The Posttranslational Processing of Sucrase–Isomaltase in HT-29 Cells Is a Function of Their State of Enterocytic Differentiation

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Abstract. The biosynthesis of sucrase–isomaltase was compared in enterocyte-like differentiated (i.e., grown in the absence of glucose) and undifferentiated (i.e., grown in the presence of glucose) HT-29 cells. Unlike differentiated cells, in which the enzyme is easily detectable and active, undifferentiated cells display almost no enzyme activity and the protein cannot be detected by means of cell surface immunofluorescence or immunodetection in membrane-enriched fractions or cell homogenates. Pulse experiments with L-[35S]methionine show that the enzyme is, however, synthesized in these undifferentiated cells. As compared with the corresponding molecular forms in differentiated cells, the high-mannose form of the enzyme in undifferentiated cells is similarly synthesized and has the same apparent Mr. However, its complex form is less labeled and has a lower apparent Mr. Pulse-chase experiments with L-[35S]methionine show that, although the enzyme is synthesized to the same extent in both situations, the high-mannose and complex forms are rapidly degraded in undifferentiated cells, with an apparent half-life of 6 h, in contrast to differentiated cells in which the enzyme is stable for at least 48 h. A comparison of the processing of the enzyme in both situations shows that the conversion of the high-mannose to the complex form is markedly decreased in undifferentiated cells. These results indicate that the absence of sucrase–isomaltase expression in undifferentiated cells is not the consequence of an absence of biosynthesis but rather the result of both an impaired glycosylation and a rapid degradation of the enzyme.

The human colon cancer cell line HT-29 is a challenging experimental model, as a very simple modification of the culture medium, namely the removal of glucose, results in a total enterocytic differentiation of the cells (32, 47, 48, 50, 51). This differentiation is characterized by a polarization of the cell monolayer with the presence of apical brush border microvilli and a high level of expression of intestinal microvillar hydrolases (19) and structural proteins like the actin-binding protein villin (4). Among these, some are already present, although at a much lower level, in undifferentiated cells: this is the case for hydrolases like aminopeptidase-N, dipeptidylpeptidase IV, or alkaline phosphatase (32) and for villin (34). The case of sucrase–isomaltase is particularly dramatic with the enzyme being present in differentiated and absent from undifferentiated cells (32, 47, 48, 51).

The mechanism(s) by which glucose modulates the differentiation of HT-29 cells is far from clear. Sucrase–isomaltase, which may or may not be expressed in HT-29 cells according to their differentiation state, therefore represents an appropriate target to further investigate the role of glucose in the differentiation of these cells. Furthermore, it can be anticipated that results from such investigations should provide a better knowledge of the overall reputation of the expression of sucrase–isomaltase. A better knowledge of this regulation is particularly needed with respect to a number of developmental or pathological situations such as the transient expression of sucrase–isomaltase in the human fetal colon (21, 52), its ectopic expression in some human colon cancers (49, 52), or its impaired expression in patients with sucrase–isomaltase malabsorption (15, 45).

Like other intestinal brush border membrane hydrolases, sucrase–isomaltase is a highly glycosylated protein (19), the biosynthesis and processing of which is the same as that of other glycoproteins (20, 38): it is co-translationally N-glycosylated in the rough endoplasmic reticulum and then processed to the Golgi apparatus where terminal N-glycosylation and O-glycosylation take place before the final insertion of the enzyme in the microvillar membrane (for review see reference 41).

Our initial purpose was to study the biosynthesis and post-translational processing of sucrase–isomaltase in differentiated HT-29 cells, using undifferentiated cells as negative control. Surprisingly, we found that sucrase–isomaltase is synthesized in undifferentiated HT-29 cells. In this case, however, the enzyme is rapidly degraded and appears to have
a glycosylation pattern which is different from that observed in differentiated cells.

