in vivo, using the real-time PCR method, with C57Bl/6J mice fed no-sugar or a glucose-rich diet for 3 days (Fig. 1A). To maintain the same caloric supply to animals, sugar was replaced by casein in the no-sugar diet. The glucose-rich diet provoked a 5-fold increase of the mRNA level of G6Pase in intestinal cells (Fig. 1A, left panel). A 2.5-fold increase of the L-pyruvate kinase mRNA level was observed in the same condition (Fig. 1A, right panel). We further analyzed the glucose-dependent expression of human G6Pase in Caco-2/TC7 cells, which were cultured on semipermeable filters to reproduce the physiological conditions of glucose supply at the basal and apical poles of intestinal cells. Serum was supplied in the lower compartment and different concentrations of glucose were applied in the basal and/or apical compartments. The
The mRNA level of G6Pase was first analyzed by Northern blot and compared with that of PEPCK (another gluconeogenic enzyme) (Fig. 1B). We observed a glucose-dependent increase of the G6Pase mRNA level. In the same conditions a slight glucose-dependent increase of the PEPCK mRNA level was also observed. Real-time PCR analysis showed that the glucose-dependent increase of G6Pase mRNA was similar whether glucose was supplied at the apical or at the basal pole of the cells, with a 5- to 6-fold increase in the presence of 25 mM glucose (Fig. 1C). In the same culture conditions, incubation of Caco-2/TC7 cells with 25 mM mannitol, used as an osmotic control, had no effect on G6Pase mRNA level as compared with the low glucose condition (0 mM apical/1 mM basal). As shown in the time course analysis (Fig. 1D), the glucose-dependent increase of the G6Pase mRNA level was detectable as soon as 4 h after incubation in a high glucose medium, and reached a plateau after 24 h incubation. Altogether, these results demonstrate that the enterocyte expression of G6Pase depends on glucose supply, which may have an impact on transcriptional and/or post-transcriptional steps.

Glucose Stabilizes G6Pase mRNA in Caco-2/TC7 Cells—To evaluate a post-transcriptional effect of glucose on G6Pase expression, Caco-2/TC7 cells were cultured in low (0 mM apical/1 mM basal) or high glucose (25 mM apical/5 mM basal) in the presence of the transcription inhibitor 5,6-dichloro-1-β-D-ribofuranosylbenzimidazole (DRB) was added to the culture medium (time 0) and total RNA was extracted at the indicated times after the addition of DRB. The mRNA level of G6Pase was determined by real-time PCR. Results are expressed, for each condition, as the percentage of the value obtained at time 0, and represent mean ± S.E. (n = 3). **, p < 0.01 as compared with low glucose condition.

Glucose Activates the Transcription of the Human G6Pase Gene via Different Glucose-responsive Regions in Caco-2/TC7 and HepG2 Cells—The effect of glucose on human G6Pase gene transcription was analyzed by transfection of different fragments of the −3970/+57 region of the human G6Pase promoter driving the luciferase reporter gene. Stably (black bars) or transient (hatched bars) transfected Caco-2/TC7 cells were cultured on semipermeable filters in low (0 mM apical/1 mM basal) or high glucose (25 mM apical/25 mM basal) conditions (Fig. 3A). A 1.5- to 2-fold increase in luciferase activity was measured in response to high glucose in Caco-2/TC7 cells transfected with the −3970/+57 and −1227/+57 fragments of the human G6Pase promoter. This stimulation by glucose was not observed with the −299/+57 region of the human G6Pase promoter in both transient and stably transfected cells. Similar results were obtained when stably transfected cells were subjected to long-term (6 days, Fig. 3A) or short-term treatment (48 h, not shown) with glucose-containing medium. A glucose-dependent transcription of G6Pase was reported using run-on experiments in hepatocytes (7). We thus analyzed the effect of glucose on the activity of the same promoter fragments of the human G6Pase gene in HepG2 cells. In stably or transiently transfected HepG2 cells, all three fragments of human G6Pase promoter (including the −299/+57 fragment) displayed glucose dependence (Fig. 3B). These results indicate that distinct molecular mechanisms account for the hepatic and intestinal response to glucose and that an enterocyte-specific glucose-responsive region is located between nucleotide −1227 and −299 on the human G6Pase promoter.
tion of the glucose metabolism (28). These three potential ARNT DNA-binding sites are homologous to the consensus DNA-binding sequence of the ARNT/AhR (aryl hydrocarbon receptor) heterodimer. Such DNA-binding sites for the ARNT protein were not found within the −299/+57 region. DNA-binding sites 3 and 4 also comprise putative DNA binding sequences for the USF (site 3 and 4), SP1 (sites 3 and 4), and CREB (site 4) transcription factors. The last DNA-binding site (site 1, between nucleotides −466 and −445) contains an E-box sequence. Such E-box sequences are known to bind basic helix loop helix transcription factors.

