Differential expression of sucrase-isomaltase in clones isolated from early and late passages of the cell line Caco-2: evidence for glucose-dependent negative regulation

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SUMMARY

The expression of the brush border-associated hydrolase sucrase-isomaltase was shown to increase from early to late passages of Caco-2 cells, concomitant with a decrease in the rates of glucose consumption. Twenty-six clones were isolated from early (P29) and late (P198) passages of the cell line. These clones show considerable and inverse differences in the levels of sucrase activities and rates of glucose consumption, without marked changes in other features of enterocytic differentiation of the cells (presence of an apical brush border, levels of expression of other brush border-associated hydrolases). Clones with low sucrase-isomaltase expression show a mosaic expression of the enzyme and a 38-fold higher rate of glucose consumption than clones with high sucrase-isomaltase expression. The clones with high expression show an homogeneous apical distribution of the enzyme and 70-fold and 35-fold higher levels of sucrase activities and sucrase-isomaltase mRNA, respectively. In contrast no differences were found from one clone to another in the enrichment of sucrase activity in brush border-enriched fractions as compared to cell homogenates. Switch to low glucose-containing medium (1 mM versus 25 mM in standard culture conditions) of cells with low sucrase-isomaltase results in an increased and more homogeneous expression of the enzyme and a tenfold augmentation of the levels of sucrase-isomaltase mRNA and sucrase activity. These results show that glucose interferes with the expression of sucrase-isomaltase in Caco-2 cells at the mRNA level.

Key words: sucrase-isomaltase, glucose consumption, Caco-2

INTRODUCTION

The major site of expression of the enzyme sucrase-isomaltase (SI) is the brush border of the small intestine (Hauri, 1988; Semenza, 1989). However SI is also expressed at significant levels in the colon under certain circumstances: in the fetus between the 12th and 25th week of gestation (Lacroix et al., 1984; Sebastio et al., 1987; Zweibaum et al., 1984), in some colon cancers (Beaulieu et al., 1990; Czernichow et al., 1989; Wiltz et al., 1991; Zweibaum et al., 1984) and in the colon cancer cell lines HT-29 (Pinto et al., 1982) and Caco-2 (Pinto et al., 1983). Both cell lines, in particular Caco-2, have been used extensively for analyzing the expression and biosynthesis of SI and other brush border hydrolases (Cross and Quaroni, 1991; Hauri et al., 1985; Le Bivic et al., 1990; Matter et al., 1990a; Matter et al., 1990b; Stieger et al., 1988; Trugnan et al., 1987; Vachon and Beaulieu, 1992). The human sucrase-isomaltase gene encodes a mRNA of 6 kb (Green et al., 1987). The complete cDNA has been recently sequenced (Chantret et al., 1992) as well as the 5′ region of the gene (Chantret et al., 1992; Wu et al., 1992). However, with the exception of the recent identification of regulatory elements that are potentially

responsible for tissue specificity (Traber et al., 1992), little is known of the mechanisms involved in the transcriptional regulation of the expression of SI.

There is circumstantial evidence that the expression of SI can be negatively regulated by glucose in HT-29 and Caco-2 cells. The malignant status of these cells results in significant alterations of glucose utilization characterized by high rates of glucose consumption and lactic acid production (for review see Weinhouse, 1982) and an abnormal glycogen storage (Rousset et al., 1979, 1984, 1985). Taking advantage of these cancer-associated metabolic properties, it has been possible to modulate the expression of sucrase-isomaltase in HT-29 and Caco-2 cells by modifying experimentally their utilization of glucose. HT-29 cells cultured under standard conditions, i.e. in the presence of glucose, do not express SI, the enzyme being expressed, at both mRNA (Chantret et al., 1992) and protein level (Huet et al., 1987; Pinto et al., 1982; Wice et al., 1985; Zweibaum et al., 1985) only when the cells are adapted to grow in a glucose-free medium. Caco-2 cells, which have a lower rate of glucose consumption and a much higher glycogen content than HT-29 cells (Rousset et al., 1979; Rousset et al., 1985) express SI even in the presence of glucose (Pinto et al.,

1983). In these cells however, treatment with forskolin (Rousset et al., 1985, 1989) and monensin (Rousset et al., 1986) results in a concomitant decrease in expression of SI and increase in glucose utilization. This repression effect is specific for SI, as it does not affect the expression of other hydrolases like aminopeptidase N, dipeptidylpeptidase-IV or alkaline phosphatase (Chantret et al., 1992; Chantret et al., 1988; Darmoul et al., 1991; Rousset et al., 1985, 1989), and appears to be the consequence of a decreased rate of transcription of the SI gene (Chantret et al., 1993). However analysis of the genetic elements involved in glucose regulation requires a better cellular model than those already available. Although HT-29 cells would seem appropriate, they express SI only at a very low level in glucose-free medium. Caco-2 cells would seem preferable since they can express SI at levels similar to those observed in the small intestine (Rousset et al., 1985). However, modulation of SI expression by drugs like forskolin or monensin makes further analysis complicated because these drugs have pleiotropic effects (Seamon and Daly, 1986; Tartakoff, 1983).

Thus clones of Caco-2 cells that differ only in the levels of expression of SI and rates of glucose consumption would be desirable. Our experience with Caco-2 cells, which have been cultured in our laboratory since 1978 (Rousset et al., 1979), has been that the level of SI, as estimated by enzyme activity assays, increases 7-fold from early (passage 29) to late passages (passage 198) (Zweibaum, 1986), this increase being concomitant with a decreased rate of glucose consumption (unpublished data). Although several groups have isolated Caco-2 cell clones (Beaulieu and Quaroni, 1991; Peterson and Mooseker, 1992; Woodcock et al., 1991), those which were analyzed for sucrase activity were derived from passage 17 and were shown to express low enzyme activities, consistent with those observed in our early passages (Beaulieu and Quaroni, 1991; Cross and Quaroni, 1991; Vachon and Beaulieu, 1992).

Our purpose was therefore to isolate clones from two very different passages of Caco-2 cells, i.e. an early (P29) and a late (P198) passage, with the hope of obtaining cell populations that would show a wide range of differences in the rates of glucose consumption and the levels of expression of SI. We show here that the levels of SI, but not of other differentiation-associated properties, observed in the 26 clones that were isolated, vary markedly from one clone to another and that these variations are inversely correlated with the rates of glucose consumption. We also show that glucose deprivation of clones with low SI and high glucose consumption results in a marked increase of SI expression.

MATERIALS AND METHODS

Cell culture

Passage 14 of the cell line Caco-2 (Fogh et al., 1977) was obtained in 1978 from Dr J. Fogh (Sloan Kettering Institute for Cancer Research, Rye, NY). Since then these cells have been maintained in the laboratory under strict conditions of culture: low density of seeding (12×10^3 cells/cm^2), 6 day passage frequency, use of Dulbecco's modified Eagle's minimum essential medium (DMEM) containing 25 mM glucose (Eurobio, Paris, France) supplemented with 20% heat-inactivated (56°C, 30 minutes) fetal bovine serum and 1% non essential amino acids (Gibco, Glasgow, Scotland), and cultured in a 10% CO_2/90% air atmosphere. The medium was changed 48 hours

after seeding, and daily thereafter, using 5 ml or 15 ml for 25 cm^2 and 75 cm^2 flasks, respectively. Frozen stocks were made every 10-20 passages from passage 26 up to passage 200. During this long period of time several batches of fetal bovine serum were used. They were tested and selected on the basis of the reproducibility of different parameters including cell growth, rates of glucose consumption, glycogen content and activity of brush border hydrolases. For experimental studies, cells were seeded in either 25 cm^2 or 75 cm^2 plastic flasks (Corning Glassworks, Corning, NY) and grown under the same conditions as described above, except that penicillin and streptomycin (100 U/ml, 0.1 µg/ml) were added to the culture medium. Fetal bovine serum was from Boehringer (Mannheim, Germany). Cells from different passages, when analyzed, were cultured concurrently. The cultures were regularly controlled for the absence of mycoplasma contamination. For growth in low glucose, DMEM devoid of glucose was used and supplemented with 20% heat-inactivated fetal bovine serum. The final concentration of glucose in the medium, due to the serum, was 1 mM. This medium was supplemented or not with uridine or inosine (2.5 mM) as reported (Wice et al., 1981, 1985).

