Presence and differential expression of SGLT1, GLUT1, GLUT2, GLUT3 and GLUT5 hexose-transporter mRNAs in Caco-2 cell clones in relation to cell growth and glucose consumption

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Seven clones from the Caco-2 cell line, three isolated from passage 29 (PD7, PD10, PF11) and four from passage 198 (TB10, TC7, TF3, TG6), all of them selected on the basis of differences in the levels of expression of sucrase–isomaltase and rates of glucose consumption, were analysed for the expression of hexose-transporter mRNAs (SGLT1, GLUT1–GLUT5) in relation to the phases of cell growth and the associated variations of the rates of glucose consumption. All clones showed a similar pattern of evolution of the rates of glucose consumption, which decreased from the exponential to the late-stationary phase, but differed, in a 1–40-fold range, in the values observed at late post-confluency. According to these values, clones could be divided into high- (PD10, PF11) and low-glucose-consuming cells (PD7, TB10, TC7, TF3 and TG6). GLUT1 and GLUT3 mRNAs were expressed in all clones and showed a similar pattern of evolution: their level decreased, from the exponential to the stationary phase, in close correlation with the decrease in rates of glucose consumption, with only high-glucose-consuming clones maintaining high levels in the stationary phase. In contrast, SGLT1, GLUT2 and GLUT5 mRNAs were only expressed, like sucrase–isomaltase mRNA, in the low-glucose-consuming clones, and their level increased from the exponential to the stationary phase, in parallel with the differentiation of the cells. GLUT4 was undetectable in all the clones. Glucose deprivation generally resulted in a discrete decrease in the levels of all transporter mRNAs in all clones, one exception being GLUT2, which in the high-glucose-consuming clones is only detectable when the cells are grown in low glucose. These clones should be ideal tools with which to study *in vitro*, at the single-cell level, how these transporters concur to the utilization and transport of hexoses and how their exclusive or co-ordinated expression is regulated.

INTRODUCTION

The entry of hexoses through the intestinal epithelium depends on the presence of brush-border membrane-associated hydrolases, such as sucrase–isomaltase, lactase and maltase–glucoamylase, which cleave the disaccharides into monosaccharides [1], and of hexose transporters, which allow the cellular uptake of monosaccharides or their exit towards the internal medium. Two categories of transporters are involved in intestinal hexose transport: the brush-border membrane-associated Na^+-dependent glucose cotransporter [2–5], which is under the control of the SGLT1 gene [6], and members of the family of facilitative hexose transporters referred to as GLUT1–GLUT5 [7,8]. Of these five transporters, only GLUT2, associated with the basolateral membrane [9], and GLUT5, associated with the brush-border membrane of enterocytes [10,11], are expressed in the small intestine, whereas GLUT1 and GLUT3 are mainly expressed in malignant epithelial cells, including colonic cells [12].

The human colon carcinoma cell line Caco-2 expresses in culture, at late confluency, the same morphological characteristics and most of the functional properties of terminally differentiated small-intestinal enterocytes (for review see [13]), including the expression of proteins involved in the terminal digestion and uptake of sugars. They express sucrase–isomaltase [14–24], the Na^+/glucose co-transporter [25], the facilitative glucose transporters GLUT1 [11,26] and GLUT3 [26], and the fructose transporter [27] GLUT5 [11,26]. However, they are also cancer cells which, as such, display an altered and generally increased utilization of glucose [28–36]. It is therefore important to distinguish which proteins are specific for the enterocyte-like differentiation of the cells and which depend on their malignant state and/or state of proliferation. It is also of interest to analyse whether some of them are involved in the malignancy-dependent alteration of glucose utilization and whether, as is the case for sucrase–isomaltase in Caco-2 cells [16–18,22,37] or for GLUT1 in other cellular systems [38–40], glucose is involved in the regulation of the expression of hexose transporters in Caco-2 cells.

