Glucose and thyroid hormone co-regulate the expression of the intestinal fructose transporter GLUT5

Miriam MATOSIN-MATEKALO*, José E. MESONERO*, Thibaut J. LAROCHE*, Michel LACASA*† and Edith BROT-LAROCHE*¹

*Unité de Recherches sur la Différenciation Cellulaire Intestinale, INSERM U178, 16 avenue Paul-Vaillant-Couturier, 97807 Villejuif cedex, France, and †Université Pierre et Marie Curie, 4 place Jussieu, 75005 Paris, France

Expression of the fructose transporter GLUT5 in Caco-2 cells is controlled by the carbohydrate content of the culture media [Mesonero, Matosin, Cambier, Rodriguez-Yoldi and Brot-Laroche (1995) *Biochem. J.* **312**, 757–762] and by the metabolic status of the cells [Mahraoui, Takeda, Mesonero, Chantret, Dussaulx, Bell, and Brot-Laroche (1994) *Biochem. J.* **301**, 169–175]. In this study we show that, in fully differentiated Caco-2/TC7 cells, thyroid hormone and glucose increase GLUT5 mRNA abundance in a dose-dependent manner. Using Caco-2/TC7 cells stably transformed with various fragments of the GLUT5 promoter inserted upstream of the luciferase reporter gene, we localized the sequences that confer 3,3',5-L-tri-iodothyronine (T_3)- and/or glucose-sensitivity to the gene. Glucose responsiveness is conferred by the –272/+41 fragment of the promoter, but it is only with the –338/+41 region that

transcription of the luciferase reporter gene is stimulated by T_3 . This 70 bp fragment from position –338 to –272 of the GLUT5 gene is able to confer T_3 /glucose-responsiveness to the heterologous thymidine kinase promoter. Electrophoretic-mobility-shift assays demonstrate that thyroid hormone receptors α and β are expressed in Caco-2/TC7 cells. They further show that the –308/–290 region of the GLUT5 promoter binds thyroid hormone receptor/retinoid X receptor heterodimers, and that glucose and/or T_3 exert a deleterious effect on the binding of the nuclear protein complex.

Key words: Caco-2/TC7 cells, GLUT5 promoter, human intestinal cells, thyroid hormone receptors, 3,3',5-L-tri-iodothyronine-responsive element.

INTRODUCTION

GLUT5 is a facilitative hexose transporter which is present in adult and fetal human small intestine, but not in the colon [1–3]. It is expressed in Caco-2 cells [2,4], a human colon cancer cell line that exhibits, after confluency, the morphology and functional characteristics of intestinal absorbing cells (reviewed in [5,6]). *In vivo*, a decrease in glucose intake does not alter GLUT5 mRNA and protein abundance [7], whereas in Caco-2 cells, a system in which sugar supplies can be fully controlled, glucose and fructose are essential activators of GLUT5 expression [8].

Thyroid hormone is a multipotent hormone that exerts effects on development, cell growth and metabolism *in vivo* (for a review, see [9]). It stimulates glucose transporter expression in liver [10] and muscle [11], as well as in the Clone 9 [12] and ARL 15 [13] hepatoma cell lines and in rat Sertoli cells [14]. Thyroid-hormone-dependent stimulation of target genes requires the interaction of thyroid hormone receptors (TRs) with specific nucleotide sequences called thyroid-hormone-responsive elements (TREs). Consensus TREs consist of two or more hexanucleotide half-sites [RGG(T/A)CA] that can be organized as direct, inverted or everted repeats. TRs belong to the ligand-dependent, DNA-interacting protein superfamily that includes receptors for steroid hormones, vitamin D_3 , retinoids and farnesoids, as well as many other receptors with unknown or no ligand (orphan receptors). Two TR genes, *TR α* and *TR β* , have been identified on human chromosomes 17 and 3 respectively, and different isoforms ($\alpha 1$, $\beta 1$, $\beta 2$) are generated by alternative

splicing. TRs bind to TREs as homo- or hetero-dimers with retinoid X receptors (RXRs) or vitamin D_3 receptors (for a review, see [15,16]). Unliganded TRs, however, can repress transcription in association with co-repressors [17,18]. Upon binding of 3,3',5-L-tri-iodothyronine (T_3), TRs can promote target gene expression, and it is proposed that T_3 binding modifies the conformation of the TR, which results in the rearrangement of an activating complex with co-activators [19].

In the present study we have used Caco-2/TC7 and Caco-2/PD7 cells, two clones that express significant levels of GLUT5 [4]. In these cells, T_3 stimulates the expression of intestinal genes involved in Na^+ /glucose co-transport, i.e. it increases the abundance of SGLT1 (Na^+ /glucose co-transporter 1) and Na^+ /K⁺-ATPase mRNAs [20,21]. It also increases the glucose consumption rate and the expression of phosphoenolpyruvate carboxykinase mRNA [21] in the cells. We investigated the effects of glucose and T_3 on the expression of GLUT5 at the levels of mRNA abundance, promoter activity and TR binding to DNA. Our results indicate a co-regulation of GLUT5 gene expression by T_3 and glucose that occurs through a TRE.

