Sugar-dependent expression of the fructose transporter GLUT5 in Caco-2 cells

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The effect of glucose and fructose and fetal bovine serum on the expression of the fructose transporter GLUT5 was studied in clone PD7 of the human colon cancer cell line Caco-2, which has been characterized previously [Chantret, Rodolosse, Barbat et al. (1994) *J. Cell Sci.* 107, 213–225; Mahraoui, Rodolosse, Barbat et al. (1994) *Biochem. J.* 298, 629–633]. Culture of the cells in dialysed serum and hexose-free media, down-regulated the expression of GLUT5, which was below detection within 3–4 days. This effect was reversed by fructose and glucose feeding of the cells. Fructose feeding yielded a 3-fold higher abundance of GLUT5 protein and mRNA as compared with that expressed in

glucose-fed cells. Cells fed normal serum exhibited an inverse hierarchy of expression, with glucose being a better inducer than fructose for the expression of GLUT5. The GLUT5 mRNA and protein abundances obtained in fructose-fed cells did not depend on the type of serum. A linear relationship between cyclic AMP (cAMP) levels and GLUT5 mRNA abundance was found in cells fed dialysed serum, whereas in cells fed normal serum, mRNA abundances were not correlated to cAMP levels. These results indicate that glucose and fructose, together with serum-related factors and cAMP, have combined effects on the expression of GLUT5 in Caco-2 cells.

INTRODUCTION

Glucose and fructose transepithelial absorption in the small intestine involves specific transport proteins located in the brush border and basolateral membrane of the enterocytes. These transporters belong to distinct families of transporters: the Na-activated transporters with the Na/glucose cotransporter, encoded by the SGLT1 gene [1,2], and two members of the GLUT facilitative transporter family [3,4] with GLUT5 [5], i.e. a brush border membrane-associated protein [6,7] shown to have a fructose transport activity in human [8] and rat [9] cells, and GLUT2 which is involved in the translocation of glucose and fructose from the cytoplasm to the bloodstream across the basolateral membrane [10,11].

Studies designed to characterize the functional adaptation of sugar transporters in animal models have provided evidence for specific dietary sugar regulation of the expression of sugar transport proteins [12–16]. Indeed fructose transport was increased in the small intestinal cells of animals fed fructose-enriched diets, this sugar being the best inducer of its own uptake [17]. These observations were confirmed at the molecular level in rat small intestine, where fructose- but not glucose-enriched diets were shown to increase the mRNA and protein abundances of GLUT5 [18–20].

The human colon carcinoma cell line Caco-2 displays in culture, after confluency, the morphological and functional features of mature enterocytes (for review see [21]), including the expression of proteins involved in the absorption of sugar nutrients: they express sucrase-isomaltase [22], the Na/glucose cotransporter SGLT1 [23], GLUT2 and GLUT5 [7,24] as well as GLUT1 and GLUT3 [24,25]. Although these are cancer cells, and as such have a peculiar metabolism of glucose, they constitute a unique human model *in vitro* to study the regulation of the expression of sugar transporters.

Little is known about the regulation of the expression of the fructose transporter in the human intestine. Forskolin, a drug

known to stimulate adenylate cyclase and increase the level of cellular cyclic AMP (cAMP) [26], was shown to increase strongly the abundances of GLUT5 protein and mRNA in Caco-2 cells [27]. This effect occurs mainly at the transcriptional level, as assessed by the activity of the reporter gene luciferase placed under the control of DNA constructs containing the promoter region of GLUT5. The cAMP responsive element sequences present in the promotor could partially explain the stimulation of transcription of the gene. Indeed their deletion only reduced, but did not abolish, the stimulation of transcription of the reporter gene, indicating that additional elements for the regulation of GLUT5 are involved [27]. The increase in cAMP content of Caco-2 cells has important consequences for the metabolism of glucose, giving an enhanced glucose consumption rate and strong glycogenolysis [28]. These alterations could be involved in the, yet unexplained, increase in the transcription of the GLUT5 gene observed under forskolin treatment [27]. Moreover, the recent observations made in our laboratory, showing that the expression of GLUT5 is strongly dependent on the level of glucose consumption of clones of Caco-2 cells [24], support the idea that monosaccharides may be important for its regulation. Hexose transporter expression in the Caco-2/PD7 clone has been reported elsewhere [24]. In these cells, as in other clones that exhibit a low glucose consumption rate in the stationary phase of growth, GLUT5 mRNA and protein are expressed after confluency in differentiated cells and can be modulated by culture of these cells in low-glucose-containing media [24].