Cloning

Caco-2 cells from passages 29 and 198 were cloned by the limited dilution technique. Exponentially growing cells were trypsinized and seeded in 96-well plates (Corning) at a dilution such that there would be less than one cell per 3 wells. Cells were cloned in a mixture (v/v) of standard culture medium and conditioned medium from cultures of passage 80 of the line. The resultant colonies were expanded and cells were set up after 5-6 passages for freezing and screening (see Results). Clones from early and late passages were named P (for *precoce*, i.e. early) and T (for *tardif*, i.e. late), respectively. The clones selected for further analysis and phenotype stability were grown under the same conditions as reported above for parental cells.

Antibodies

Monoclonal antibodies (mAbs) HBB 2/614/88 raised against human intestinal SI, and HBB 3/775/42 against dipeptidylpeptidase-IV (Hauri et al., 1985) were obtained from Dr H. P. Hauri (Biozentrum, Basel, Switzerland); mAb Caco 3/73 against Caco-2 SI (Quaroni, 1986) was obtained from Dr Andrea Quaroni (Cornell University, Ithaca, NY); mAbs mglu1, mglu2 and mglu3 against human intestinal SI (Green et al., 1988) were obtained from Dr D. Swallow (MRC Biochemical Genetics Unit, London). Rabbit polyclonal antibodies L458 and L459 were raised against purified Caco-2 SI (Trugnan et al., 1987). Rabbit polyclonal antibodies against porcine villin (Robine et al., 1985) were obtained from Dr D. Louvard (Institut Pasteur, Paris).

Immunofluorescence

Indirect immunofluorescence was performed concurrently on the unpermeabilized cell layer and on frozen cryostat sections from the same cultures according to the same protocol as described earlier (Rousset et al., 1989). Cell layers and cryostat sections were fixed for 15 minutes at room temperature in 3.5% paraformaldehyde in Ca^{2+}/Mg^{2+}-free PBS. Anti-rabbit and anti-mouse fluorescein-coupled sheep antiglobulins were from Institut Pasteur Production (Marnes-la-Coquette, France).

Electron microscopy

Transmission electron microscopy was performed, according to established techniques, on late postconfluent cultures (day 21) of cells grown in 25-cm^2 plastic flasks. Samples embedded in Epon were re-embedded to make sections perpendicular to the bottom of the flask. Scanning electron microscopy was performed on cultures grown on plastic coverslips.

Glycogen and glucose consumption assays

For glycogen assays the cells were harvested, 16 hours after the

medium changes, with 0.25% trypsin in 3 mM EDTA in Ca^{2+}/Mg^{2+}-free PBS and centrifuged (950 *g*, 5 minutes, 4°C) for subsequent extraction and measurement with anthrone as previously reported (Rousset et al., 1979; Rousset et al., 1984). Glucose consumption was determined by measuring the concentration of glucose in the medium 16 hours after the medium changes, using the glucose oxidase technique and a Beckman Glucose Analyzer 2. Proteins were measured by the method of Lowry (1951).

Enzyme activity assays

Enzyme activities were measured on the cell homogenates or on brush border-enriched fractions (Schmitz et al., 1973) as previously reported (Rousset et al., 1989). Sucrase (EC 3.3.1.48) activity was measured according to Messer and Dahlqvist (1966), dipeptidylpeptidase-IV (DPP-IV) (EC 3.4.14.5) according to Nagatsu et al. (1976), using 1.5 mM glycyl-L-proline-4-nitroanilide as substrate, aminopeptidase N (APN) (EC 3.4.11.2) according to Maroux et al. (1973), using L-alanine-*p*-nitroanilide as substrate, alkaline phosphatase (EC 3.1.3.1) according to Garen and Levinthal, 1960), with *p*-nitrophenylphosphate as substrate, and lactase (EC 3.2.1.23) according to Messer and Dahlqvist (1966), with lactase as substrate, in the presence of 0.2 mM *p*-chloromercuribenzoate as inhibitor of endogeneous acid β-galactosidase (Asp and Dahlqvist, 1972). Proteins were assayed with the BCA protein assay reagent (Pierce, Rockford, IL). Results are expressed as milliunits/mg of protein. One unit is defined as the activity that hydrolyzes 1 μmole of substrate per minute at 37°C.

RNA analysis

Total RNA was isolated by extraction with guanidium isothiocyanate and centrifugation through a CsCl gradient (Chirgwin et al., 1979). Samples of total RNA, denatured in 1 mM glyoxal (Thomas, 1980), were immobilized on nylon (Hybond N, Amersham), 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 to the radiolabelled probes for 24 hours at 42°C in the presence of 40% formamide and 10% dextran sulfate. Blots were washed twice in 2× SSC, 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 70°C for 15 minutes. Quantitation was achieved with a densitometric scanner (model Mark III CS; Joyce, Loebl and Co., Ltd., Gatehead, UK).

cDNA probes

Sucrase-isomaltase mRNA was detected with SI2 (Green et al., 1987), obtained from Dr D. Swallow (MRC, London), DPP-IV with DPI-101 (Darmoul et al., 1990), and villin with V19 (Pringault et al., 1986), obtained from Dr D. Louvard (Institut Pasteur, Paris). Actin was detected with cDNA probe pA2 (Cleveland et al., 1986). Probes were ^{32}P-labelled using megaprime DNA labeling kit (Amersham).

RESULTS

Passage-related general characteristics of parental Caco-2 cells

The general characteristics of parental cells at the passages used for cloning (P29 and P198) are shown in Fig. 1. The main differences observed in cells from passage 198 as compared with cells from passage 29, are an increased growth rate (doubling time being 24 hours versus 34 hours) and a higher cell density at late postconfluency (25×10^6 cells versus 12×10^6 cells per 25 cm[2]) (Fig. 1A), a 7-10 fold increase in the level of sucrase activity (Fig. 1 B,C) associated with a similar increase in the level of SI mRNA (not shown), and a 10-fold decrease
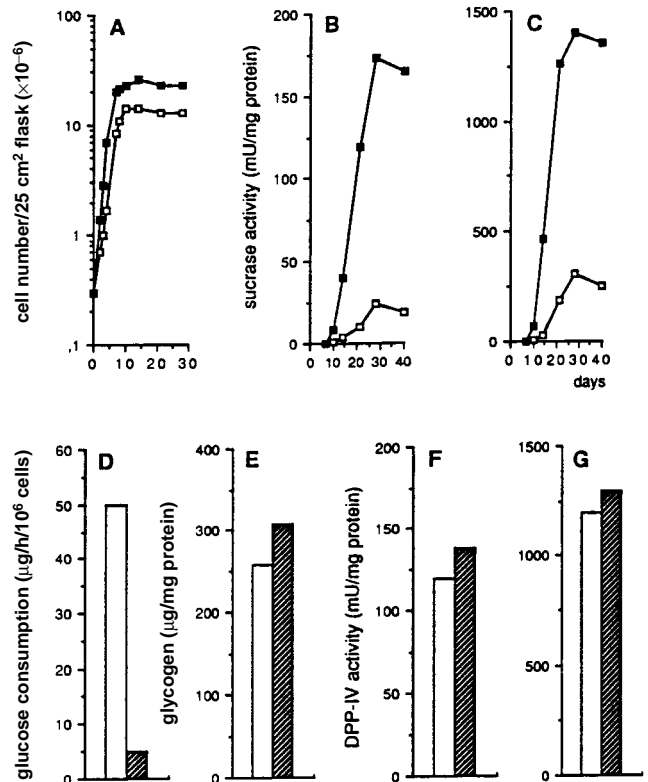
Fig. 1. General characteristics of early (P29) versus late passage (P198) of Caco-2 cells. Upper panel: (A) growth curves; (B) and (C) growth-related variations of sucrase activity in the cell homogenate (B) and in a brush border-enriched fraction (C); open symbols correspond to P29, closed symbols to P198. Lower panel: values in post-confluent cultures (day 20) of (D) glucose consumption rates; (E) glycogen content; (F) and (G) DPP-IV activities in the cell homogenate (F) and in a brush border-enriched fraction (G); open and hatched columns correspond to P28 and P198, respectively. Results shown are from a single culture of each passage. Similar results were obtained with 5 different cultures of the same passages cultured at different periods of time after thawing of our frozen stock.