EXPERIMENTAL

Cell culture

This paper will describe results obtained with Caco-2/TC7 cells; identical results were obtained in parallel with Caco-2/PD7 cells. The cells were cultured and maintained as described

Abbreviations used: T_3 , 3,3',5-L-tri-iodothyronine; TRE, thyroid-hormone-responsive element; RXR, retinoid X receptor; TR, thyroid hormone receptor; TK, thymidine kinase; SIFR, sucrase–isomaltase factor, repressor.

¹ To whom correspondence should be addressed (e-mail laroche@infobiogen.fr).

```

-338/-299  5'-TCGACATCTTTGAGGTCTCTAGTGGCCCAATCTGTTGCCCTCAG-3'
-310/-272                                5'-TGTTGCCCTCAGATTACCTGGTGCATGTAGGGTGGAGGAC-3'
-308/-290                                5'-TGCCCTCAGATTACCTGG-3'
-308/-290M                                5'-TGgtCTCAGATTAggTGG-3'
DR4      5'-TCGATCAGGTACAGGAGGTGAG-3'
SIFR     5'-TCCCAGAGAGAAACATTTATGTAACTA-3'

```

Figure 1 Oligomers used in the present study

previously [22] in Dulbecco's modified Eagle's medium (Eurobio, Paris, France) containing 25 mM glucose, 1% non-essential amino acids (Gibco, Glasgow, Scotland, U.K.), 100 units/ml penicillin, 100 µg/ml streptomycin and 20% (v/v) heat-inactivated (30 min; 56 °C) fetal bovine serum (Boehringer, Mannheim, Germany) (G^{25,N} medium). Glucose-free (G^{0,d}) and glucose-supplemented (G^{25,d}) media were also prepared containing 20% (v/v) heat-inactivated serum that was dialysed against saline (molecular mass cut-off 6000–8000 Da). These culture conditions were applied as described previously [8], after the differentiation process that depends on serum factors is fully established for at least 5 days. All cells were harvested 24 h after a medium change. T₃ (Sigma, St. Louis, MO, U.S.A.) was added as a 1 mM stock solution in 0.1 M NaOH. Addition of up to 1 µM T₃ did not affect cell viability or growth parameters [21].

cDNA probes and Northern blot analysis

The cDNA probes used in this study were: GLUT5 (pJHT5), obtained from G. I. Bell (Howard Hughes Medical Institute, University of Chicago, Chicago, IL, U.S.A.); villin V19 [23], from D. Louvard (Institut Pasteur, Paris, France); and SI2 [24]. The luciferase probe was the *EcoRI*–*KpnI* fragment from PTZ-Luc [25]. Total RNA was isolated using guanidium isothiocyanate and centrifugation through a CsCl gradient [26]. Glyoxal-denatured samples of total RNA were Northern-blotted as described previously [27]. Hybridizations of ³²P-labelled probes to membranes, washing procedures and exposure of X-ray films were carried out as described in [8].

Expression of human GLUT5-reporter-gene constructs in Caco-2 cells

The 2.5 kb *EcoRI*–*MluI* fragment from pGT5/pGL2 (–2500/+41) containing the promoter region of GLUT5 was used [27] to generate 5' deletion constructs using the *HinfI* (–385), *SfaI* (–338) and *PvuII* (–272) restriction sites. The GLUT5-promoter-luciferase unit was further inserted into the multi-cloning site of the episomal p205-GTI plasmid, which contains a hygromycin-resistance gene for the selection of transformed cells [28]. In some constructs the thymidine kinase (TK) promoter was inserted downstream of the –385/–272 and –338/–272 fragments of the GLUT5 promoter.

Plasmid DNAs were purified twice on CsCl/ethidium bromide density gradients [29]. Dividing Caco-2/TC7 cells (seeded at 12 × 10³ cells/cm² in 78 cm² Petri dishes; Corning Glassware Co.) were transfected with 15 µg of plasmid DNA, using the calcium phosphate precipitation procedure as described in [27]. Cells were allowed to reach confluence and then selected twice with hygromycin (500 µg/ml final concentration). As hygromycin affects metabolism [30], transformed cells were grown without hygromycin for one passage, even though it did not affect GLUT5 mRNA abundance (results not shown).

Luciferase assays

Cell layers were rinsed with cold Ca²⁺/Mg²⁺-free PBS and lysed in 25 mM Tris/phosphate buffer, pH 7.8, containing 15% (v/v) glycerol, 8 mM MgCl₂, 1 mM EDTA, 1% Triton X-100, 1 mM dithiothreitol and 0.4 mM PMSF. The extracts were prepared at day 16 of culture. Luciferase activities were assayed using 20 µl of the 13000 g supernatant with the Luciferase Assay System (Promega) and a Lumat LB9501 luminometer (Berthold). The protein content of the samples was quantified with the BCA protein assay reagent (Pierce, Rockford, IL, U.S.A.). The plasmid luciferase and genomic sucrose-isomaltase DNAs were assayed as described in [31]. Luciferase activities are expressed as relative light units/s per mg of protein, and were corrected for the number of copies of the plasmid in the cells.