This study describes the effect of the dietary monosaccharides, glucose and fructose, on GLUT5 mRNA and protein abundances in the Caco-2/PD7 clone.

MATERIALS AND METHODS

Cell culture

The parental Caco-2 population [22] was obtained from the late Dr. J. Fogh (Memorial Sloan Kettering Cancer Center, Rye,

Abbreviations used: DMEM, Dulbecco's modified Eagle's minimum essential medium; DPPIV, dipeptidyl-peptidase IV; cAMP, cyclic AMP; ECL, enhanced chemiluminescence; FBS, fetal bovine serum.

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NY, U.S.A.) and has been cloned recently in the laboratory. The isolation, characterization and stability of the clones have been reported elsewhere [29]. The PD7 clone was used in this study because of its high relative levels of expression of the hexose transporter mRNA SGLT1, GLUT2 and GLUT5 [24]. The cells were seeded at 12×10^3 cells/cm² in 25 cm² plastic flasks (Corning Glassworks, Corning, NY, U.S.A.) and cultured in a 10% CO₂/90% air atmosphere in Dulbecco's modified Eagle's minimum essential medium (DMEM) (Eurobio, Paris, France) containing 25 mM glucose. The media were supplemented with 20% heat-inactivated (30 min, 56 °C) fetal bovine serum (FBS; Boehringer, Mannheim, Germany) and 1% non-essential amino acids (Gibco, Glasgow, Scotland, U.K.). This 'standard' medium was used to maintain the cell line which was passaged every 6 days. Under all culture conditions, the medium (0.2 ml/cm²) was changed 48 h after seeding and daily thereafter. Unless specified, all cells were harvested 24 h after a medium change.

For the purpose of this study, the cells were grown either in normal heat-inactivated FBS, yielding a final 1 mM glucose concentration, or in heat-inactivated serum that was dialysed (*M_r* cut-off 6000–8000) against saline and contained less than 100 µM glucose, yielding a final glucose concentration below 10 µM. Dialysed serum conditions were applied after day 10 of culture in the standard medium, so that the differentiation process was under way. Cells were then switched to the three different hexose conditions in dialysed serum. Two different lots of FBS were used. Culture media containing different sugars were made using hexose-free (Hf) DMEM which was supplemented with 25 mM glucose (G) or fructose (F). Detailed protocols of the cultures are given in the legends of the Figures.

cDNA probes and Northern blot analysis

The cDNA probe pHJT5/hGLUT5 (1.9 kb insert) was obtained from G. I. Bell (Howard Hughes Medical Institute, University of Chicago, Chicago, IL, U.S.A.) and DPI-101 (2.5 kb insert) (DPPIV; EC 3.4.14.5) from D. Darmoul [30]. Probes were ³²P-labelled using a Megaprime DNA labelling kit (Amersham).

RNA analysis were performed on cells harvested at the indicated days of the culture. Total RNA was isolated using guanidium thiocyanate and centrifugation through a CsCl gradient [31]. Glyoxal-denatured samples of total RNA were fractionated by electrophoresis on 1% (w/v) agarose gels and subsequently transferred to Hybond*-N membranes (Amersham). Hybridization of the membranes with ³²P-labelled probes was carried out as described [32]. The membranes were washed using high-stringency conditions with a final 15 min wash in 0.1 × SSC/0.1% (v/v) SDS (SSC = 0.15 M NaCl/0.015 M sodium citrate) at 65 °C before exposure of X-ray film.