Materials and Methods

Cells and Culture Conditions

HT-29 cells (passage 143) were obtained from J. Fogh (Memorial Sloan-Kettering Cancer Center, Rye, NY) and routinely grown in DME (Eurobio, France) supplemented with 10% inactivated (56°C, 30 min) FBS (Boehringer Mannheim GmbH, Mannheim, Germany) in 25-cm² plastic flasks (Corning Glass Works, Corning, NY) in an atmosphere of 10% CO₂/90% air at 37°C. For experimental purposes, the cells were switched from the standard culture medium to the same medium devoid of glucose, supplemented with 10% of the same inactivated FBS but dialyzed, and with either 2.5 mM inosine (differentiation-permissive conditions) or 2.5 mM inosine plus 25 mM glucose (control undifferentiated cells). Glucose-free medium supplemented with inosine (47) was chosen rather than glucose-free medium supplemented with galactose (32) or totally hexose-free medium (51) because, unlike these two other conditions, the cells are readily adaptable to inosine without any impairment of cell growth and viability while exhibiting the same pattern of enterocytic differentiation (48). Cells were seeded at 1 X 10⁶ cells per flask, passed each week, and tested between passages 6 to 8 under the experimental conditions (i.e., a number of passages which has been found to be needed for the differentiation to reach its maximal expression as assessed by electron microscopy and enzyme activity assays (unpublished results)). The ultrastructural morphology of differentiated and undifferentiated cells (not shown) was as previously reported (32, 47, 48, 51). Except when indicated, all experiments were performed after 18-19 d in culture (i.e., 10 d after confluence), this period corresponding to the maximum rate of enzyme biosynthesis in differentiated cells.

Preparation of Cellular Extracts

The cell culture medium was removed, the cell layer rinsed three times with ice-cold saline, snap-frozen by blotting of the flasks on a bath of liquid nitrogen, and stored at -70°C. For preparation of cell homogenates, the cells were scraped at 4°C in 2 ml Tris-mannitol buffer (2 mM Tris, 50 mM mannitol), pH 7.1, supplemented with a cocktail of protease inhibitors containing antitain (1 μg/ml), benzamidine (7.5 μg/ml), phenylmethanesulfonyl fluoride (1 mM), pepstatin (1 μg/ml), and aprotonin (10 μg/ml). The cells were then disrupted in a conical grinding tube and sonicated as already described (32, 50). Brush border-enriched fractions (P2) were prepared from the cell homogenates according to Schmitz et al. (39) as previously reported (32, 50). Briefly, CaCl₂ was added to the homogenate to a final concentration of 18 mM. After 10 min at 4°C, the homogenate was centrifuged (950 g, 10 min, 4°C) to yield a small pellet (P2 fraction) enriched in brush border membranes which was resuspended in 10 mM K+ phosphate buffer, pH 6.8.

Enzyme Assays

Enzyme activities were measured in the cell homogenates and P2 fractions. Sucrase (EC 3.2.1.48) activity was determined according to Messer and Dahlqvist (26), aminopeptidase-N (EC 3.4.11.2) according to Marouz et al. (25), using l-alanine-p-nitroanilide as substrate, and dipeptidylpeptidase IV (EC 3.4.14.5) according to Nagatsu et al. (29) using glycyl-l-proline-4-nitroanilide as substrate. The enzyme activities are expressed as milliunits (μU) per mg of proteins. One unit is defined as the activity that hydrolyzes 1 μmol of substrate per min at 37°C.

Antibodies

Monoclonal antibody HBB 2/64/88 (16) (from ascites fluid) specific for sucrase-isomaltase from normal human small intestine was obtained from H. P. Hauri (Biocenter of the University of Basel, Basel, Switzerland). Polyclonal rabbit antibodies produced against sucrose-isomaltase from human blood group O small intestine were obtained from N. Triadou (43) (Hôpital Necker-Enfants Malades, Paris, France). Polyclonal antibodies against sucrase-isomaltase from another enterocyte-like cell line, Caco-2 (48), were produced in the laboratory by immunizing rabbits with sucrose-isomaltase purified from brush border membranes from Caco-2 cells, according to N. Triadou (43). These antibodies will be referred in this paper as antibodies No. 1, No. 2, and No. 3, respectively.