Because of its heterogeneity, the parental Caco-2 population does not allow simple answers to these questions. This is made even more complicated by the observation that glucose utilization, as well as the expression of proteins involved in the absorption of sugars, have been shown to vary with the number of passages of the cell line: indeed, the expression of sucrase–isomaltase [41] and of GLUT5 [11] increases from early to late passages, concurrently with a marked decrease in the rate of glucose consumption [41].

Based on these observations, we have recently isolated clones from early (P 29) and late (P 198) passages of the Caco-2 cell line [41]. These clones, when analysed at late post-confluency, are very similar as to cell polarity, presence of an apical brush border and levels of expression of villin, dipeptidyl-peptidase IV, aminopeptidase N or alkaline phosphatase [41]. However, they differ dramatically, in a 40–50-fold range, in their rates of glucose consumption and levels of expression of sucrase–isomaltase, which was shown to be negatively regulated by glucose [41].

Seven of these clones were analysed for the expression of

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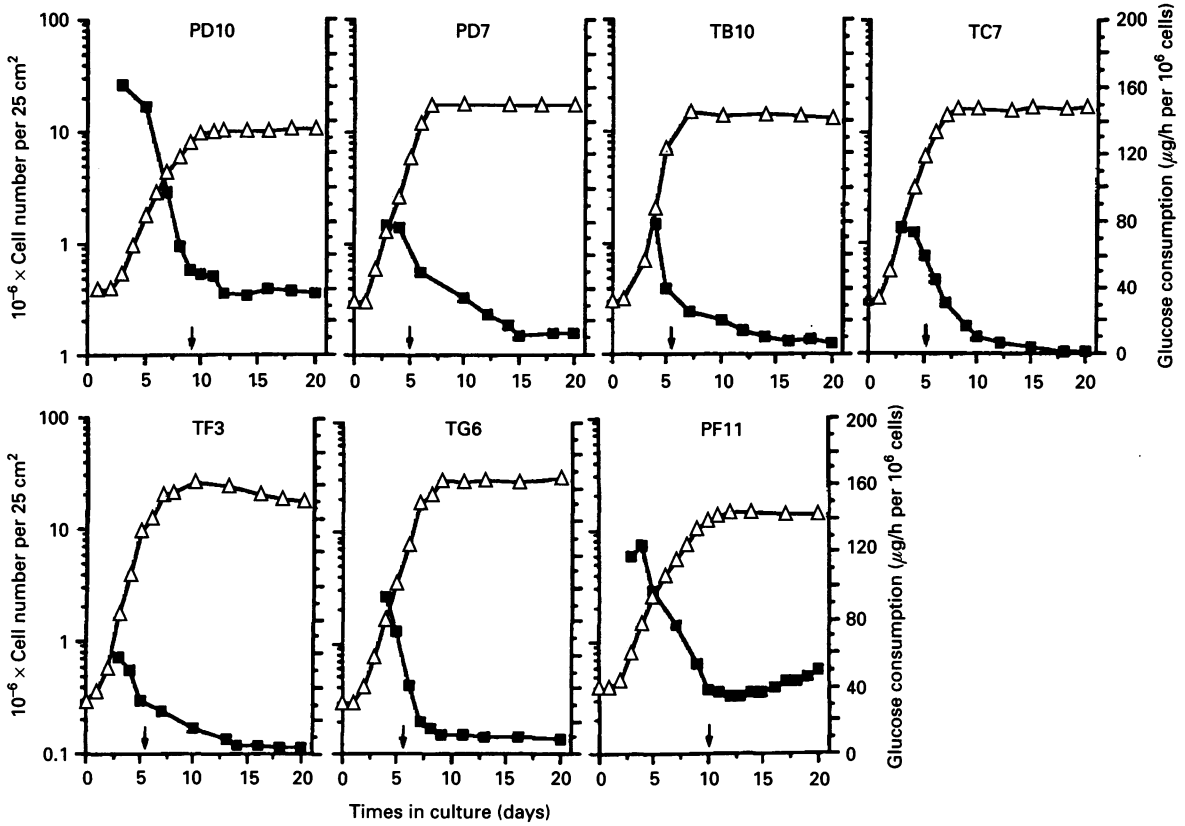

Figure 1 Growth characteristics and glucose-consumption rates of Caco-2 cell clones

Growth curves (△) and growth-related glucose-consumption rates (■) of Caco-2 cell clones. Results are the means of five to six passages analysed between passages 5 and 20. S.D. values (not shown) were less than 10%. Arrows indicate days of confluency. Note that two clones, PD10 and PF11, maintain at late confluency high glucose-consumption rates (high-glucose-consuming clones) as compared with the other five clones (low-glucose-consuming clones).