in the rate of glucose consumption (Fig. 1D). These differences are associated with morphological changes characterized by a diminution in the number and size of domes from early to late passage (not shown), without modification of the electrical properties of the cells (E. Grasset, unpublished results) and changes in the organization of the apical brush border. Cells from passage 29 are highly heterogeneous with a number of cells showing microvilli, which are organized in clumps distributed on the cell surface (Fig. 2) and which are adjoined at their apical end as previously reported (Peterson and Mooseker, 1992; Pinto et al., 1983). Cells from late passages are more homogeneous (Fig. 2). These differences are also detected by indirect immunofluorescence staining of SI on the cell layer surface, which shows a more homogeneous distribution of SI in cultures of late passages as compared with early ones (Fig. 2). In contrast no differences could be observed in the levels of glycogen (Fig. 1E) or activity of DPP-IV (Fig. 1F,G).
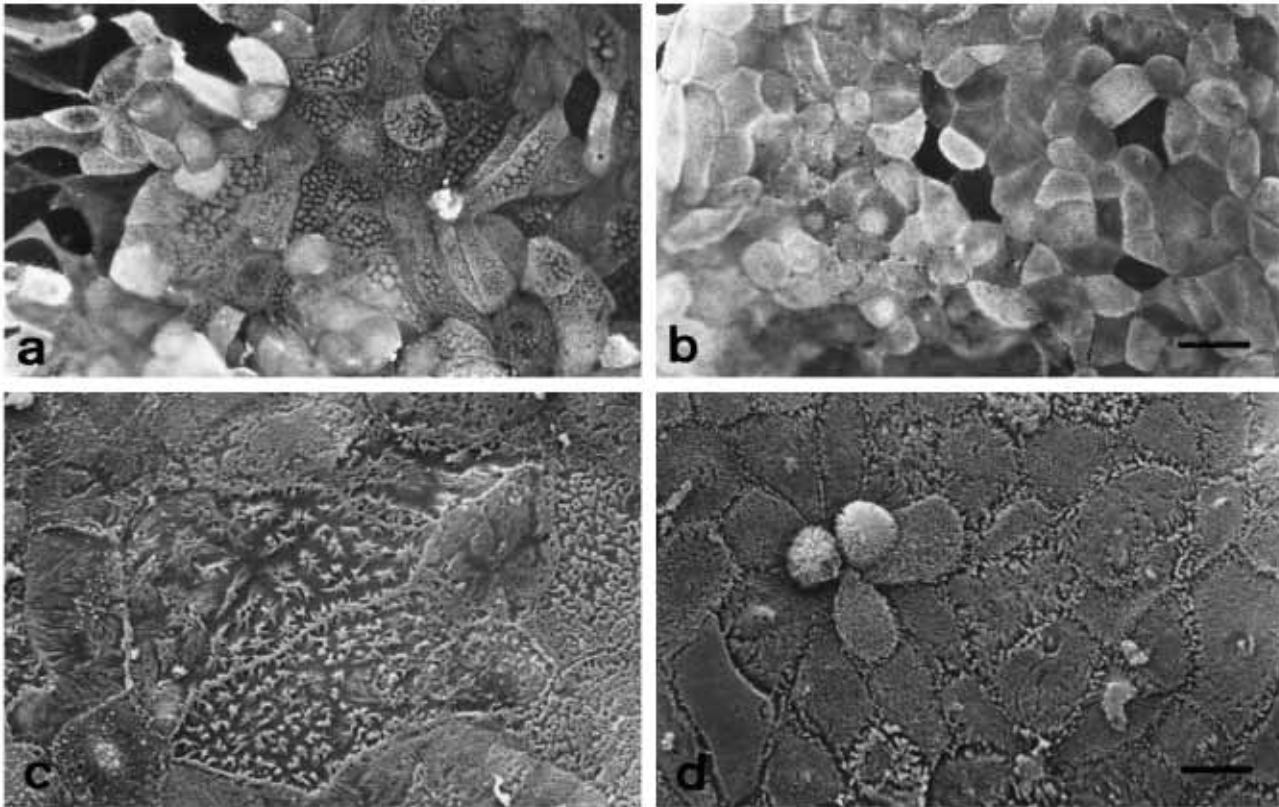
Fig. 2. Upper panel: indirect immunofluorescence staining of sucrase-isomaltase with mAb 2/614/88 on the cell surface of late post-confluent cultures (day 20) of early (a) and late (b) passages of Caco-2 cells; the picture in (a) corresponds to a cluster of SI-positive cells whereas (b) is representative of the totality of the cell surface; a similar pattern was obtained with all anti-SI antibodies (see Materials and Methods). Bar, 17.5 µm. Lower panel: scanning electron microscopy of corresponding cultures of early (c) and late (d) passages. Bar, 8 µm.

Comparative analysis of hydrolase activities and glucose consumption rates in Caco-2 clones

Twenty-six clones were obtained, 8 from the passage 29 cells and 18 from passage 198. Postconfluent cultures of the clones (passage 6, day 20) were analyzed for the rates of glucose consumption and the activities of brush border-associated enzymes in the cell homogenates. As shown in Fig. 3, the levels of sucrase activities varied considerably from one clone to another, ranging from 0.32 to 145 mU/mg protein; the rates of glucose consumption also varied considerably, ranging from 1.26 to 50 μg/h/10^6 cells. It can be seen that there is a remarkable inverse correlation between these parameters. In contrast there was relatively less variability in the activities of DPP-IV, APN, lactase and alkaline phosphatase. The variation in expression of SI was confirmed by immunofluorescence. Clones with high sucrase activity showed an intense and homogeneous apical staining, as shown in Fig. 4i,k,m,o. Clones with low sucrase activity showed a weaker intensity of staining that was restricted to clusters of cells as shown in Fig. 4a,c,e,g. In contrast DPP-IV and villin were expressed to the same extent in all the clones, as shown in Fig. 4. It is interesting to note that all clones derived from passage 198 had a high level of expression of SI and a low rate of glucose consumption, whereas only one of eight clones derived from passage 29 (clone PD7) showed these same characteristics, all the other ones showing low levels of SI and high rates of glucose consumption.

Characterization of 4 selected clones with respect to growth-related variations of SI expression and rates of glucose consumption

Four clones, 3 derived from passage 29 (PD7, PD10 and PF11) and 1 from passage 198 (TC7) were selected for further analysis on the basis of differences in the levels of sucrase activity, rates of glucose consumption and morphology of postconfluent cultures (Fig. 5). All four clones exhibited a well-organized brush border at late confluency (Fig. 6). These clones were analyzed for cell growth and growth-related variations of sucrase and DPP-IV activities, rates of glucose consumption, and levels of SI, DPP-IV and villin mRNAs.