Indirect immunofluorescence

Indirect immuno-fluorescence was performed on cell monolayers grown on glass coverslips fixed for 15 min at room temperature in 3.7% paraformaldehyde in Ca²⁺- and Mg²⁺-free phosphate buffered saline (PBS⁻), as previously reported (51). Monoclonal antibodies against sucrase-isomaltase were used at a dilution of 1:100 in PBS⁻. Rabbit anti-sucrase-isomaltase antibodies against human small intestine were used at 50 μg/ml in PBS⁻. Rabbit antiserum against sucrase-isomaltase from Caco-2 cells were used at a dilution of 1:100 in PBS⁻. Anti-rabbit fluorescein-coupled sheep antiguinea pig antibodies (Institut Pasteur Productions, Paris, France) and anti-mouse fluorescein-coupled rabbit antiguinea pig antibodies (Cappel Laboratories, Cochranville, PA) were used as reported (51).

Labeling with L-[35S]Methionine

Before l-[35S]methionine labeling, the culture medium was replaced by 5 ml of methionine-free medium (Institut Jacques Boy, Reims, France) supplemented with 2.5 mM inosine and 10% inactivated dialyzed FBS in the presence or absence of 25 mM glucose, according to the differentiation culture conditions. The cells were incubated in this medium for two consecutive periods of 30 min at 37°C with an intermediate change. This medium was then removed and replaced by 2 ml of the same methionine-free medium containing 750 μCi of l-[35S]methionine (800 Ci/mmol; Amersham Corp., Arlington Heights, IL). Cells were incubated in this medium at 37°C for 3 h. Although the volume of the medium was reduced to 2 ml during the pulse labeling, the amount of glucose in the medium of glucose-grown cells was regularly in excess with regard to the glucose consumption requirement of the cells (i.e., 0.46 ± 0.08 μmol/h per mg of protein [35] for an average cell protein content of 14 ng per flask), with the concentration of glucose in the medium at the end of the 3-h labeling period ranging from 15 to 18 mM. At the end of the labeling period, the medium was removed and the cells were either rinsed three times with ice-cold saline, snap-frozen, and stored at -70°C (pulse experiments) or further incubated for the indicated periods in complete medium containing 10 mM unlabeled methionine (pulse-chase experiments).

Imunoisolation of Sucrase-Isomaltase and SDS PAGE

Sucrase-isomaltase was immunoprecipitated using monoclonal and polyclonal anti-sucrase-isomaltase antibodies and a protein A-Sepharose assay as described by Hauri et al. (16). A 30% stock solution of protein A-Sepharose (Pharmacia Fine Chemicals, Uppsala, Sweden) in 40 μl of 0.1% (wt/vol) BSA in 100 mM sodium phosphate buffer, pH 8 (buffer 1) was added to 200 μl of buffer 1 containing the antibodies at the same dilutions as for immuno-fluorescence. The preparation was incubated for 2 h at room temperature on a horizontal Lab-shaker (New Brunswick Scientific Co., Edison, NJ) at 14 rpm. The beads were then washed twice with 1 ml buffer 1 and once with 1 ml of 100 mM sodium phosphate buffer, pH 8, containing 1% (wt/vol) Triton X-100 and 0.2% (wt/vol) BSA (buffer 2). The beads were incubated (90 min, 4°C, under constant shaking) with 200 μl of 1 mg/ml protein preparation of cell homogenates, first solubilized in 1% Triton X-100 and subsequently washed three times with 1 ml buffer 2, twice with 1 ml 100 mM phosphate buffer, pH 8, and once with 1 ml 10 mM phosphate buffer, pH 8. The antigen-antibody complex was then solubilized in the electrophoresis sample buffer (2% SDS, 100 mM dithiothreitol) at 100°C for 4 min. Proteins were then run on 7.5% SDS polyacrylamide slab gels. The material loaded in each slot always corresponded to the same amount of cellular protein in order to allow quantitative comparisons. The gels were either stained with silver nitrate (27) or, for l-[35S]methionine-labeled enzyme, visualized by fluorography using Amido Black (Amersham Corp.). In no case did radioactive material get stuck on top of the gels. Quantitation of sucrase-isomaltase labeling was achieved by scanning the bands of fluorograms, using a densitometric scanner (model Mark III CS; Joyce, Loebl and Co., Ltd., Gateshead, England). Control immunoprecipitations were done using protein A-Sepharose without antibodies. In this case, no band could be evidenced on the gels (data not shown).