Glucose Increases the Binding of Nuclear Proteins on Site 4 of the Human G6Pase Promoter—EMSAs were performed with a set of radiolabeled oligonucleotide probes corresponding to DNA-binding sites 1, 2, 3, and 4 (Table I) and with nuclear protein extracts from villus intestinal cells of mouse fed a standard carbohydrate diet (C) containing 51% of complex sugar, a glucose-rich (G), or a no-sugar (NS) diet. Among the DNA-binding sites analyzed, only site 4 (between nucleotides −1157 to −1133) displayed obvious modifications of DNA-protein complexes according to the presence of sugars (Fig. 4A). Incubation of site 4 with nuclear extracts from mice fed sugar diets (lanes C and G) gave rise to four major bands that markedly increased in these diet conditions as compared with no sugar diet (lane 2 versus 1 and 3). Supershift experiments showed that the three slowest migrating bands were abolished using anti-ARNT (lanes 4 and 5), anti-CREB (lanes 8 and 9), or anti-USF (lanes 6 and 7) antibodies. The fourth band that has the fastest migration was also abolished after incubation with anti-ARNT antibodies (lanes 4 and 5). These observations show that ARNT was present as two complexes, a major ARNT complex corresponding to the fastest migrating band and a minor ARNT complex corresponding to the slowest migrating band. They also suggest that, in the minor complex, ARNT was associated with proteins that provoke a slower migration. Interestingly, we noted that the addition of CREB antibody reduced also the intensity of the minor ARNT complex (lanes 8 and 9). Anti-HNF4α antibodies, used here as controls, did not modify the binding pattern of site 4 (lanes 10 and 11). The binding patterns of site 1 (Fig. 4B), site 2, and site 3 (not shown) were not modified by the presence of sugar, nor by anti-ARNT antibodies (not shown). Recently, Collier et al. (29) have shown, in rat hepatocytes, that the transcription factor c-myc was necessary for the maximal glucose-dependent increase of the G6Pase mRNA level. We have analyzed the presence of c-myc in the protein-DNA complexes obtained with the −1157/−1133 fragment in gel-shift experiments. The addition of a specific anti-c-myc antibody did not provoke any modification of the DNA-protein complexes (data not shown). These results suggested that the −1157/−1133 region of the human G6Pase gene, which binds ARNT, CREB, and USF transcription factors, is involved in the intestinal specific glucose-dependent transcription of the human G6Pase gene.