Western blot analysis

Cell homogenates were prepared as described [7]. Briefly, the cells were scraped in cold 2 mM Tris/HCl, pH 7.1, and 50 mM mannitol containing 1 mM PMSF, 0.02% (v/v) sodium azide and 25 µg/ml benzamidine as protease inhibitors. The cells were disrupted with a conical grinding tube and sonicated (15 s, 60 W). Protein samples (20 µg) were solubilized in Laemmli buffer as modified by Haspel et al. [33] and electrophoresed under denaturing conditions in 10% SDS-slab gels. Molecular-mass markers (Rainbow Markers, Pharmacia) were run in parallel. Proteins were transferred to Hybond-ECL membranes (Amersham) by electroblotting, fixed and stained for visuali-

zation with 0.25% (w/v) Ponceau S in 5% trichloroacetic acid. The membranes were then allowed to react with the anti-(human GLUT5) antibody (ra1668/9; 1:2000 dilution) prepared and characterized in our laboratory [7]. The primary antibody was detected using an anti-(rabbit Ig) antibody from goat (Pasteur Institute, Paris, France) and the luminol ECL detection system (Amersham).

Biochemical assays: glycogen content, glucose- and fructose-consumption rates, lactate production, DPPIV activities and cAMP concentrations

For glycogen assays, the cells were quickly rinsed with cold Ca²⁺/Mg²⁺-free PBS and scraped off the plastic support for subsequent extraction and measurement with anthrone, as previously reported [34]. Glucose consumption was determined by measuring the concentration of glucose in the medium 24 h after the medium change, using the glucose oxidase technique and a Beckman Glucose Analyzer 2. Fructose consumption rates were measured with the glucose/fructose assay kit (Boehringer, Mannheim, Germany) and protein with the BCA protein assay kit using BSA as a standard (Pierce, Rockford, IL, U.S.A.). DPPIV activities were assayed by measuring the hydrolysis of 1.5 mM glycyl-L-proline-4-nitroanilide, as described by Nagatsu et al. [35]. For cAMP assays the culture flasks were quickly emptied, frozen in liquid nitrogen and stored at –70 °C. Monolayers were extracted with ice-cold ethanol and spun at 2000 *g* for 15 min at 4 °C. Dried extracts were assayed with the Biotrak enzyme immunoassay system (Amersham, Les Ulis, France).

RESULTS

Glucose or fructose is required for the expression of GLUT5 mRNA

After 10 days of culture under the standard conditions, i.e. normal serum and 25 mM glucose, total glucose deprivation was

Figure 1 Differential expression of GLUT5 mRNA in Caco-2/PD7 cells cultured in the absence or presence of glucose or fructose

Cells were grown under the standard conditions of culture, i.e. 25 mM glucose and normal heat-inactivated FBS until day 10, and subsequently switched to dialysed serum and either hexose-free- (Hf), 25 mM glucose- (G) or fructose- (F) DMEM. Dot blots were made with 10 µg of total RNA and hybridized with GLUT5 and DPPIV cDNA probes respectively as indicated. Note the absence of modification of the level of DPPIV mRNA abundances under the three conditions of culture.

Figure 2 Reversibility of GLUT5 mRNA expression in glucose-deprived cells re-fed glucose or fructose

Cells were cultured in the standard medium up to day 10. They were totally deprived of glucose from day 10 to day 20 and subsequently cultured in either hexose-free (Hf) or 25 mM glucose (G) or fructose (F) media from day 20 to day 30. Northern blots of 20 μ g of total RNA were hybridized with GLUT5 and DPPIV cDNA probes respectively. *20⁸ stands for RNA extracts taken 8 h after the medium was changed.

obtained by feeding the cells with glucose-free DMEM and dialysed serum. A rapid decrease of GLUT5 mRNA abundance, down to levels below detection, was observed within the first 4 days after the medium change (Figure 1). On the contrary, cells fed glucose and fructose and dialysed serum exhibited measurable GLUT5 mRNA abundances (Figure 1). However, GLUT5 mRNA abundance was significantly higher in fructose- than in glucose-fed cells. Stabilized levels were observed as soon as day 16, i.e. 5 days after the change in the sugar composition of the media. The abundance of DPPIV mRNA did not vary and was therefore used as an internal standard for Northern blot analysis. In order to test the reversibility of the down-regulation of GLUT5 mRNA expression in hexose-free media, cells were switched back to glucose or fructose media (Figure 2). GLUT5 mRNAs were detectable within 8 h, with fructose-fed cells exhibiting a higher abundance than glucose-fed cells. Conversely, in fructose-fed cells which express continuously high levels of GLUT5 (Figure 1), sugar deprivation as well as glucose feeding, down-regulated the expression of GLUT5 to a level identical with that obtained in cells fed the same medium continuously (results not shown).