Immunoblotting

Immunoblotting analysis of sucrase-isomaltase in brush border-enriched fractions (P2) was performed as previously described (51). Briefly, 60 μg

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of cellular proteins were separated on 7.5% SDS polyacrylamide slab gels in the buffer system of Laemmli (22) and transferred to nitrocellulose sheets by the method of Burnette (5). The sucrase-isomaltase complex was identified using the same antibodies as those used for immunofluorescence. The antigen–antibody complexes were detected with the peroxidase technique (12).

Results

The Expression of Sucrase–Isomaltase Is Severely Impaired in Undifferentiated HT-29 Cells

Sucrase–isomaltase is present in differentiated cells grown in glucose-free medium supplemented with inosine: the enzyme is detectable on the apical surface of the cells as shown with immunofluorescence by the binding of anti–sucrase–isomaltase antibodies (Fig. 1) and by immunoblotting of a brush border–enriched fraction (P2) (Fig. 2 A). Furthermore, the enzyme is active (Table I) in both the cell homogenate and the brush border–enriched fraction (P2), with a P2/H enrichment of 6.5-fold. It must be noted that the activity of the enzyme, although present in these differentiated cells, is 10–30 times lower than that found in Caco-2 cells (48) or in the normal small intestine (44). Despite this low activity, it can be detected in the cell homogenate by silver staining of SDS PAGE of immunoprecipitates (Fig. 2 B). It appears as a band migrating with an apparent Mr at 210 kD, that presumably corresponds to the complex form of the enzyme detected in the pulse–labeling experiments. It has to be mentioned that a faint faster migrating band can also be detected, which is so far unidentified. In undifferentiated cells, in contrast, it is impossible to detect the presence of sucrase–isomaltase with the same methods; i.e. immunofluorescence (Fig. 1), immunoblotting (Fig. 2 A), or silver stain.

Figure 1. Indirect immunofluorescence staining of sucrase–isomaltase in (A) differentiated HT-29 cells grown in glucose-free medium supplemented with 2.5 mM inosine and (B) undifferentiated HT-29 cells grown in the same medium supplemented with 25 mM glucose. Immunofluorescence was performed after 30 d in culture at passage 6 under the experimental conditions. Anti–sucrase–isomaltase antibody No. 3 was used (see Materials and Methods). Note the absence of immunoreactive material in B and the positive staining in A which corresponds to an apical binding of the antibody. Also note that only 50–60% of differentiated cells (A) exhibit a positive apical staining. Similar results were obtained with antibody Nos. 1 and 2. Bar, 40 µm.

Figure 2. Immunodetection of sucrase–isomaltase after SDS PAGE of cellular extracts from (A) differentiated and (B) undifferentiated HT-29 cells (passage 6 under the experimental culture conditions). In A, a P2 fraction of cells grown for 20 d was run on SDS polyacrylamide slab gels and analyzed after transfer onto nitrocellulose sheet by immunological detection with anti–sucrase–isomaltase antibody No. 3. In B, sucrase–isomaltase immunoisolated with the same antibody from the cell homogenates of cells grown for 30 d was run on SDS polyacrylamide slab gels and stained with silver nitrate. Note the absence of detectable sucrase–isomaltase in undifferentiated cells and the apparent Mr, of the band observed in differentiated cells at ≈210 kD, which corresponds to the complex glycosylated form of the enzyme.
Border-associated Hydrolases in Differentiated and Undifferentiated HT-29 Cells

The activity of sucrase in these cells is almost absent and there is almost no P2/H enrichment (Table I).