SGLT1 [6] and GLUT1–GLUT5 [7,8] mRNA levels. This study was done in relation to the growth-related process of differentiation of the cells [41,42], the differences and variations of glucose consumption and modifications of glucose supply.

MATERIALS AND METHODS

Cell culture

The parental Caco-2 cell line [43] was obtained from the late Dr. Jorgen Fogh (Memorial Sloan Kettering Cancer Center, Rye, NY, U.S.A.). The isolation, characterization and stability of the clones obtained from early (labelled P/letter/number) and late passages of the cell line (labelled T/letter/number) have been reported [41]. Of the seven clones used in this study, three originated from passage 29 (PD7, PD10 and PF11), and four from passage 198 (TB10, TC7, TF3 and TG6). Cells were seeded at 12×10^3 cells/cm^2 in 25 or 75 cm^2 plastic flasks (Corning Glassworks, Corning, NY, U.S.A.) and cultured in a CO_2/air (1:9) atmosphere in Dulbecco's modified Eagle's minimum essential medium (DMEM) (Eurobio, Paris, France), containing 25 mM glucose, supplemented with 20% inactivated (30 min, 56 °C) fetal-bovine serum (Boehringer, Mannheim, Germany) and 1% non-essential amino acids (Gibco, Glasgow, Scotland, U.K.). The same culture conditions were used for growth in low glucose, except that DMEM was devoid of glucose, the final concentration of glucose in the medium being 1 mM, due to the presence of serum. For growth in strictly hexose-free medium, DMEM devoid of glucose was supplemented with 2.5 mM inosine [44] and dialysed serum. In all culture conditions the medium (0.2 ml/cm^2) was changed 48 h after seeding, and daily thereafter. For maintenance purposes the clones were passaged every 6 days.

Growth curves and glucose-consumption assays

For growth curves the cells were detached with 0.25% trypsin in 3 mM EDTA in Ca^{2+}/Mg^{2+}-free PBS and counted with an haemocytometer. Glucose consumption was determined by measuring the concentration of glucose in the medium 16 h after changes of medium, by using the glucose oxidase technique and a Beckman Glucose Analyzer 2.

RNA analysis

RNA analysis was performed on cells harvested at the indicated days of the culture 24 h after the medium change. Total RNA was isolated by extraction with guanidium isothiocyanate and centrifugation through a CsCl gradient [45]. Portions of total RNA samples were denatured in 1 mM glyoxal [46] and immobilized on nylon filters (Hybond N, Amersham) by using a dot-blot manifold (Gibco BRL), or fractionated by electrophoresis on 1%-agarose gels and transferred to Hybond N. Dot-blots and Northern blots were prehybridized at 42 °C in the presence of 50% formamide and hybridized for 24 h at 42 °C in