PD10 and PF11 cells have a lower growth rate (doubling time 34-36 hours) than PD7 and TC7 (doubling time 20-24 hours) and a lower cell density at late confluency ($11\pm1\times10^6$ versus $16\pm1.5\times10^6$ cells per 25 cm^2) (Fig. 7). The four clones exhibited the same classical postconfluent increase of the activities of sucrase and DPP-IV and decrease in the rate of glucose consumption (Fig. 7). No significant differences in the levels of DPP-IV activity were observed between the clones. In contrast dramatic differences were observed in sucrase activity and rates of glucose consumption with two clones, PD10 and PF11, showing a much higher rate of glucose consumption and a much lower activity of sucrase than the other two, whatever the time-point examined (Fig. 7). The values observed in the four clones at day 20 illustrate well the negative relationship between the level of sucrase activity and the rate of glucose
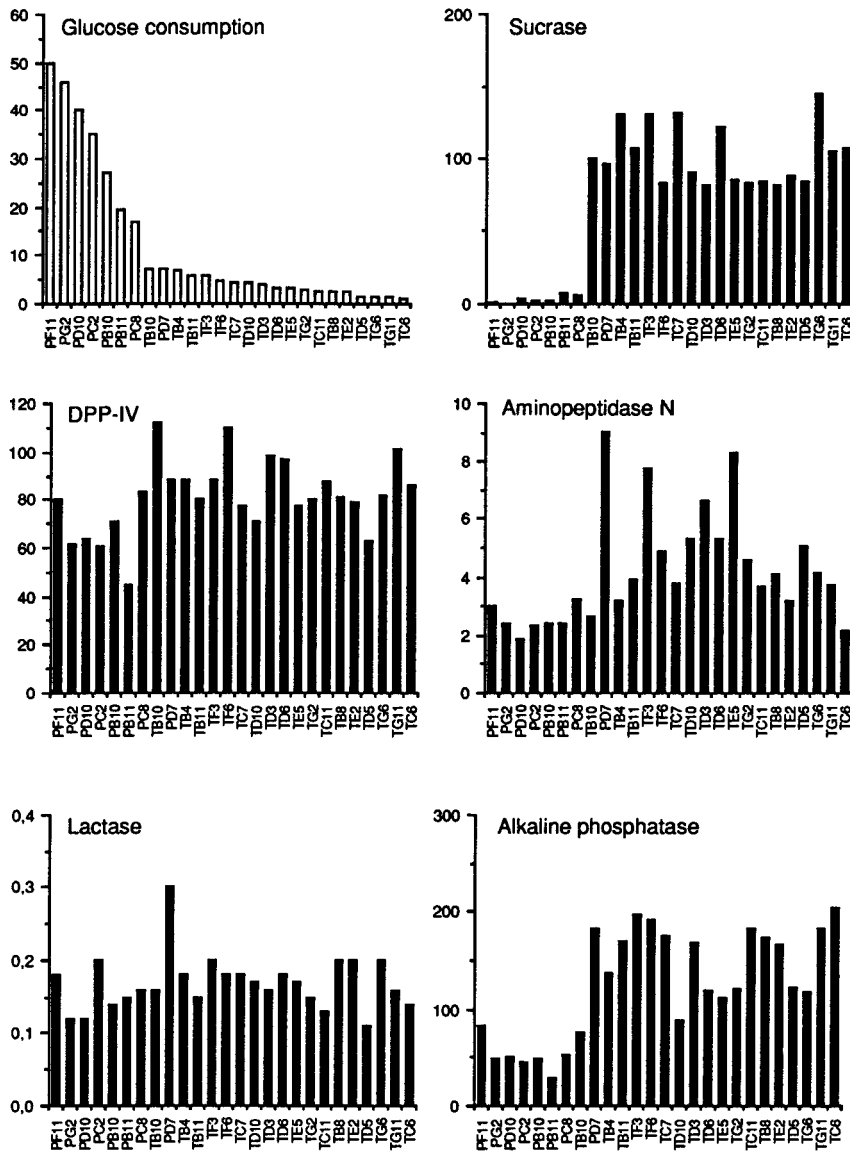
Fig. 3. Rates of glucose consumption ($\mu g/h/10^6$ cells) and levels of activity of brush border-associated enzymes (mU/mg protein) in cell homogenates from post-confluent cultures (day 20) of the 26 clones isolated from passages 28 and 198 of Caco-2 cells. Results are sorted according to decreasing values of glucose consumption rates. Note the marked negative correlation between the rates of glucose consumption and the activities of sucrase. Results are from single cultures.

consumption (Fig. 8), with a 70-fold higher level of sucrase activity in TC7 than in PF11 (235 versus 3.3 mU/mg protein in cell homogenates), paralleled by a 38-fold lower rate of glucose consumption (1.3 versus 50 $\mu g/h/10^6$ cells). The variations in the rates of glucose consumption from one clone to another are correlated with similar variations in the rates of lactic acid production (not shown) and contrast with the much lower variations in the levels of glycogen content (Fig. 9). The levels of activity of DPP-IV (not shown) and of sucrase in cell homogenates and brush border-enriched fractions, measured at day 20 of different passages (up to passage 20), were found to be stable in all clones (Fig. 10). It must be noted that, despite the enormous differences in sucrase activities, there is no difference between the clones in the ratio brush border enriched fraction/cell homogenate (Fig. 10).

The low activity of sucrase in PD10 and PF11 cells is associated with a low level of expression of the protein, as detected by immunofluorescence analysis of the cell layer: only a proportion of cells, which are grouped in clusters, show an apical expression of SI (see Fig. 4). These SI-positive clusters represent approximately 40% and 70% of the cell layer in PF11 and PD10 cells, respectively, and they never reach a higher proportion, even after 30 days in culture. By contrast the entire apical surface of PD7 and TC7 cells expresses SI, with a much more intense staining than in PF11 and PD10 cells (Fig. 4). Unlike SI, DPP-IV and villin are expressed to the same extent in the 4 clones (Fig. 4).