The presence of increasing amounts of normal serum in the culture media of Caco-2 cells increases the expression of GLUT5 (results not shown). To compare further GLUT5 mRNA abundance in relation to hexose and type of serum (normal versus dialysed) in the culture media, 12 parallel subcultures were made using the different combinations of medium changes (see the legend to Figure 3). The cells were harvested at day 30 of culture, 24 h after the medium change. In dialysed serum the GLUT5

Figure 3 Variations of GLUT5 mRNA abundance as a function of serum and/or hexose content of the media

Northern blots were made with 15 μ g of total RNA extracted from cells harvested at day 30. Dialysed-serum-fed cells were cultured from day 10 to day 20 in either hexose-free (group 1) or 25 mM glucose- (group 2) or fructose- (group 3) DMEM and from day 20 to 30 were placed in glucose- (G), fructose- (F) or hexose-free- (Hf) DMEM. Normal serum-fed cells (group 4) were cultured for 30 days with G-, F-, or Hf-DMEM. Note that identical culture media yield similar GLUT5 mRNA abundances, regardless of the past culture conditions in dialysed serum.

Figure 4 GLUT5 protein abundance in glucose-deprived cells as compared with fructose- and glucose-fed cells

Hexose-free-fed (Hf), glucose-fed (G) and fructose-fed (F) cells were grown as described in Figure 1. Western blots were made with 20 μ g of cell homogenates according to the procedure described in the Materials and methods section.

mRNA abundance is related to the type of sugar added to DMEM. Indeed, cells express more GLUT5 mRNA when fed fructose or glucose compared with cells deprived of any hexose source, the latter exhibiting very low GLUT5 mRNA abundances. This hierarchy of expression, fructose > glucose > hexose-free, was identical in cells harvested earlier at day 20 of culture after a single medium change at day 10 (results not shown). Therefore GLUT5 mRNA abundance is rapidly modulated by sugars in Caco-2 cells and is stabilized at a specific, sugar-defined, level of expression regardless of the sugar composition of the media in which cells were grown during the preceding 10-day period (Figure 3, groups 1, 2 and 3).

The presence of normal serum in the culture medium altered the regulation of the GLUT5 gene by hexoses (Figure 3, group

Figure 5 Variations in GLUT5 protein abundance as a function of serum and/or hexose content of the media

Western blots were made with 20 μ g of cell homogenates at day 30 of culture. Cells were cultured as described in the legend of Figure 3. Group 1 (Hf), group 2 (G) and group 3 (F) indicate the culture conditions from day 10 to day 20, in dialysed serum; group 4 stands for continuous normal-serum cultures. Letters indicate glucose- (G), fructose (F) and hexose-free- (Hf) DMEM used at the moment of harvest.

4). Although cells grown in fructose- or glucose-DMEM and normal serum expressed the highest amounts of GLUT5 mRNA, a significant expression was found in hexose-free-fed cells (Figure 3 group 4). Moreover, and differing from the hierarchy of stimulation found in dialysed serum, cells fed glucose and normal serum expressed the highest abundance of GLUT5 mRNA. This change in the potency of stimulation of the transcription of the GLUT5 gene by monosaccharides suggests that additional serum-dependent, yet unknown mechanisms of regulation, are necessary to obtain the full expression of GLUT5 in Caco-2 cells.