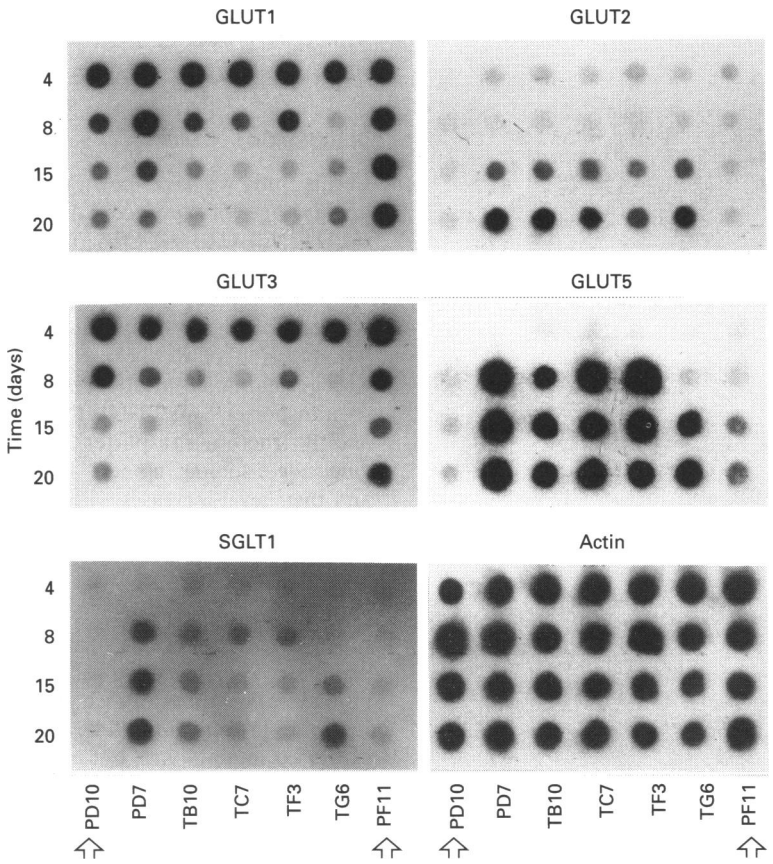

Figure 2 Growth-related expression of hexose-transporter mRNA in Caco-2 clones

Dot-blot analysis of the growth-related variations in levels of hexose-transporter mRNAs in Caco-2 cell clones (passages 6–7) and of actin used as control. The same amount of total RNA (20 μg) was laid on each spot. This is a representative experiment made at passage 6 or 7. Similar results were obtained with cells taken at passage 20. Arrows indicate high-glucose-consuming clones (see Figure 1).

the presence of 40% formamide and 10% dextran sulphate. Blots were washed for 4 × 5 min in 2 × SSC (SSC = 0.15 M NaCl/0.015 M sodium citrate)/0.1% SDS at room temperature, then once in 0.1 × SSC/0.1% SDS at 50 °C, and once in 0.1 × SSC/0.1% SDS at 65 °C for 15 min.

cDNA probes

The cDNA probes SGLT1 (2.0 kb insert), pGEM4Z-HepG2GT GLUT1 (1.75 kb insert), pBS-HTL210/hGLUT2 (2.4 kb insert), phMGT-31GLUT3 (2.2 kb insert), phJHT-3GLUT4 (1.7 kb insert) and phJHT5/hGLUT5 (1.9 kb insert) were obtained from G. I. Bell (Howard Hughes Medical Institute, University of Chicago). Actin was detected with cDNA probe pA2 [47], sucrase–isomaltase with cDNA probe SI2 [48] and dipeptidyl-peptidase IV with DPI-101 [49]. Probes were ^{32}P-labelled by using a Megaprime DNA-labelling kit (Amersham).

RESULTS

Growth curves and growth-related variations of glucose-consumption rates

The differentiation features of the seven clones selected have been reported [41]. The pattern of cell growth and the variations of glucose consumption rates during the period of culture analysed (20 days) for the seven clones are reported in Figure 1. A similar pattern of evolution of glucose-consumption rates, in relation to the phases of cell growth, was observed in all clones and was characterized by an overall decrease from the exponential to the late-stationary phase of growth (Figure 1). The rates of glucose consumption measured on day 20, i.e. when they are stabilized at their lower level (Figure 1), allow us to classify the clones into two groups: a group of high-glucose-consuming clones, including PD10 and PF11, which on day 20 show glucose-consumption rates of 39 ± 2 and 50 ± 3 μg/h per 10^6 cells; and a group of low-glucose-consuming clones, with PD7, TB10, TC7, TF3 and TG6, which consume 12.3 ± 1.5, 7.0 ± 0.5, 1.3 ± 0.5, 3.7 ± 1.0 and 9.0 ± 1.0 μg/h per 10^6 cells respectively.