Gel mobility-shift assays

Nuclear proteins were extracted from between 1 × 10⁸ and 1 × 10⁹ Caco-2/TC7 cells at day 16, as described in [32]. Two overlapping oligonucleotides, corresponding to the –338/–299 and –310/–272 sequences of the GLUT5 promoter (Figure 1), as well as the overlapping –308/–290 sequence, were synthesized (Eurobio, Les Ulis, France). The SIFR ('sucrase-isomaltase factor, repressor') E4BP4 binding sequence from the sucrase-isomaltase gene was used as an unrelated competitor ([33]; see Figure 1). [³²P]dCTP-end-labelled probes (0.2 ng) were challenged with 2 µg of nuclear protein extract from control or T₃-treated Caco-2 cells in the presence of 1 µg of poly(dI-dC) and binding buffer containing 1.5 mM Hepes, pH 7.9, 0.1 mM EGTA, 0.1 mM EDTA, 40 mM KCl, 0.5 mM dithiothreitol and 5% (v/v) glycerol. Increasing amounts of nuclear proteins (1, 2 and 3 µg) in the assay increased the abundance of the shifted band, without modifying its profile (results not shown). Supershifts were carried out using: (1) a TRα-specific antibody directed against the N-terminal sequence of the A/B domain of the receptor (kindly donated by O. Chassande and J. Samarut, ENS, Lyon, France); (2) an anti-TRβ1 antibody (J52; Santa Cruz Biotechnology) that does not cross-react with TRα; (3) an anti-RXR antibody (4RX-1D12; provided by P. Chambon and C. Rochette-Egly, IGBMC, Strasbourg, France); and (4) two IgG2bs directed against peptides comprising residues 371–380 and 16–25 respectively of p53 [34] (kindly given by T. Soussi, Institut Curie, Paris, France). Unrelated antibodies were used as controls: a polyclonal antibody to the C-terminus of GLUT5 (1169/3-G5) and a monoclonal ascitic anti-Lewis A antibody (7LE; from R. Oriol, INSERM U178, Villejuif, France).

RESULTS

T₃ increases GLUT5 mRNA abundance in Caco-2/TC7 cells

Caco-2/TC7 cells were treated chronically with T₃ during the growth and differentiation process of this enterocytic-like cell line, i.e. treatment was started at day 2 after seeding, and was

Figure 2 T_3 -dependent stimulation of GLUT5 mRNA abundance in Caco-2/TC7 cells

Standard conditions of culture (i.e. normal serum and 25 mM glucose) were applied to Caco-2/TC7 cells. Left panel: growth-dependent increase in GLUT5 and villin expression in cells treated with 1 μ M T_3 . Right panel: GLUT5 and villin mRNA expression as a function of T_3 dose in 20-day-cultured cells. Exposure times were 2 h and 5 h for villin and GLUT5 respectively.

maintained up to day 20. Before confluency, which takes place around day 6 under our culture conditions, GLUT5 was not expressed and could not be induced by T_3 treatment (results not shown). T_3 treatment of the cells during the early differentiation period (days 7–12) did not alter GLUT5 expression; indeed, it was only in fully differentiated cells (i.e. after day 12) that T_3 treatment increased GLUT5 mRNA levels strongly (4–5-fold) (Figure 2, upper left panel). The hormone did not affect cell differentiation, as evaluated morphologically under the microscope and functionally by the expression of the villin gene (Figure 2, lower panels). T_3 treatment affected the three transcripts of GLUT5 (5.1, 2.8 and 2.0 kb) equally. Stimulation was dose-dependent, and reached a maximum at 100 nM T_3 (Figure 2, upper right panel).

T_3 increases transcription through a 67 bp fragment present in the GLUT5 gene promoter

To study the possible effect of T_3 on GLUT5 gene transcription and to investigate the presence of a TRE in the GLUT5 promoter, different fragments of this region were placed upstream of the luciferase reporter gene. The basal transcriptional activities were similar, independent of the deletion end points, and T_3 stimulated the expression of the reporter gene 4–5-fold in all constructs that contained the –338/–272 region of the promoter (Figure 3). A 4-fold stimulation was also observed with T_3 when the –385/–272 or the –338/–272 fragment was inserted upstream of the TK promoter. The stimulation by T_3 was only 2-fold with the TK promoter alone, the –272/+41 GLUT5 promoter or the promoterless luciferase construct (Figure 3). The –338/–272 GLUT5 promoter region thus contains T_3 -responsive elements that can increase the response of the heterologous TK promoter to T_3 . Transformed and parental cells exhibited identical T_3 -dependent increases in GLUT5 mRNA abundance (results not shown), indicating that transfection and cell selection with hygromycin did not alter the T_3 transduction pathways.