Control of the abundance of GLUT5 protein

GLUT5 protein was assayed by Western blotting (Figure 4). After a transient increase of the protein in all samples (day 13), probably due to a time-lag in the response of protein biosynthesis after the medium changes, the abundance of the GLUT5 protein paralleled that of mRNA, suggesting that its expression is not regulated at the translational level (Figure 5). This observation holds for cells that re-express GLUT5 mRNA, although the protein re-expression was delayed (results not shown). Transmission scanning of the Northern and Western blots confirmed that protein levels are correlated with mRNA abundance regardless of the serum used to grow the cells (Figure 6a).

Hexose consumption rates, lactate production and glycogen and cAMP content in late post-confluent cells

The glucose- and fructose-consumption rates, glycogen content and lactate production of the cells at day 30 of culture were measured to establish whether the expression of GLUT5 could be correlated to the variation of these parameters. Hexose-deprived cells lost essentially all (90%) their glycogen (Table 1) within 48 h. Conversely, glycogen stores were replenished after 48 h when glucose or fructose were provided to the cells (results not shown). However, fructose-fed cells contained 25% less glycogen than glucose-fed cells (Table 1) regardless of the type of serum used. In normal serum, glycogen stores in hexose-deprived cells were 50% that of glucose-fed cells and did not fall to, essentially, zero as in dialysed serum, presumably due to the presence of 1 mM glucose in normal serum (Table 1). Similar observations were made in cells harvested in the early post-confluent stage of growth (day 10) and in the late stationary phase (day 25) (results not shown). Hexose consumption rates and lactate production at day 30 of culture were proportional, although the rate of lactate production was lower in dialysed serum-fed cells compared with cells fed normal serum.

For cells fed dialysed serum, a linear relationship was obtained when GLUT5 mRNA abundance was plotted against cAMP

Figure 6 (a) Protein and mRNA relationship in Caco-2 cells and (b) plot of GLUT5 mRNA abundance as a function of cAMP

(a) Plot of GLUT 5 protein abundance (arbitrary units) as a function of mRNA abundance (arbitrary units) obtained by transmission scanings of the X-ray films corresponding to 4–6 independent cell cultures. Linear regression parameters: $y = -5.7851 + 1.1043x$, $R = 1$. (b) Data from four independent cell cultures with two lots of FBS were normalized to the amount of cAMP obtained in 25 mM glucose and dialysed FBS. Linear regression parameters: $y = -8.018 + 1.1527x$, $R = 1$. Normal serum (squares); dialysed serum (circles). Cell feeding: glucose (hatched symbols), fructose (closed symbols) or hexose-free (open symbols).

(Figure 6b). Cells fed fructose contained ten times more cAMP than hexose-deprived cells. Glucose yielded an intermediate level of cellular cAMP and mRNA abundance (Table 1). On the contrary, no such correlation is found for the effect of glucose or fructose on the expression of GLUT5 in normal serum. Indeed the highest expression of GLUT5 mRNA occurs in glucose-fed

Table 1 Biochemical parameters of cells taken at day 30 of culture

Glucose and fructose consumption rates and lactate production are given in $\mu\text{g}/\text{mg}$ of protein per h. DPPIV activities are given in m-units/mg of protein, cAMP concentration in fmol/mg of protein and glycogen in $\mu\text{g}/\text{mg}$ of protein. Data represent means $\pm$ S.E.M. for (*n*) independent cultures. nd, not determined.

Serum	DMEM	Sugar consumption rates	Lactate	DPPIV	Glycogen	cAMP
Dialysed	Glucose	41.1 $\pm$ 2.3 (18)	39.7 $\pm$ 1.7 (18)	170 $\pm$ 11 (7)	423 $\pm$ 25 (16)	549 $\pm$ 61 (5)
	Fructose	67.0 $\pm$ 7.9 (16)	38.7 $\pm$ 2.3 (18)	164 $\pm$ 17 (6)	333 $\pm$ 28 (13)	1936 $\pm$ 224 (5)
	Hexose-free	nd	nd	179 $\pm$ 28 (4)	32 $\pm$ 5 (13)	177 $\pm$ 3 (5)
Normal	Glucose	77.5 $\pm$ 2.3 (6)	65.93 $\pm$ 2.7 (6)	150 $\pm$ 4 (4)	328 $\pm$ 29 (4)	1819 $\pm$ 230 (3)
	Fructose	114.0 $\pm$ 6.6 (6)	57.93 $\pm$ 2.3 (6)	174 $\pm$ 3 (3)	261 $\pm$ 13 (5)	2717 $\pm$ 47 (5)
	Hexose-free	nd	nd	192 $\pm$ 9 (4)	184 $\pm$ 24 (5)	1971 $\pm$ 341 (3)