Presence and growth-related variations of expression of hexose-transporter mRNAs

As shown in Figure 2, the presence of hexose-transporter mRNAs differs from one clone to another, and their level of expression varies, in relation to the rates of glucose consumption and to the phases of cell growth, from one transporter to another. GLUT1 and GLUT3 are present at a high level in all clones in the exponential phase of growth; it decreases from the exponential (day 4) to the late-stationary phase of growth (day 20) in the low-glucose-consuming clones, whereas it remains high in the high-glucose-consuming clones. In contrast, SGLT1, GLUT2 and GLUT5 mRNAs are absent from exponentially growing cells, being only detectable after confluency (Figure 2). Unlike GLUT1 and GLUT3, their presence is associated with the low-glucose-

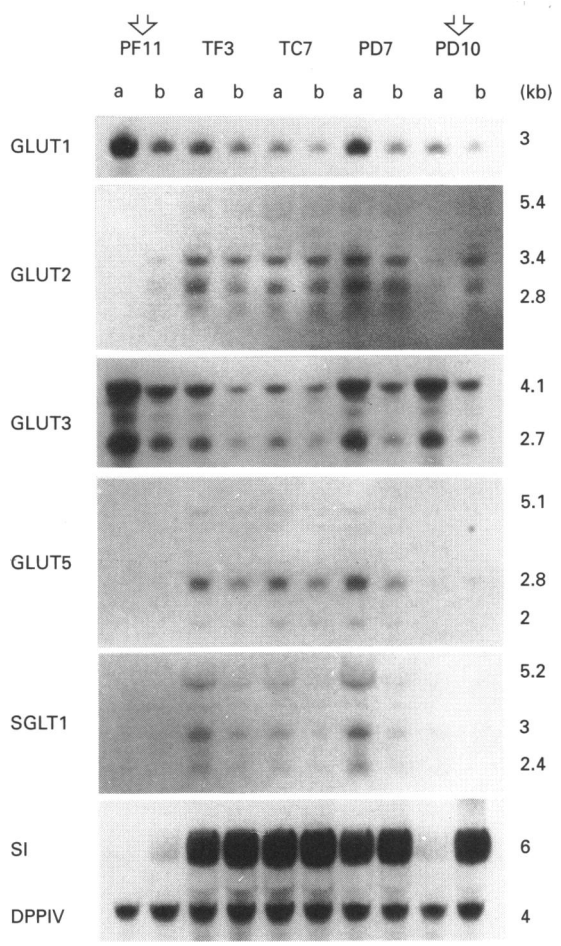

Figure 3 Effect of glucose deprivation on the expression of hexose-transporter mRNA in Caco-2 clones

Northern-blot analysis of hexose-transporter mRNAs in post-confluent cultures (day 20) of Caco-2 cell clones grown in the presence of 25 mM (a) or 1 mM (b) glucose. The same amount (30 μg) of total RNA was laid on each lane. Sucrase–isomaltase (SI), which increases dramatically in PD10 and PF11 cells grown in low glucose, and dipeptidylpeptidase IV (DPPIV), which is not modified [41], were used as internal controls. This is a representative experiment made at passage 15 of the Caco-2 clones. Arrows indicate high-glucose-consuming clones (see Figure 1).

consuming clones, whereas they are absent or poorly expressed in the high-glucose-consuming clones. GLUT4 was not detectable in any of the clones.

Effect of glucose deprivation on the expression of hexose-transporter mRNAs

To investigate a possible role for glucose in the regulation of the expression of hexose transporters, five clones, two of them from the high-glucose-consuming group (PD10, PF11), and three from the low-glucose-consuming group (PD7, TC7, TF3), were analysed for the effect of a decreased glucose supply (1 mM; see the Materials and methods section). In this experiment, cells were grown from day 1 in low-glucose medium and compared, after 20 days in culture, with cells grown in 25 mM glucose. The number of cells at day 20 was the same in both culture conditions (results not shown). As shown in Figure 3, this decrease in glucose supply generally results in a decrease in the levels of SGLT1, GLUT1, GLUT2, GLUT3 and GLUT5 and the appearance of GLUT2 in high-glucose-consuming clones. Similar results were obtained with cells grown in strictly hexose-free medium (results not shown).