Glucose is required for the response of GLUT5 to T_3

GLUT5 mRNA is virtually absent from glucose-deprived ($G^{0.4}$) Caco-2 cells ([8]; and Figure 4). Its level increased progressively with glucose concentration (1–25 mM) in the culture medium, and reached a plateau around 5 mM glucose (Figure 4A). The promoter activities of the –272/+41 and –338/+41 GLUT5

Figure 3 The T_3 -responsive element of GLUT5 is located in the –338/–272 region of the promoter

Standard (G^{25N}) conditions of culture were applied to transformed Caco-2/TC7 cells. The extracts were prepared at day 16. Luciferase activities (relative light units; means $\pm$ S.D.) obtained with the –2500/+41, –385/+41, –338/+41 and –272/+41 GLUT5-promoter–luciferase constructs and the –385/–272 and –338/–272 TK-promoter–luciferase constructs in control (empty bars) or T_3 -treated (solid bars) cells were normalized to the number of copies of the plasmid. The inset presents an expanded view of the data obtained in the absence of the promoter.

fragments, revealed by measuring luciferase mRNA (Figure 4B) and activity (Figure 4C), were also glucose-dependent up to a concentration of 5 mM. T_3 increased the mRNA levels transcribed from the endogenous GLUT5 gene (Figure 4A), as well as from the luciferase gene (Figure 4B) driven by the –338/+41 fragment. The latter observation is consistent with the luciferase activity data (Figure 4C). The T_3 stimulation, however, is lost in the absence of the –338/–272 element. A parallel study using the TK promoter construct containing the –338/–272 fragment of the GLUT5 gene yielded the same glucose- and T_3 -dependence (results not shown). The luciferase activity of the TK promoter construct alone was insensitive to both glucose and T_3 in cells cultured with dialysed serum (results not shown), suggesting that some factor that is stimulatory to the TK promoter and is present in normal serum has been lost.

Protein binding to the –338/–272 promoter region of GLUT5 depends on glucose and T_3 cell culture conditions

The 67 bp region (–338/–272) that is involved in the glucose/ T_3 -dependent transcriptional response was split into two overlapping oligomers (Figure 1). Both the –338/–299 and –310/

Figure 4 Glucose and T_3 are required for efficient transcriptional activity of the GLUT5 promoter

Cells were transformed with the $-338/+41$ (□, ■) or the $-272/+41$ (●, ○) region of the GLUT5 promoter. Controls (○, □) and chronically T_3 -treated (■, ●) cells were taken at day 16 after a 6-day culture in the appropriate glucose-supplemented culture media ($G^{0-25,0}$) before measurement of luciferase activities (C). Total RNA was extracted from the $-338/+41$ -transformed cells. Northern blots were prepared with 15 μ g of total RNA and hybridized with the GLUT5 (A) and luciferase (LUC) (B) cDNA probes, as described in the Experimental section. X-ray films were exposed for 9 h and 72 h for GLUT5 and luciferase respectively. Luciferase activity and mRNA abundance were both saturated at a concentration of 5 mM glucose.

-272 probes formed DNA complexes with Caco-2 cell nuclear protein extracts (Figure 5). Surprisingly, the amounts of complexes were reduced in T_3 -treated cells (compare lanes 1 and 2, and lanes 10 and 11, in Figure 5). The complexes completely disappeared upon the addition of a 100-fold molar excess of the homologous oligonucleotides (Figure 5, lanes 3/4 and 12/13), but not of the unrelated SIFR element (Figure 5, lanes 5/6 and 16/17). DNA-protein interactions involving the distal $-338/-299$ probe were fully displaced by the unlabelled proximal $-310/-272$ oligonucleotide (Figure 5, lanes 8 and 9). A reciprocal competitive effect, however, was not observed, as an excess of $-338/-299$ oligonucleotide did not affect protein binding on the $-310/-272$ probe (Figure 5, lanes 14 and 15). This suggests the existence of a high-affinity binding site in the $-310/-272$ fragment and of a weaker site in the $-338/-299$ fragment. Alternatively, the complex may bind preferentially to the sequence common to both fragments.

Electromobility-shift assays using an oligonucleotide ($-308/-290$) that overlapped each of the other two were therefore performed. Figure 6 (upper panel) shows that the profiles obtained with the three probes were very similar, and depended on the culture conditions of the cells. With $G^{0,d}$ cell extracts (Figure 6, lane 0,d), the probe was almost completely shifted and formed a single band, the intensity of which was not altered by T_3 treatment of the cells. With extracts from glucose-fed cells (Figure 6, lanes 25,d), a less abundant and T_3 -sensitive complex was formed with the three probes ($-338/-299$, $-308/-290$ and $-310/-272$). A comparable decrease in the bandshift was produced by the addition of 100 nM T_3 during the incubation of control nuclear extracts with the probe (results not shown). The bandshift was dramatically decreased when cells were grown in a medium containing normal serum and 25 mM glucose (Figure

Figure 5 Interactions between Caco-2 nuclear protein and GLUT5 promoter DNA are modulated by T_3

Nuclear extracts were prepared using $G^{25,d}$ cells at day 16 of culture, as described in the Experimental section. T_3 treatment was applied throughout the culture. Electromobility-shift assays of the $-338/-299$ and $-310/-272$ oligonucleotide probes were carried out with 2 μ g of nuclear extract from control [NE/ T_3 (-)] or T_3 -treated [NE/ T_3 (+)] cells. The end-labelled probes were pre-incubated with a 100-fold molar excess of the $-338/-299$ or $-310/-272$ oligonucleotide or the SIFR element, an unrelated oligonucleotide from the promoter of the sucrase-isomaltase gene. 'Free probe' represents an incubation lacking nuclear extracts.