cells, where the cAMP level did not differ from that obtained in fructose- or hexose-free-fed cells (Table 1).

DISCUSSION

Glucose, fructose and serum are involved in the regulation of the fructose transporter GLUT5 in the Caco-2/PD7 clone. In dialysed serum, fructose was the best stimulator of GLUT5 expression, whereas glucose- and hexose-deprived cells exhibited low and essentially no expression respectively. This hierarchy was different in normal serum-fed cells where glucose is the best inducer. Fructose, however, yielded essentially the same level of GLUT5 mRNA abundance in dialysed and normal serum. Serum therefore mainly affects the expression of GLUT5 in glucose-fed cells, indicating that Caco-2 cells utilize glucose and fructose differently even though both sugars are used as energy and carbon sources [36]. In effect, Caco-2 cells produce lactate and synthesize glycogen from fructose or glucose. The effect of glucose and fructose on GLUT5 protein and mRNA abundances was reversible and specific, since DPPIV mRNA abundance and activity were not modified under any of the culture conditions used in this study.

Reversible fructose enhancement of the mRNA and protein abundance of GLUT5 have also been demonstrated in rats fed fructose-enriched diets [18–20], indicating that a common mechanism of control of the expression of the fructose transporter is shared by human and rat intestinal cells. However, in rat small intestine, glucose did not appear to induce the transcription of the GLUT5 gene [18–20]. It cannot be excluded that the stimulation provided by blood glucose-supplies could be sufficient to induce GLUT5 expression at a basal level of abundance even though the control diet is devoid of any hexose [18].

GLUT5 protein abundance in cells fed monosaccharides paralleled that of mRNA, indicating that the expression of the gene is not regulated at the translational level in Caco-2 cells. Protein and mRNA abundances exhibit different time-courses of regulation, a longer time being necessary to obtain a stabilized level of protein than that necessary to stabilize mRNA levels (compare GLUT5 mRNA and protein abundances in Figures 1 and 4). These results are in contrast with those obtained in rat small intestine where the fructose-enriched diet had a 3- to 4-fold stronger effect on protein abundance than on mRNA levels [19,20]. Fructose-enriched diets induced stimulated fructose uptake 2.5-fold in the rat [13,17], a value similar to that obtained for mRNA abundance but lower than that for protein. In human small intestine, not only fructose but also glucose, stimulates fructose uptake (reviewed in [37]), suggesting that rodents and normal human enterocytes or Caco-2 cells may display different

mechanisms for the control of the abundance of the fructose carrier.

The question now arises of the identification of the signal(s) that are responsible for the increase in GLUT5 mRNA and protein abundances in human intestinal cells. The answers presently offered are: the metabolic hypothesis, supported by the difference of expression observed in high- and low-glucose-consuming Caco-2 clones which express low and high GLUT5 mRNA abundances respectively [24]; and the hypothesis that a fructose and/or glucose sensor could be involved in the expression of hexose transporters, as proposed for SGLT1 in sheep [38,39] and rodents [40,41] small intestine. Our results do not favour one or the other of these proposals because GLUT5 is reversibly modulated in differentiated cells which were seeded in glucose media.