DISCUSSION

In this study we have shown that clones isolated from early and late passages of the Caco-2 cell line express differently GLUT1, GLUT2, GLUT3, GLUT5 and SGLT1 mRNAs. Their presence, as well as their level of expression, vary from one clone to another and from one transporter to another, in relation to the phases of cell growth and the rates of glucose consumption.

Expression of the hexose transporters which are associated with normal small-intestinal enterocytes, namely the Na^+/glucose co-transporter SGLT1 [4], the glucose transporter GLUT2 [9] and the fructose transporter GLUT5 [10,11], is restricted to low-glucose-consuming clones. They are concomitantly expressed and their level increases with time in culture. This time-related increase in GLUT2, GLUT5 and SGLT1 correlates with the onset and maintenance of the morphological and functional differentiation of Caco-2 cells [42] and is similar to that generally observed for other proteins which are either associated with the brush-border membrane such as sucrase–isomaltase, dipeptidyl-peptidase IV [41] and alkaline phosphatase [50], or with the basolateral membrane, such as the vasoactive-intestinal-peptide receptor [51,52]. GLUT1 and GLUT3 are concomitantly expressed in all clones, but exhibit, as compared with GLUT2, GLUT5, SGLT1 and sucrase–isomaltase [41], inverse kinetics of expression in relation to cell growth: they are highly expressed in exponentially growing cells. They decrease after confluency in low-glucose-consuming clones, whereas they remain high in high-glucose-consuming clones. It is noteworthy that there is an apparently exclusive relationship between the expression of GLUT1 and GLUT3 on the one hand and of SGLT1, GLUT2 and GLUT5 on the other hand.

On the basis of the expression of GLUT1 and GLUT3 in human tumours of the digestive tract [12], in the parental Caco-2 cells [26] and in BeWo and HepG2 cells (cited in [26]), it has been suggested that GLUT1 and GLUT3 are over-expressed in malignant epithelial cells. The present results further suggest that both transporters could be specifically involved in the alteration of glucose utilization associated with the malignant phenotype. Indeed, there is a close correlation between the level of expression of GLUT1 and GLUT3 and the rates of glucose consumption. The observed growth-related decrease in GLUT3 mRNA is in contradiction with the observation by Harris et al. [26], who showed, in parental Caco-2 cells, that the level of the protein GLUT3 increased after confluency. This apparent discrepancy may be due to the fact that anti-GLUT3 antibodies also recognize actin [53], which is the main cytoskeleton protein of brush-border microvilli.

Although there is an apparent correlation between the rates of glucose consumption and the level of expression of transporter mRNAs, this correlation being positive for GLUT1 and GLUT3 and negative for SGLT1, GLUT2 and GLUT5, modifications of the glucose supply differently affect the expression of hexose transporters. Indeed, glucose deprivation generally results in a decrease in the level of expression of most transporters, the only exception being GLUT2, which is not detectable in high-glucose-consuming clones grown in 25 mM glucose, but is present when they are grown in low glucose. It should be noted that this increase in GLUT2 parallels that in sucrase–isomaltase [41], suggesting that GLUT2, like sucrase–isomaltase, may be negatively regulated by glucose. The decrease in GLUT1 mRNA in cells grown in low glucose contrasts with the increase in GLUT1

mRNAs in myocytes and NIH 3T3 fibroblasts [40] or primary cultures of rat glial cells [38] or skeletal-muscle cells [39] grown under the same conditions of low glucose as here, this suggesting that, depending on their tissue origin and normal or malignant phenotype, cells may behave differently as to the glucose-dependent regulation of GLUT1.

Altogether, these clones should be valuable models to analyse *in vitro*, at the single-cell level, how hexose transporters concur to the transport and utilization of hexoses and how their expression is regulated.

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