6, lanes 25,N). Altogether, these results indicate that the fragment containing nucleotides -308 to -290 includes the necessary sequence for protein binding to the DNA, and further suggest that the formation of the complex depends on the presence of glucose and/or T_3 .

The specificity of this binding was assessed by true competition experiments (Figure 6, lower panel) performed using the $-310/-272$ sequence as a probe and with increasing concentrations of either the unrelated SIFR or the oligonucleotide $-308/-290$ 20-mer element of the GLUT5 promoter. As expected, SIFR was inert, and the wild-type $-308/-290$ GLUT5 fragment competed fully for the probe. When compared with the effect of the wild-type element, a 9-fold higher amount of a $-308/-290$ mutated element (Figure 1) was required to obtain half-inhibition of the binding of the probe.

TR and RXR bind to the $-308/-290$ sequence of the GLUT5 promoter

In order to identify the protein partners in the complexes, we carried out supershift assays using antibodies directed against polypeptides that are known to bind TREs, i.e. TR α , TR β 1 and RXR, and with anti-p53 antibodies [15,16,35]. Antibodies to p53 did not supershift the complexes formed with the $-308/-290$ fragment or the TRE consensus DR4 probe (see Figure 1), indicating the absence of p53 from the complexes (results not shown). Two bands were formed with the DR4 probe and nuclear extracts from $G^{25,d}$ cells (Figure 7, lanes 9–12 and 21–24). The anti-TR α antibody supershifted the slowest-migrating band of DR4 complexes (Figure 7, lanes 10 and 12). T_3 treatment of the cells did not alter the profile of the bandshift obtained with DR4 (Figure 7, compares lane 9 and 10 with lanes 11 and 12).

Figure 6 Restriction of the effects of glucose and T_3 on nuclear protein interactions to the $-308/-290$ element of the GLUT5 promoter

Upper panels: electromobility-shift assays were carried out with the $-338/-299$, $-310/-272$ and $-308/-290$ labelled oligonucleotides. Nuclear protein extracts (NE) were prepared from cells cultured in the presence (+) or absence (–) of $1 \mu\text{M}$ T_3 in standard medium (25,N) or in medium containing dialysed serum in the absence (0,d) or presence (25,d) of glucose. Lower panels: true competition (probe and competitor added together to the incubation mixture) of the binding of the ^{32}P -labelled $-310/-272$ probe (10 nM) was challenged with increasing concentrations (0.2–100-fold molar excess) of the unlabelled $-310/-272$ element, SIFR, the wild-type $-308/-290$ oligonucleotide or $-308/-290$ oligonucleotide that was mutated on the TRE element ($-308/-290\text{M}$). Nuclear extracts were from $G^{25,d}$ cells.

$\text{TR}\alpha$ could also supershift the $-308/-290$ probe (Figure 7, lanes 2, 6 and 8). Two striking differences, however, were apparent in the gel shifts obtained with this probe as compared with DR4. First, a single band was obtained with control nuclear extracts from $G^{25,d}$ cells (Figure 7, lanes 1–8, 13–20 and 25–30). Secondly, a supershift with anti- $\text{TR}\alpha$ antibodies was hardly detectable when T_3 was present (compare lanes 2 and 4 in Figure 7). Glucose starvation of the cells greatly increased the amount of bound nuclear protein, but did not change the relative abundance of $\text{TR}\alpha$ in the complex (Figure 7, compare lanes 5 and 6 with lanes 7 and 8).

$\text{TR}\beta$ could also bind the $-308/-290$ probe (Figure 7, lane 27). However, neither $\text{TR}\alpha$ nor $\text{TR}\beta$, nor a combination of the two (lane 28), could totally supershift the complexes formed with nuclear protein from glucose-fed cells. The comparison of $\text{TR}\alpha$ with $\text{TR}\beta$ supershifts further suggests that $\text{TR}\alpha/\text{RXR}$ heterodimers are favoured over $\text{TR}\beta/\text{RXR}$ heterodimers for binding to the $-308/-290$ GLUT5 probe.