The GLUT5 gene is up-regulated by cAMP [27]. Under the standard conditions of culture, i.e. normal serum and 25 mM glucose, the presence of neuropeptides that activate adenylate cyclase yield constitutive and permanent activation of the transcription of the gene. This effect is in contrast to that seen in PD7 cells cultured in dialysed as compared with normal serum. Indeed the cAMP levels of glucose-fed cells are lowered by two-thirds in dialysed serum, thus explaining the sharp decrease in GLUT5 mRNA abundance observed in response to a medium change from normal to dialysed serum. In fructose-fed cells, cAMP levels were 10-fold higher than those obtained in hexose-free DMEM and yielded high mRNA and protein abundances which did not depend on the type of serum. In dialysed serum only, the expression of GLUT5 was directly related to the increase in cAMP concentrations in the cells. In normal serum, glucose was the best inducer, although cAMP levels are essentially not modified by addition of glucose to the culture media. Normal serum contains not only adenylate-cyclase activators but also small molecules which may be important co-factors for the metabolic fate of glucose. Therefore we conclude that not only cAMP but also the utilization of glucose by the cells is involved in the expression of GLUT5.

More work is obviously needed to compare and contrast the results obtained in Caco-2 cells and normal human small intestinal absorbing cells. Nevertheless, the present study describes a first attempt to understand the molecular mechanisms by which the GLUT5 transporter is regulated by sugar in human cells. Our results demonstrate that monosaccharides and cAMP are essential for the expression of the GLUT5 gene in Caco-2 cells. Fructose induction seems to rely exclusively on the increment of cAMP and of its transduction pathways, whereas additional intermediates are required for the full activation of the expression of GLUT5 by glucose.