## Evidence that the variations of SI expression occur at the mRNA level

Fig. 11 shows the analysis of SI expression at the mRNA level and demonstrates that the protein variations observed in relation to cell growth, and the differences between the clones, are associated with concomitant variations and differences in the levels of SI mRNA. The differences in the levels of SI mRNA, which are 35-fold higher in TC7 than in PF11 cells on day 20, are exclusively quantitative, as substantiated by northern blotting analysis, which does not show any difference in the size of SI mRNA from one clone to another (Fig. 12). These quantitative differences are specific
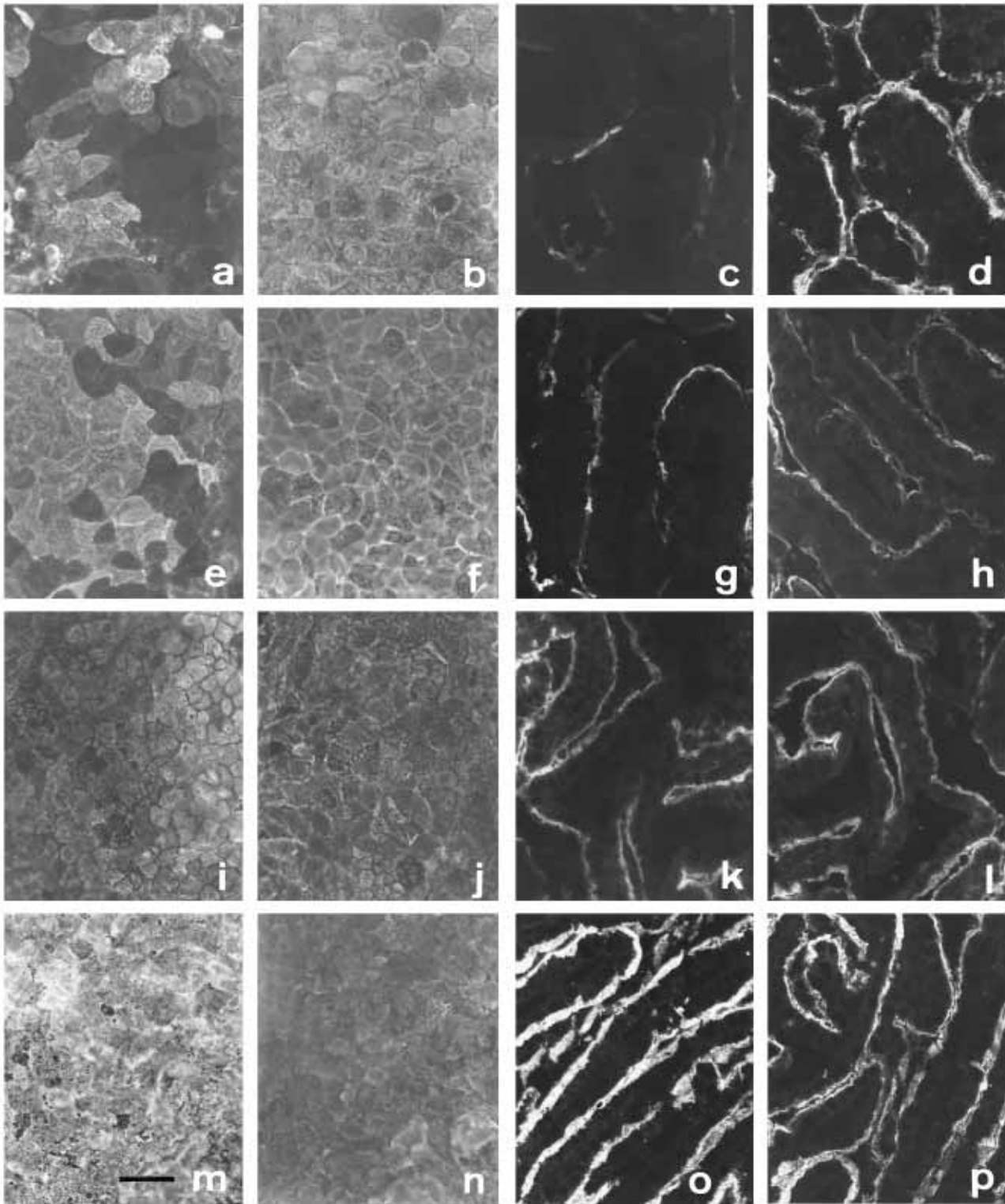
Fig. 4. Indirect immunofluorescence detection of sucrase-isomaltase, DPP-IV and villin on the cell surface and in cryostat sections of the cell layer of postconfluent (day 20) PF11 (a-d), PD10 (e-h), PD7 (i-l) and TC7 cells (m-p). Cultures were from passage 8 of the clones. Sucrase-isomaltase was detected with mAb 2/614/88 on the cell surface (a,e,i,m) and on cryostat sections (c,g,k,o); similar patterns were obtained with all anti-SI antibodies. Note that only a proportion of PF11 and PD10 cells express SI, in contrast to PD7 and TC7 cells, which all express the enzyme. DPP-IV (b,f,j,n) is present on the cell surface of all cells. (d,h,l,p) expression of villin in cryostat sections; note the homogeneous apical expression of villin in all the clones. Bar, 50 μm.
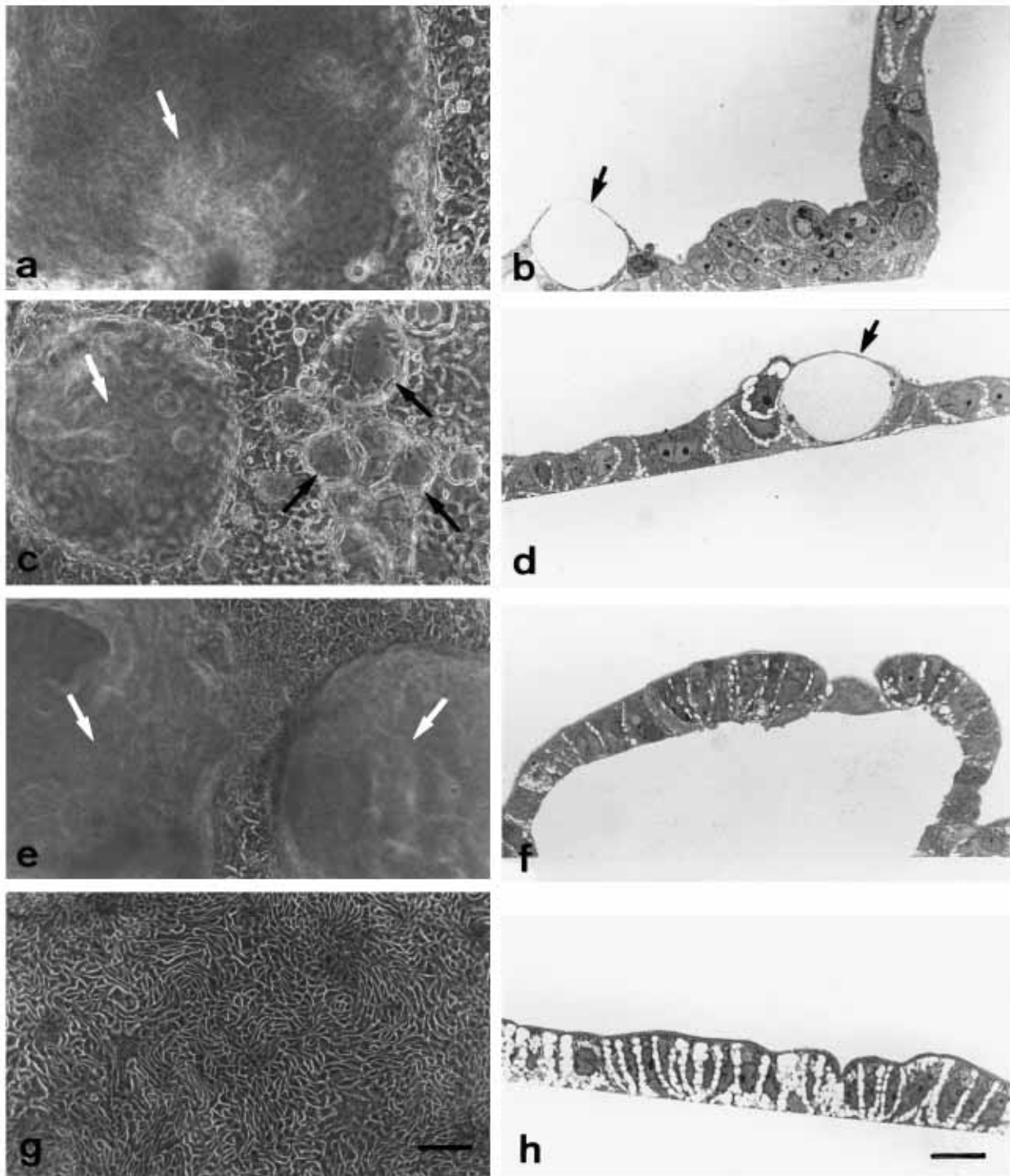
Fig. 5. Phase-contrast microscopy and thin sections of postconfluent cultures (day 20) of passage 6 of clones PF11 (a,b), PD10 (c,d), PD7 (e,f) and TC7 (g,h). PF11 cells are characterized by the formation of numerous (15-20 per cm^2) and huge domes (650-750 µm in diameter) and the presence of a few bubbles (20 per cm^2) limited by a thin bridge covered, at electron microscopy, with an apical brush border (not shown); note in (b) the edge of a dome. PD10 cells form a smaller number (5-10 per cm^2) of large domes (300-400 µm in diameter) and show a considerable number of bubbles (80-100 per cm^2). PD7 forms an homogeneous layer of cells with 5-10 domes per cm^2 ranging in diameter from 300 to 400 µm, but without bubbles; note in (f) a section of a dome. TC7 forms a very regular monolayer with only a few domes appearing between days 10 and 15 and disappearing thereafter. White arrows, domes; black arrows, bubbles. Left figures: bar, 100 µm; right figures: bar, 25 µm.
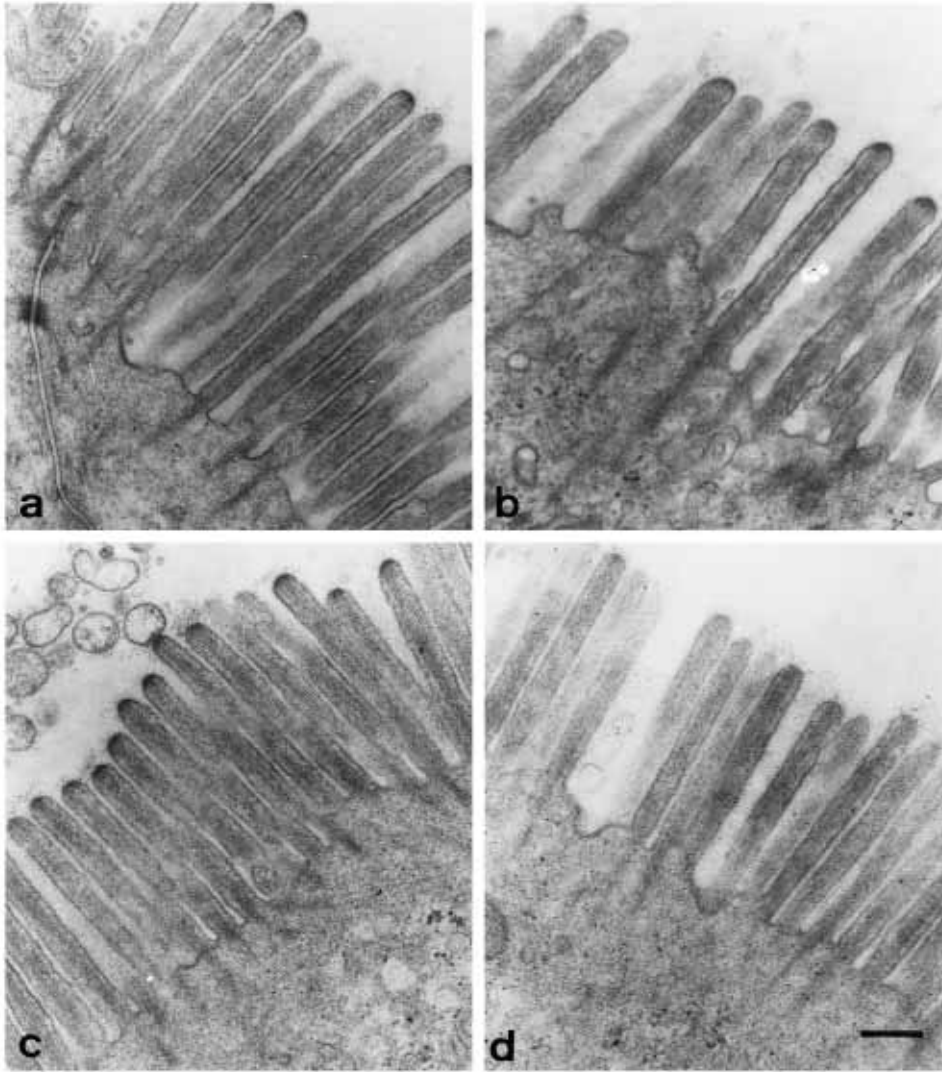
Fig. 6. Transmission electron microscopy of the apical brush border of (a) PF11, (b) PD10, (c) PD7 and (d) TC7 postconfluent (day 20) cells corresponding to the same cultures as in Fig. 5. Bar, 0.4 μm.