RXR was also a constituent of the complexes, and was present within the faster-migrating band formed with the DR4 probe in the absence (Figure 7, lanes 21 and 22) or in the presence (lanes 23 and 24) of T_3 treatment. In all instances, i.e. the absence or presence of glucose in the cell cultures and/or T_3 treatment, the anti- RXR antibody fully supershifted the $-308/-290$ probe

Figure 7 The nuclear protein complex contains $\text{TR}\alpha$ and RXR

The ^{32}P -labelled $-308/-290$ GLUT5 promoter fragment and TRE-DR4 consensus oligonucleotide were used. Nuclear extracts (cells) are shown as 25,d and 0,d for glucose-fed and glucose-starved cells respectively. T_3 was either a treatment for cells (25,d,t3; top panels) or a component of the incubation mixture of DNA and nuclear protein extracts (T_3 : + and –; middle panels). The antibodies were pre-incubated for 40 min at room temperature with either $\text{TR}\alpha$ (lanes 2, 4, 6, 8, 10 and 12) or RXR (4RX-1D12) at a 1:20 final dilution (lanes 14, 16, 18, 20, 22 and 24) before the addition of the labelled probe. In the bottom panel, in order to detect the presence of $\text{TR}\beta$, the probe was pre-incubated for 20 min at room temperature before the addition of antibodies against $\text{TR}\beta$ alone (lane 27), $\text{TR}\beta$ plus $\text{TR}\alpha$ (lane 28), or RXR (lane 30). The non-relevant monoclonal antibody 7LE (anti-Lewis A) (lane 29) was used at 1:20 dilution to assess the non-specific effects of ascites on protein binding. Note that a 7-fold (98 h) overexposure of the X-ray film was used to compare the bandshift of the $-308/-290$ probe in $G^{25,d}$ (lanes 5/6 and 13–16) and $G^{0,d}$ (lanes 7/8 and 17–20) cell extracts.

(Figure 7, lanes 13–20), indicating that all of the complexes contained RXR -associated heterodimers.

DISCUSSION

In vivo, dietary fructose controls the expression of the GLUT5 gene in intestinal and kidney absorbing cells, and glucose feeding is unable to up-regulate the expression of the gene [7,36–38]. However, *in vivo*, cells are also continuously nourished by blood glucose, and we suggest that this supply accounts for the basal expression of GLUT5. In the enterocytic Caco-2/TC7 cell model, monosaccharides appear to be essential for the basal expression of GLUT5, and we have shown previously that GLUT5 is not

expressed in glucose-starved cells [8]. Therefore glucose may play a role in the regulation of the expression of GLUT5 *in vivo*, and our results favour such a hypothesis. In streptozotocin-induced diabetes, the expression of GLUT5 is increased in intestinal cells [39,40]. However, hyperglycaemia is probably not the sole mediator of this effect, as other plasma parameters are perturbed.

We report here that T_3 increases GLUT5 mRNA abundance 4–5-fold compared with that in control cells, in a glucose-dependent manner. This T_3 /glucose effect involves a transcriptional level of regulation, and the sequence located within the proximal –338 bp upstream from the transcription start site was sufficient for the T_3 -induced response. Interestingly, the response of the GLUT5 promoter in Caco-2 cells is saturated at 5 mM glucose, a concentration that matches interprandial blood-glucose levels in healthy animals. Within this region, a 67 bp fragment (–338/–272) confers positive T_3 /glucose-responsiveness to both the GLUT5 promoter and the heterologous TK promoter. Gel-shift studies showed that the –338/–299 and –310/–272 regions, and subsequently the smaller –308/–290 region, bind nuclear proteins, and that the glucose nutritional status of the cells interferes with binding. Indeed, more complexes are formed with nuclear protein extracts from glucose-deprived than from glucose-fed cells. T_3 has no effect on the binding of nuclear proteins from glucose-deprived cells. In contrast, it greatly decreases DNA–protein interactions obtained using glucose-fed cells. Nuclear protein binding is thus inversely correlated with the transcriptional activities of GLUT5 promoter constructs. This decrease was not associated, under our experimental conditions, with the organization of new protein complexes on the –308/–290 fragment or on the larger –338/–299 and –310/–272 regions. The 67 bp region probably contains DNA-interacting elements that are involved in inhibition of the transcriptional machinery and which act in concert with, as yet unidentified, putative activators.

Among the genes involved in lipid metabolism that are regulated both by glucose and T_3 , *Spot14* has been extensively studied (for a review, see [41]). A far upstream region (FUR) containing a cluster of TREs (Fur10, Fur11 and Fur12) has been shown to be involved in the response of the gene to T_3 /glucose [42]. An analysis of the –338/–272 region of the GLUT5 gene reveals the presence of four TRE half-sites. They are organized either as an IR4 inverted repeat (AGGTCTCTAGTGGCCC) located at –330/–315, or as a DR4 direct repeat (AGGTAA TCTGAGGGCA) at positions –292/–307 (Figure 1). The latter TRE/DR4 motif closely matches the Fur10 motif present in the *S14* gene (AGGGCAGCTGAGGTTA) [42]. In cells overexpressing the TR receptor, the Fur10 motif binds both TRs and RXRs, and confers T_3 -responsiveness to the heterologous TK promoter.