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REFERENCES

- 1 Hediger, M., Coady, M. J., Ikeda, T. S. and Wright, E. M. (1987) *Nature* (London) **330**, 379–381
- 2 Wright, E. M. (1993) *Annu. Rev. Physiol.* **55**, 575–589
- 3 Bell, G. I., Kayano, T., Buse, J. B. et al. (1990) *Diabetes Care* **13**, 198–208
- 4 Bell, G. I., Burant, C. F., Takeda, J. and Gould, G. W. (1993) *J. Biol. Chem.* **268**, 19161–19164
- 5 Kayano, T., Burant, C. F., Fukumoto, H. et al. (1990) *J. Biol. Chem.* **265**, 13276–13282
- 6 Davidson, N. O., Hausman, A. M. L., Ifkovits, C. A. et al. (1992) *Am. J. Physiol.* **262**, C795–C800
- 7 Mahraoui, L., Rousset, M., Dussaulx, E., Darmoul, D., Zweibaum, A. and Brot-Laroche, E. (1992) *Am. J. Physiol. (Gastrointest. Liver Physiol. 26)* **263**, G312–G318
- 8 Burant, C. F., Takeda, J., Brot-Laroche, E., Bell, G. I. and Davidson, N. O. (1992) *J. Biol. Chem.* **267**, 14523–14526
- 9 Rand, E. B., Depauli, A. M., Davidson, N. O., Bell, G. I. and Burant, C. F. (1993) *Am. J. Physiol. (Gastrointest. Liver Physiol. 27)* **264**, G1169–G1176
- 10 Thorens, B., Cheng, Z.-Q., Brown, D. and Lodish, H. F. (1990) *Am. J. Physiol.* **259**, C279–C285
- 11 Cheeseman, C. I. (1993) *Gastroenterology* **105**, 1050–1056
- 12 Ferraris, R. P. and Diamond, J. M. (1986) *J. Membr. Biol.* **94**, 77–82
- 13 Bode, C., Eisenhardt, J. M., Haberich, F. J. and Bode, J. C. (1981) *Res. Exp. Med.* **179**, 163–168
- 14 Karasov, W. H. and Diamond, J. M., eds. (1987) in *Physiology of the Gastrointestinal Tract*, pp. 1489–1497, Raven Press, New York
- 15 Karasov, W. H. and Diamond, J. M. (1983) *Am. J. Physiol. (Gastrointest. Liver Physiol. 8)* **245**, G443–G462
- 16 Ferraris, R. P. in (1994) *Physiology of the Gastrointestinal Tract*, vols. 1 and 2, (Johnson, L. R., ed.), 3rd edn., pp. 1821–1844, Raven Press, New York
- 17 Solberg, D. H. and Diamond, J. M. (1987) *Am. J. Physiol.* **252**, G574–G584
- 18 Miyamoto, K., Hase, K., Takagi, T. et al. (1993) *Biochem. J.* **295**, 211–215
- 19 Inukai, K., Asano, T., Katagiri, H. et al. (1993) *Endocrinology* **133**, 2009–2014
- 20 Burant, C. F. and Saxena, M. (1994) *Am. J. Physiol.* **267**, G71–G79
- 21 Zweibaum, A., Laburthe, M., Grasset, E. and Louvard, D. (1991) in *Intestinal Absorption and Secretion. Handbook of Physiology, Section 6. The Gastrointestinal System* (Field, M. and Frizzell, R. A., eds.), vol. IV, pp. 223–255, American Physiological Society, Bethesda
- 22 Pinto, M., Robine-Léon, S., Appay, M. D. et al. (1983) *Biol. Cell.* **47**, 323–330
- 23 Blais, A., Bissonnette, T. and Berteloot, A. (1987) *J. Membr. Biol.* **99**, 113–125
- 24 Mahraoui, L., Rodolosse, A., Barbat, A. et al. (1994) *Biochem. J.* **298**, 629–633
- 25 Harris, D. S., Slot, J. W., Geuze, H. J. and James, D. E. (1992) *Proc. Natl. Acad. Sci. U.S.A.* **89**, 7556–7560
- 26 Seamon, K. B., Padgett, W. and Daly, J. W. (1981) *Proc. Natl. Acad. Sci. U.S.A.* **78**, 3363–3367
- 27 Mahraoui, L., Takeda, J., Mesonero, J. et al. (1994) *Biochem. J.* **301**, 169–175
- 28 Rousset, M., Laburthe, M., Pinto, M. et al. (1985) *J. Cell Physiol.* **123**, 377–385
- 29 Chantret, I., Rodolosse, A., Barbat, A. et al. (1994) *J. Cell Sci.* **107**, 213–225
- 30 Darmoul, D., Lacasa, M., Chantret, I., Swallow, D. and Trugnan, G. (1990) *Ann. Hum. Genet.* **54**, 191–197
- 31 Chirgwin, J. M., Przybyla, A. E., McDonald, R. J. and Rutter, W. J. (1979) *Biochemistry* **18**, 5294–5299
- 32 Thomas, P. S. (1980) *Proc. Natl. Acad. Sci. U.S.A.* **77**, 5201–5205
- 33 Haspel, H. C., Wilk, E. W., Birnbaum, M. J., Cushman, S. W. and Rosen, O. M. (1986) *J. Biol. Chem.* **261**, 6778–6789
- 34 Rousset, M., Chevalier, G., Rousset, J. P., Dussaulx, E. and Zweibaum, A. (1979) *Cancer Res.* **39**, 531–534
- 35 Nagatsu, T., Hino, M., Fuyamada, H. et al. (1976) *Anal. Biochem.* **74**, 466–476
- 36 Ellwood, K. C., Chatzidakis, C. and Failla, M. L. (1993) *Proc. Soc. Exp. Biol. Med.* **202**, 440–446
- 37 Rumessen, J. J. and Gudmand-Hoyer, E. (1986) *Gut* **27**, 1161–1168
- 38 Freeman, T. C., Wood, I. S., Sirinathsinghji, D. J. S., Beechey, R. B., Dyer, J. and Shirazi-Beechey, S. P. (1993) *Biochim. Biophys. Acta* **1146**, 203–212
- 39 Dyer, J., Scott, D., Beechey, R. B., Care, A. D., Abbas, S. K. and Shirazi-Beechey, S. P. (1994) in *Mammalian Brush Border Membrane Proteins II*, (Lenze, M. D., Grand, R. G. and Naim, H. Y., eds.), pp. 65–72, Thieme Verlag, Stuttgart, New York
- 40 Ferraris, R. P., Villenas, S. A., Hirayama, B. A. and Diamond, J. (1992) *Am. J. Physiol.* **262**, G1060–G1068
- 41 Ferraris, R. P. and Diamond, J. (1992) *Am. J. Physiol.* **262**, G1069–G1073