for SI, since they do not affect DPP-IV (Figs 11, 12) or villin (Fig. 11). Results similar to those obtained for PD7 and TC7 were also observed with clones TB10, TF3, and TG6 (not shown).

## Glucose deprivation results in an increased expression of sucrase-isomaltase in clone PF11

Clone PF11 was chosen to analyze the effect of a glucose deprivation on the expression of SI. For these experiments we took advantage of previous observations that showed that inosine and uridine support growth and viability of malignant epithelial cells in totally glucose-free medium, i.e. supplemented with dialyzed serum (Wice et al., 1981, 1985). Because of the growth-related variations of expression of the enzyme, a prerequisite for these experiments is that the culture conditions should not modify the growth curve. Since the use of dialyzed serum results in a decreased growth rate (not shown), we used normal serum, which provides a small amount of glucose (final concentration: 1 mM under our culture conditions), but does not modify the growth of the cells and the general appearance of the cell layer, i.e. the number and the size of domes, as compared to control cells.

The time taken by the cultures to consume this small amount of glucose decreases from 8 hours at day 3, down to 2 hours after day 8. Permanent growth of PF11 cells under such conditions of low glucose supply results in a progressive increase in the level of sucrase activity to values of 10-fold higher than in control cells at day 20 (Fig. 13). This increased expression of the enzyme is also confirmed by immunofluorescence, which shows a more intense staining of SI, along with a much higher number of cells expressing the enzyme in these cultures (Fig. 14). This increased expression of SI in cells grown in low glucose is correlated with an 11- to 13-fold increase in the level of SI mRNA at day 20, without modification of the level of expression of DPP-IV and actin (Fig. 15). Similar results were obtained with cells cultured in 1 mM glucose without supplementation with uridine or inosine (not shown); under these culture conditions, however, the growth rate was diminished, with confluency being attained two days later than in the control cells (not shown). Similar results were obtained with PD10 cells (not shown). In a separate experiment PF11 cells at late confluency (day 15) were switched to low glucose. The level of SI mRNA, analyzed after 5 days, shows a 4-fold increase (Fig. 15).
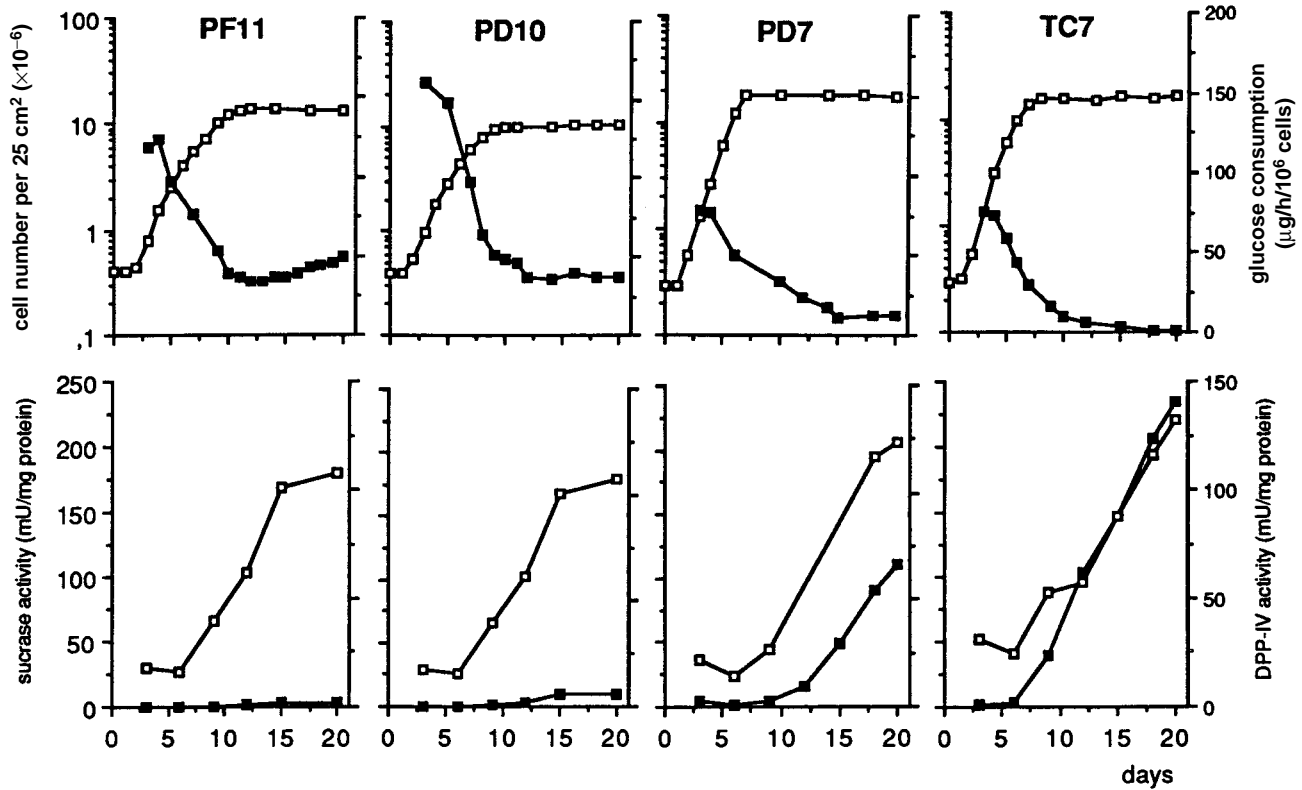
Fig. 7. Growth-related general characteristics of PF11, PD10, PD7 and TC7 clones. Upper panel: growth curves (open symbols) and glucose consumption rates (closed symbols); results are the means of 5 passages analyzed between passages 6 and 20; s.d. (not shown) were less than 10%. Lower panel: activity of sucrase (closed symbols) and DPP-IV (mU/mg protein) in cell homogenates from passage 8 of the clones.
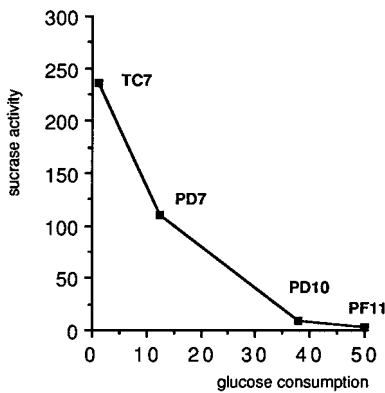
Fig. 8. Negative correlation between the rates of glucose consumption and the levels of sucrase activity in the cell homogenates from postconfluent (day 20) TC7, PD7, PD10 and PF11 cells.
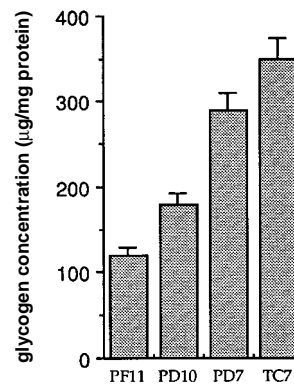

Fig. 9. Glycogen content in postconfluent (day 20) PF11, PD10, PD7 and TC7 cells. Cells were harvested 16 hours after medium change, i.e. when the concentration of glucose in the medium remains higher than 10 mM, even in PF11 cells, which have the highest rate of glucose consumption. Results are means of 5 different passages.