Our results show that the complexes formed with the –308/–290 region of the promoter included TR α /RXR and TR β /RXR heterodimers, although the latter complexes were less abundant. These receptors are spontaneously expressed in Caco-2/TC7 cells; they bind the GLUT5 promoter and were present whatever the glucose and/or T_3 -treatment culture protocol used to produce the nuclear protein extracts. Anti-RXR antibodies completely supershifted the complexes. In contrast, anti-TR antibodies only partially affected the bandshift in glucose-fed cells, indicating that other proteins are probably associated with RXRs. Interestingly, TR α was able to supershift more complex formed with protein extracts from glucose-starved than from glucose-fed cells. As far as we know, the regulation of the GLUT5 promoter by T_3 differs from that described for other T_3 target genes identified. Classically, in the absence of T_3 , TR/RXR heterodimers bind TREs and can repress promoter activity

(reviewed in [15,16]). TR α /RXR heterodimers bind DNA in association with auxiliary co-repressors or co-activators of the so-called thyroid receptor auxiliary protein (TRAP) family. Upon T_3 loading, co-repressors are released, to the benefit of activator binding (see [17–19], and references therein). Our results with the GLUT5 promoter region do not fit this model, since T_3 loading *in vivo* or *in vitro* dissociates the protein complex from the TRE. The mechanism by which T_3 activates the GLUT5 promoter also differs in that glucose-feeding of Caco-2/TC7 cells interferes dramatically with the binding of the TR/RXR dimers to the probes. The metabolism of glucose is necessary for the activation of the GLUT5 promoter (results not shown). Conversely, there is no need for a T_3 metabolic effect in order to obtain this decrease in TR/RXR binding. Indeed, the hormone can be added either directly to the culture medium of the cells or to the nuclear extracts.

The gene encoding GLUT5 is thus a T_3 - and glucose-responsive gene. We have shown that the –308/–290 motif in the GLUT5 promoter constitutes a new glucose/ T_3 -specific responsive element. More studies are needed in order to identify the other partners of the TR/RXR heterodimer, and to determine how the metabolism of glucose influences transcription. Whether or not the same promoter element(s) are involved in the regulation of transcription of the GLUT5 gene by fructose and glucose is currently under investigation.

We are grateful to Dr. A. Muñoz and Dr. O. Chassande for their critical reading of the manuscript. We thank our antibody providers: Dr. T. Soussi (Institut Curie, Paris, France) for anti-p53 antibodies, Dr. J. Samarut and O. Chassande (E.N.S., Lyon, France) for the anti-TR α antibody, Dr. J. Chambon and Dr. C. Rochette-Egly (IGBMC, Strasbourg, France) for the anti-RXR antibody, and Dr. R. Oriol for the anti-Lewis A anti-gene antibody. Our warm thanks go to E. Dussaulx for her help with cell culture, and to A. Barbat for photographic work. This work was supported in part by Université Paris XI and by grants from the Association pour la Recherche sur le Cancer. M.M.-M. is a fellow of the M.R.E.S.; J.E.M. was supported by an INSERM Poste Vert grant.

REFERENCES

- Davidson, N. O., Hausman, A. M. L., Ifkovits, C. A., Buse, J. B., Gould, G. W., Burant, C. F. and Bell, G. I. (1992) *Am. J. Physiol.* **262**, C795–C800
- Mahraoui, L., Rousset, M., Dussaulx, E., Darmoul, D., Zweibaum, A. and Brot-Laroche, E. (1992) *Am. J. Physiol.* **263**, G312–G318
- Kayano, T., Burant, C. F., Fukumoto, H., Gould, G. W., Fan, Y. S., Eddy, R. L., Byers, M. G., Shows, T. B., Seino, S. and Bell, G. I. (1990) *J. Biol. Chem.* **265**, 13276–13282
- Mahraoui, L., Rodolosse, A., Barbat, A., Dussaulx, E., Zweibaum, A., Rousset, M. and Brot-Laroche, E. (1994) *Biochem. J.* **298**, 629–633
- Zweibaum, A., Laburthe, M., Grasset, E. and Louvard, D. (1991) *Handb. Physiol. Sect. 6 Gastrointest. Syst.* **4**, 223–255
- Delie, F. and Rubas, W. (1997) *Crit. Rev. Ther. Drug Carrier Syst.* **14**, 221–286
- Burant, C. F. and Saxena, M. (1994) *Am. J. Physiol.* **267**, G71–G79
- Mesonero, J., Matosin, M., Cambier, D., Rodriguez-Yoldi, M.-J. and Brot-Laroche, E. (1995) *Biochem. J.* **312**, 757–762
- Brent, G. A. (1994) *N. Engl. J. Med.* **331**, 847–853
- Weinstein, S. P., O'Boyle, E., Fisher, M. and Haber, R. S. (1994) *Endocrinology* **135**, 649–654
- Casla, A., Rovira, A., Wells, J. A. and Dohm, G. L. (1990) *Biochem. Biophys. Res. Commun.* **171**, 182–188
- Kuruville, A. K., Perez, C., Ismail-Beigi, F. and Loeb, J. N. (1991) *Biochim. Biophys. Acta* **1094**, 300–308
- Weinstein, S. P., Watts, J., Graves, P. N. and Haber, R. S. (1990) *Endocrinology* **126**, 1421–1429
- Ullisse, S., Jannini, E. A., Pepe, M., De Mattei, S. and D'Armiento, M. (1992) *Mol. Cell. Endocrinol.* **87**, 131–137
- Lazar, M. A. (1993) *Endocr. Rev.* **14**, 184–193
- Gandrillon, O., Rasle, A. and Samarut, J. (1995) *Int. J. Oncol.* **6**, 215–231
- Hörlein, A. J., Näär, A. M., Heinzel, T., Torchia, J., Gloss, B., Kurokawa, R., Ryan, A., Kamei, Y., Söderström, M., Glass, C. K. and Rosenfeld, M. G. (1995) *Nature (London)* **377**, 397–404