As the DNA binding sequences of these transcription factors appeared greatly overlapping within site 4 (see Table I), we have tried to determine more precisely the relative affinities of these proteins for the native site 4 sequence using mutated probes (Table II). These mutated probes were designed within the sequence (−1151/−1137) that contains the DNA-binding sites for ARNT, CREB, and USF proteins. As shown in Fig. 5, the m1 mutated probe abolished binding of CREB protein while strongly increasing the binding of USF and of the minor ARNT complex. The m2 mutated probe decreased the binding of CREB, USF, and the major ARNT complex and increased the binding of the minor ARNT complex. The m3 and m4 mutated probes abolished the minor ARNT complex without affecting the major ARNT complex and increased the binding of CREB

| DNA-binding site | Nucleotide position | Sequence | Transcription factors (position) |
|------------------|---------------------|----------|---------------------------------|
| Site 1           | −466/−455           | 5′-GGGATTCAGCGGTGACCCACCC-3′ | USF (−459/−454) |
| Site 2           | −597/−586           | 5′-GTTAGCACTGCACGAAAATTT-3′  | AhR/ARNT (−590/−575) |
| Site 3           | −652/−618           | 5′-CCTGCGCTAGCAACAGCCTCGGC-3′ | AhR/ARNT (−646/−631) |
| Site 4           | −1157/−1133         | 5′-GCCAGGGCATGTCGCTCCTGTCG-3′ | USF (−1138/−1143) |

* Located in the minus strand.
Transcription of Human G6Pase Gene in Intestinal Cells—To determine the functional role in the glucose-dependent transcription of the human G6Pase gene, site 4 has been deleted within the −1227/+57 fragment, generating the 1227/site4/wild-type fragment. The presence of ARNT siRNA did not modify the reporter gene expression as compared with untreated cells (14.4 ± 0.5 versus 16.1 ± 2.4 light units/s/μg of protein for the first cell population (black bars) and 7.4 ± 0.15 versus 7.3 ± 0.8 light units/s/μg of protein for the second cell population (gray bars)). By contrast, when cells were cultured in the presence of glucose, ARNT siRNA reduced by 50–60% the increase of the reporter gene transcription as compared with untreated cells. These results indicate that the transcription factor ARNT is involved in the glucose-dependent transcription of the human glucose-6-phosphatase gene in Caco-2/TC7 cells.

**DISCUSSION**

Enterocytes, the major cell population of intestinal epithelium, ensure the transfer of nutrients from the lumen to the blood, contributing to lipids and sugars homeostasis. It was thus of importance to specifically analyze the regulation of the expression of enzymes involved in the metabolism of nutrients in these cells, among which glucose-6-phosphatase exerts a strategic position in cell energy metabolism. We demonstrate a glucose-dependent regulation of G6Pase gene expression in enterocytes through both post-transcriptional and transcriptional mechanisms, the latter involving the ARNT transcription factor.

In Caco-2/TC7 cells, the activation of G6Pase expression by glucose, which could be observed as soon as after the 4-h treatment, was maximal after 24 h and was in accordance with the effect of fasting on G6Pase expression as described in rat small intestine (30). We have observed that, in the absence of glucose, the half-life of G6Pase mRNA (t½ = 60 min) in enterocytes was in the same range as that previously reported in isolated hepatocytes (t½ = 90 min) (7). Extracellular glucose caused an important increase in G6Pase mRNA stability in Caco-2/TC7 cells (Fig. 2) as already reported in hepatocytes (7). Although the increase of G6Pase mRNA stability could be sufficient to account for an increase in G6Pase mRNA level, we have observed a supplementary effect of glucose leading to activation of G6Pase gene transcription in Caco-2/TC7 enterocytes. This effect requires the −1157/−1133 region of the human G6Pase promoter, whereas in the hepatic HepG2 cell line, the −299/+57 region of the human G6Pase promoter is sufficient for glucose activation. Localization of the glucose-respon-
Insulin on its target genes (34). Recently, Krones and glucose metabolism (32, 33) and it mediates the effect of glucose signaling in the regulation of L-pyruvate kinase. Demonstrated the presence of a cross-talk between oxygen and cultured in low (1 mM) or high (25 mM) glucose conditions and assayed at 1 in the absence of ARNT siRNA and was reported anti-E-cadherin (E-cad) stained using an anti-ARNT antibody and then re-probed with an experiment for each cell population. Data were from three independent experiments for each cell population.