DISCUSSION

The twenty-six clones isolated from early and late passages of the Caco-2 cell line differ dramatically in the levels of expression of SI and rates of glucose consumption. By contrast, the other parameters associated with the enterocytic differentiation of the cells or their metabolism are much less variable.

The inverse relationship between the level of SI expression and the rate of glucose consumption was further analyzed in 4 clones selected on the basis of extreme and intermediate values of both parameters. The analysis showed that: (a) the growth-related increase in SI expression is associated with a growth-related decrease of the rates of glucose consumption; (b) the

levels of sucrase activity differ 70-fold between the lowest and the highest, in strict negative correlation with a 40-fold variation of the rates of glucose consumption; (c) the growth-related variations and the differences in sucrase activities from one clone to another are closely correlated with the levels of SI mRNA, which, on day 20, differ 35-fold, this contrasting with no difference in the levels of DPP-IV and villin mRNAs; (d) the level of expression of SI is closely correlated with the proportion of cells that express the enzyme: in clones with low SI (PF11 and PD10) only some of the cells express the enzyme, whereas in clones with high SI (PD7 and TC7) the enzyme is present in all the cells; (e) the large variations in the rates of glucose consumption have little effect on glycogen storage,
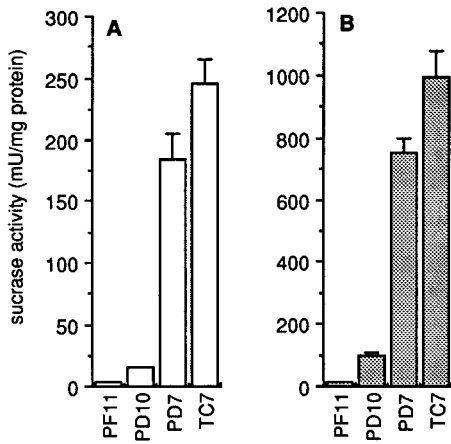
Fig. 10. Activities of sucrase in cell homogenates (A) and brush border-enriched fractions (B) from postconfluent cultures (day 20) of PF11, PD10, PD7 and TC7 Caco-2 cell clones. Results are means and s.d. of 3 to 5 different passages ranging from 6 to 20.

which varies only in a 1 to 3-fold range. Altogether these results are indicative per se of a negative relationship between the rate of glucose consumption and the expression of SI only. The hypothesis that SI expression can be negatively regulated by glucose is further supported by the results obtained with clone PF11: lowering the glucose concentration in the culture results in a dramatic increase in SI expression.

Previous studies with parental cells treated with forskolin and monensin have shown that the drug-dependent decrease in SI expression results from a decrease in the rate of transcription of the gene (Chantret et al., 1993). The results reported here show a close correlation between the sucrase activities and the SI mRNA levels suggesting that the differences in SI expression observed from one clone to another occur at the transcriptional level or result from modifications of RNA stability, but do not involve post-translational events. Studies are currently in progress to determine whether transcriptional differences are responsible for the differences in SI expression

between the clones, and whether a glucose-sensitive sequence of the gene is involved in this regulation. In this respect these clones appear as much more valuable experimental models than HT-29 cells to answer such questions. Indeed, although HT-29 cells adapted to glucose-free medium express SI, they express the enzyme at a much lower level than PF11 and PD10 clones grown in the presence of glucose with, furthermore, in contrast to these clones, less than 50% of the cells expressing the enzyme in glucose-free medium (Pinto et al., 1982; Zweibaum et al., 1985, Huet et al., 1987).

It will also be of interest to determine what causes the differences in glucose utilization. In a study performed with clones isolated from an early passage of Caco-2 cells, Beaulieu and Quaroni (1991) have observed an inverse relationship between the amounts of TGF-α/EGF-like activity released in the culture medium and the levels of SI expression, suggesting an autocrine-like regulation of SI expression. This was further confirmed by observations showing that treatment with EGF of a Caco-2 clone with high SI expression resulted in a dramatic decrease of the expression of the enzyme (Cross and Quaroni, 1991). Whether the differences in the level of SI expression in these different clones, or the effect of EGF, are also associated with changes in the rates of glucose consumption has not yet been investigated.

Also of interest is the mosaic expression of SI in clones with low SI expression. Such mosaic expression of SI in clonal populations has also been observed in HT-29 cell clones (Huet et al., 1987) and in other Caco-2 clones (Beaulieu and Quaroni, 1991; Peterson and Mooseker, 1992; Vachon and Beaulieu, 1992). It has also been reported in Caco-2 cells for endopeptidase-24.11 (Howell et al., 1992). It is not a phenomenon restricted to cultured cells, as it has been observed in the human small intestine for example for lactase (Maiuri et al., 1991). This phenomenon remains unexplained, but may well be a reflection of local differences in metabolic regulation. In our culture conditions for PF11 and PD10 cells, it is a stable phenomenon. This contrasts with the observation of Vachon and Beaulieu (1992) that the mosaic pattern in their cell clone Caco-2/15 is transient, disappearing at late confluency. This discrepancy may be explained by the characteristics of SI
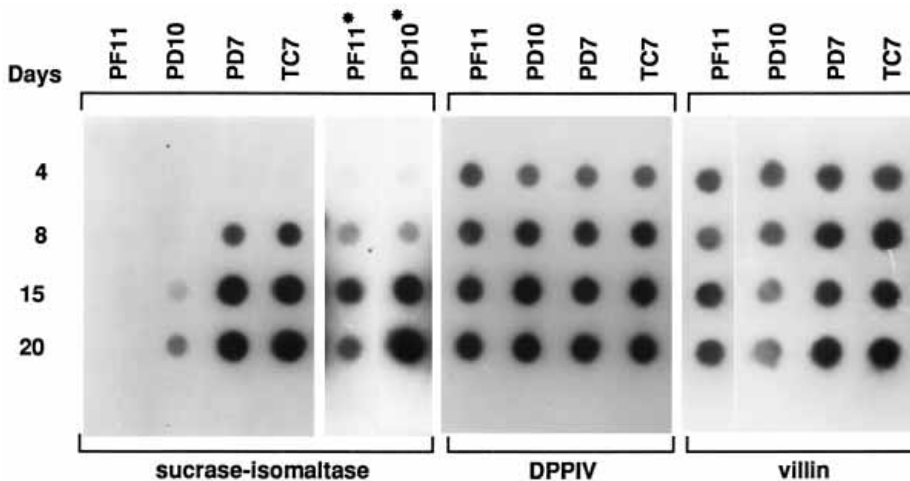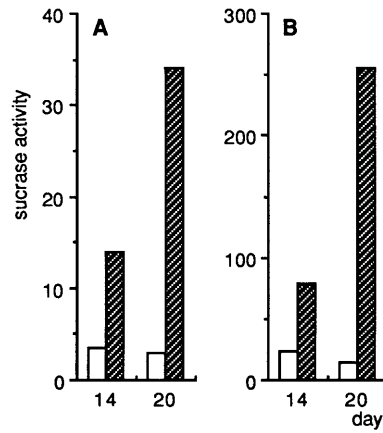

Fig. 11. Growth-related variations of the levels of SI, DPP-IV and villin mRNAs analyzed by dot blot in PF11, PD10, PD7 and TC7 cells. The same amount of total RNA (20 µg) was laid on each spot. In order to visualize the low levels of SI mRNA in PF11 and PD10 cells the film was exposed for 48 hours (∗), instead of 5 hours in left panel. Note the growth related increase of SI and DPP-IV mRNA, with SI being not yet expressed at day 4, in contrast to DPP-IV. Also note the low level of SI mRNA in PF11 and PD10 cells. In contrast to SI and DPP-IV, villin is equally expressed in all cells

independently from cell growth. SI mRNA levels on day 20, as deduced from scanning analysis, are 35- and 14-fold higher in TC7 than in PF11 and PD10 cells, respectively. The same filter was used for SI and DPP-IV, and a different filter for villin.
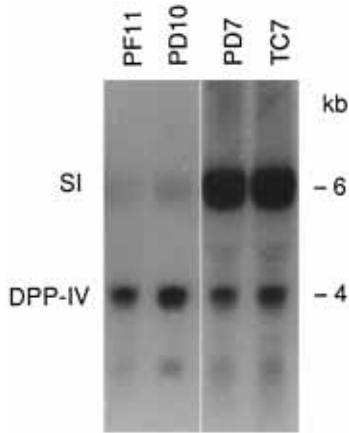
Fig. 12. Northern blot analysis of SI and DPP-IV with total RNA (20 μg) from post-confluent (day 20) cells.