- 18 Chen, J. D. and Evans, R. M. (1995) *Nature* (London) **377**, 454–457
- 19 Zhang, J., Zamir, I. and Lazar, M. A. (1997) *Mol. Cell. Biol.* **17**, 6887–6897
- 20 Giannella, R. A., Orlowski, J., Jump, M. L. and Lingrel, J. B. (1993) *Am. J. Physiol.* **265**, G775–G782
- 21 Matosin-Matekalo, M., Mesonero, J. E., Delezay, O., Poirée, J. C., Ilundain, A. A. and Brot-Laroche, E. (1998) *Biochem. J.* **334**, 633–640
- 22 Chantret, I., Rodolosse, A., Barbat, A., Dussaulx, E., Brot-Laroche, E., Zweibaum, A. and Rousset, M. (1994) *J. Cell Sci.* **107**, 213–225
- 23 Pringault, E., Arpin, M., Garcia, A., Finidori, J. and Louvard, D. (1986) *EMBO J.* **5**, 3119–3124
- 24 Green, F., Edwards, Y., Hauri, H. P., Povey, S., Ho, M. W., Pinto, M. and Swallow, D. (1987) *Gene* **57**, 101–110
- 25 Trotot, P., Mechali, F., Blangy, D. and Lacasa, M. (1994) *Virology* **202**, 724–734
- 26 Chirgwin, J. M., Przybyla, A. E., McDonald, R. J. and Rutter, W. J. (1979) *Biochemistry* **18**, 5294–5299
- 27 Mahraoui, L., Takeda, J., Mesonero, J., Chantret, I., Dussaulx, E., Bell, G. I. and Brot-Laroche, E. (1994) *Biochem. J.* **301**, 169–175
- 28 Sary, A., James, M. R. and Sarasin, A. (1989) *J. Virol.* **63**, 3837–3843
- 29 Sambrook, J., Fritsch, E. F. and Maniatis, T. (1989) *Molecular Cloning: A Laboratory Manual*, 2nd edn., Cold Spring Harbor Laboratory Press, Cold Spring Harbor
- 30 Rodolosse, A., Barbat, A., Chantret, I., Lacasa, M., Brot-Laroche, E., Zweibaum, A. and Rousset, M. (1998) *Am. J. Physiol.* **274**, G931–G938
- 31 Rodolosse, A., Chantret, I., Lacasa, M., Chevalier, G., Zweibaum, A., Swallow, D. and Rousset, E. (1996) *Biochem. J.* **315**, 301–306
- 32 Shapiro, D. J., Sharp, P. A., Wahli, W. W. and Keller, M. J. (1988) *DNA* **7**, 47–55
- 33 Wu, G. D., Chen, L., Forslund, K. and Traber, P. G. (1994) *J. Biol. Chem.* **269**, 17080–17085
- 34 Legros, Y., Lafon, C. and Soussi, T. (1994) *Oncogene* **9**, 2071–2076
- 35 Baht, M. K., Yu, C. L., Yap, N., Zhan, Q., Hayashi, Y., Seth, P. and Cheng, S. Y. (1997) *J. Biol. Chem.* **272**, 28989–28993
- 36 Ferraris, R. P. and Diamond, J. (1989) *Annu. Rev. Physiol.* **51**, 125–141
- 37 Ferraris, R. P., Villenas, S. A., Hirayama, B. A. and Diamond, J. (1992) *Am. J. Physiol.* **262**, G1060–G1068
- 38 Ferraris, R. P. and Diamond, J. (1992) *Am. J. Physiol.* **262**, G1069–G1073
- 39 Corpe, C. P., Basaleh, M. M., Affleck, J., Gould, G., Jess, T. J. and Kellett, G. L. (1996) *Pflügers Arch.* **432**, 192–201
- 40 Castello, A., Guma, A., Sevilla, L., Furriols, M., Testar, X., Palacin, M. and Zorzano, A. (1995) *Biochem. J.* **309**, 271–277
- 41 Towle, H. C. and Kaytor, E. N. (1997) *Annu. Rev. Nutr.* **17**, 405–433
- 42 Liu, H. C. and Towle, H. C. (1994) *Mol. Endocrinol.* **8**, 1021–1037

Received 16 November 1998/6 January 1999; accepted 5 February 1999