Increase of the normalized luciferase activity measured in high glucose of G6Pase expression in hepatic and intestinal cells, which may indicate different transcription mechanisms involved in glucose-dependent regulation of genes implicated in angiogenesis necessary for tumor progression (35, 36). An important role for this enzyme during the postprandial state in a tissue where the major function is the trans-epithelial transport of nutrients. A role for the G6Pase system in the intestinal glucose transport has been suggested from studies performed in GLUT2 null mice (42) and in humans with congenital GLUT2 deficiency (Fanconi-Bickel syndrome) (43). In these conditions, GLUT2 null mice (42) and in humans with congenital GLUT2 deficiency have been reported to show a defect in glucose transport (43). This suggests that the molecular mechanisms involved in glucose-dependent gene transcription may differ from one tissue to another.

The importance of CREB in the control of glucose homeostasis has also been reported (40). CREB belongs to the basic domain-leucine zipper superfamily and is involved in cAMP-responsive signaling. CREB has been proposed to regulate enzymes of glucose metabolism such as PEPCK (40). Interestingly, Ebert and Bunn (41) showed that ARNT heterodimers were able to interact with CREB-1 to modulate gene expression. Based on our results, we suggest that a cooperation of ARNT (in association or not with its dimerization partners) with USF and CREB factors on the glucose-dependent regulation of G6Pase gene transcription exists in intestinal cells.

The activation by glucose of G6Pase expression in the small intestine through specific mechanisms raises the question of a role for this enzyme during the postprandial state in a tissue where the major function is the trans-epithelial transport of nutrients. A role for the G6Pase system in the intestinal glucose transport has been suggested from studies performed in GLUT2 null mice (42) and in humans with congenital GLUT2 deficiency (Fanconi-Bickel syndrome) (43). In these conditions, the trans-epithelial transport of glucose via the G6Pase system would compensate for the lack of GLUT2 activity leading to an apparent normal transport of glucose through enterocytes. In agreement with this hypothesis we recently observed a large increase in G6Pase mRNA level in the small intestine of GLUT2 null mice compared with wild-type animals (2). This G6Pase pathway may thus coexist with the well described GLUT2-dependent pathway and could contribute up to 15% of total trans-epithelial transport of glucose (42).

During the postprandial period, G6Pase could also regulate utilization of glucose 6-phosphate by several metabolic pathways such as triglyceride and glycolysis synthesis. In the liver it has been proposed that the induction by glucose of G6Pase expression may play a role in the control of glycolysis synthesis by avoiding excess storage in the postprandial period in this tissue (5). The inhibition or the overexpression of G6Pase in vito and in cellular models supported such a hypothesis. In humans, a defect in G6Pase system activity (named Glycogen Storage Disease type 1) is characterized by several severe metabolic disorders including a marked hypoglycemia, glycogen accumulation in the liver, kidney, intestine, hyperlipidemia and lactic acidemia (for re-
view, see Ref. 44). Accordingly, the overexpression of G6Pase in rat liver provoked several abnormalities associated with early stages of non-insulin-dependent diabetes mellitus, including glucose intolerance, hyperinsulinemia, and decreased hepatic glyco- 

gen content. These animals also exhibited significant lower cir- 

nus and lipoprotein metabolism in enterocytes via the assembly and the secretion of chylomicrons during the postprandial period, a physiological regulatory role of G6Pase in the control of sugars and lipoprotein metabolism in enterocytes needs to be considered.

Acknowledgments—We thank Michel Lacasa for helpful discussions and the French researcher’s movement “Sauvons la Recherche” for psychological support.

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Intestinal Glucose-dependent Expression of Glucose-6-phosphatase: INVOLVEMENT OF THE ARYL RECEPTOR NUCLEAR TRANSLOCATOR TRANSCRIPTION FACTOR

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J. Biol. Chem. 2005, 280:20094-20101.
doi: 10.1074/jbc.M502192200 originally published online March 14, 2005

Access the most updated version of this article at doi: 10.1074/jbc.M502192200

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