Fig. 13. Effect of decreased glucose supply (1 mM) as compared to standard culture conditions (25 mM glucose) on the levels of sucrase activity in cell homogenates (A) and brush border-enriched fractions (B) in postconfluent PF11 cells. Open columns, control cells; hatched columns, cells grown in low glucose medium supplemented with inosine (see Materials and Methods). Results are from a single experiment. The same results were obtained with low glucose medium supplemented or not with uridine.

expression in Caco-2/15 cells and the culture conditions used. Caco-2/15 cells, although showing the highest expression of SI in their series, in fact shows levels of enzyme activity (Cross and Quaroni, 1991; Vachon and Beaulieu, 1992) that are very similar to those observed in PD10 cells. Thus it can be postulated that these cells have a high rate of glucose consumption similar to that of PD10 cells. The disappearance of the mosaic expression of SI in late post-confluent Caco-2/15 cells resembles what is observed when PF11 and PD10 cells are grown in low glucose and could conceivably be a culture artifact. Indeed the culture conditions used for Caco-2/15 cells (10 ml of medium for 75 cm² changed twice a week, versus 15 ml of medium changed daily in our culture conditions) probably do not meet the glucose consumption requirements
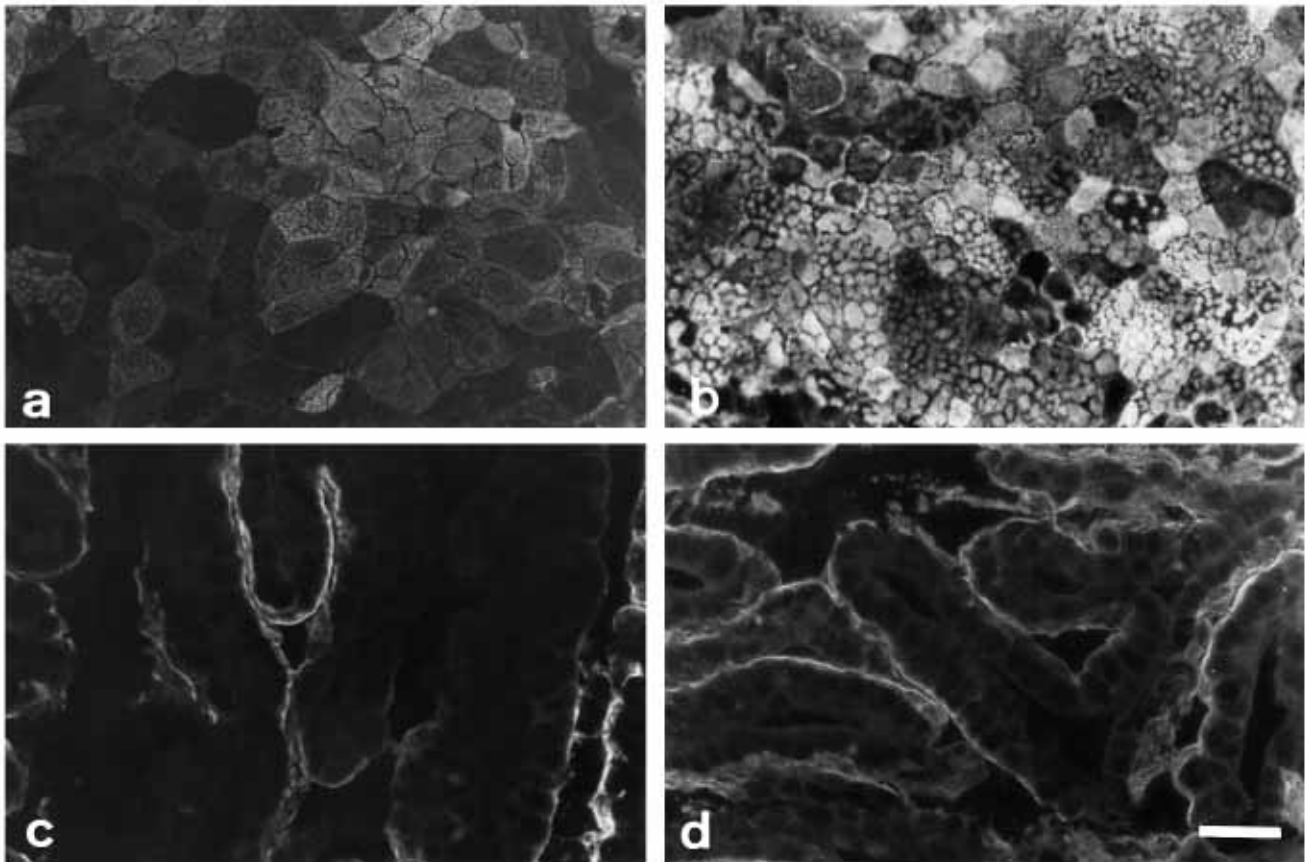
Fig. 14. Indirect immunofluorescence staining of SI with mAb 2/614/88 on the cell surface (upper figures) and in cryostat sections (lower figures) of postconfluent (day 20) PF11 cells grown in the presence of 25 mM (a,c) or 1 mM glucose plus inosine (b,d). Same results as in (b) and (d) were obtained in cells cultured in 1 mM glucose plus uridine. The same pattern of expression of SI was observed with all the anti-SI antibodies of this study. Bar, 40 μm.

glucose concentration (mM)
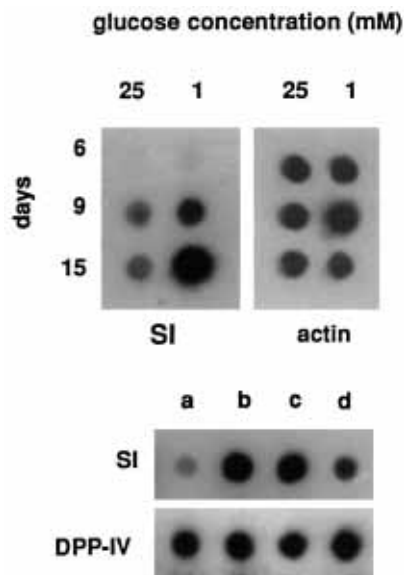
Fig. 15. Effect of low glucose (1 mM) on the level of SI mRNA in PF11 cells as compared to standard culture conditions (25 mM). A higher amount of total RNA (30 μg) than in Fig. 12 was laid on each spot. The same filters were dehybridized and rehybridized with actin or DPP-IV cDNAs as internal controls. Upper panel: growth-related expression of SI mRNA in cells cultured in the presence of 25 mM or 1 mM glucose plus inosine. Lower panel: expression of SI mRNA in postconfluent cultures (day 20) of cells grown from the first day on in 25 mM glucose (a), or 1 mM glucose plus inosine (b) or uridine (c). In (d) the cells were grown in 25 mM glucose from the first day on, and switched on day 15 and onwards to 1 mM glucose plus inosine. Increases in the levels of SI mRNA, as deduced from scanning analysis of the dots, were 13-, 11-, and 4-fold higher in (b), (c) and (d), respectively, than in (a).

of the cells, which are consequently deprived of glucose most of the time.

The present results are also of significance in relation to the cell line Caco-2 itself. The 18 clones isolated from passage 198 had a high growth rate (doubling time 24 hours) and a high level of SI expression. In contrast 7 of 8 clones isolated from passage 29 had a lower growth rate (34 hours) and a low level of expression of SI. Only one clone from passage 29, PD7, was similar to the clones from passage 198 as to its growth rate and level of SI expression. This would suggest that the parental population contains a small proportion of cells that grow more rapidly and express a much higher level of SI than the majority of the cells. This small population progressively increases in number, from passage to passage. These faster growing cells may also show changes in expression of other differentiation-associated markers. Indeed this is the case for the fructose transporter GLUT5, the expression of which increases, like that of SI, with the number of passages (Mahraoui et al., 1992) and which is highly expressed in clones isolated from passage 198 and in PD7 cells, but not in PD10 and PF11 cells (Mahraoui et al., unpublished data).

Altogether the results reported here show that these clones should be good models to further investigate and characterize potential glucose-sensitive regulatory elements of the human SI gene.

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