Sucrase–Isomaltase Is Synthesized in Undifferentiated Cells

To gain further insight into the mechanism of the regulation of sucrase–isomaltase expression, this protein was labeled with [35S]methionine and immunoisolated from both cell populations. Surprisingly, in a first experiment performed with antibody No. 3 (i.e., the polyclonal antibody raised against sucrase–isomaltase from Caco-2 cells), we found evidence of sucrase–isomaltase protein synthesis in undifferentiated cells. To make sure that this result was not an artefact due to the particular antibody used, the same experiment was repeated using all three anti-sucrase–isomaltase antibodies. As shown in Fig. 3, these three antibodies gave identical results, demonstrating that sucrase–isomaltase protein is synthesized in undifferentiated cells as well as in differentiated cells, though there are some quantitative and qualitative differences. A comparative scanning analysis of the amount of labeled sucrase–isomaltase present after 3 h indicates that in undifferentiated cells there is up to two-thirds of that measured in differentiated cells (data not shown). Identification of the two bands, in both differentiated and undifferentiated cells, using endo H and endo F enzymatic digestions (not shown), gave similar results as those previously reported for sucrase–isomaltase in the cell line Caco-2 by Hauri et al. (16) and by us (36, 46), with the lower band corresponding to the high-mannose type (endo H and endo F sensitive) and the upper band of the complex type (endo H resistant but endo F sensitive). The size of the two forms of sucrase–isomaltase in differentiated (A) and undifferentiated (B) HT-29 cells (passage 6 under the experimental conditions, day 20 in culture). Cells were labeled with L-[35S]methionine for 3 h and then a chase was performed with complete medium containing 10 mM unlabeled methionine. At the indicated intervals sucrase–isomaltase was immunoisolated from the cell homogenate with antibody No. 3 and run on SDS polyacrylamide slab gels. The same amount of cellular proteins was used for each immunoisolation. Lane numbers (1–6) refer to 0, 3, 6, 12, 24, and 48 h of chase, respectively. Similar results were obtained in two separate independent experiments from different passages (passages 6–8 in the experimental culture conditions). Quantification of the total labeling of the enzyme in both conditions and of the proportion of the two bands are reported in Figs. 5 and 6.

Table I. Specific Enzyme Activities of Brush Border-associated Hydrolases in Differentiated and Undifferentiated HT-29 Cells

|                     | Differentiated cells | Undifferentiated cells |
|---------------------|----------------------|------------------------|
|                     | Homogenate*          | P2 fraction†           | Homogenate*          | P2 fraction†           |
|                     | mU/mg                | mU/mg                  | mU/mg                | mU/mg                  |
| Sucrase             | 5.0                  | 32.4                   | 0.3                   | 0.6                    |
| Dipeptidyl         |                      |                        |                       |
| peptidase IV       | 51.3                 | 268.6                  | 30.8                  | 124.2                  |
| Amino               |                      |                        |                       |
| peptidase N        | 11.8                 | 24.3                   | 12.1                  | 12.8                   |

Results are expressed as mU/mg of protein (see Materials and Methods) and were obtained from cells grown for 30 d. The values are the mean of five separate independent experiments performed between passages 6 and 8 under the experimental culture conditions. SDs, not shown, are <10%.

* Whole cell homogenate.
† Brush border-enriched fraction.

Table II. Comparison of the Percentages of the Two Molecular Forms of Sucrase–Isomaltase, Immunoisolated from Differentiated and Undifferentiated HT-29 Cells with Three Different Antibodies

| Antibody No.* | Differentiated cells | Undifferentiated cells |
|---------------|----------------------|------------------------|
|               | Complex form | High mannose form | Complex form | High mannose form |
| 1             | 29.8       | 70.2                 | 16.8        | 83.2               |
| 2             | 37.8       | 62.2                 | 16.2        | 83.3               |
| 3             | 35.5       | 64.5                 | 17.6        | 82.4               |

Fluorograms from Fig. 3, A and B, were scanned and the surface of the peaks corresponding to the upper (complex) and the lower (high-mannose) bands were calculated. Each value represents the percentage of the intensity of each band relative to the sum of the two bands in each lane. Results are the mean of two separate independent experiments with the variation being <10%.

* Anti-sucrase–isomaltase antibodies are as defined in Materials and Methods.
crase-isomaltase is consistent with the notion that they are uncleaved forms of sucrase-isomaltase as observed in small intestinal pig explants (6, 7) and Caco-2 cells (16, 36, 46). However, as shown in Fig. 3, A and B, it has to be pointed out that sucrase-isomaltase seems to be different in the two cell populations: the migration of the complex form is regularly faster in undifferentiated cells with an apparent Mr at 206 kD as compared with 208 kD in differentiated cells (Fig. 3). In addition, the proportion of this complex form is lower in undifferentiated cells (Table II). This suggests that the glycosylation of sucrase-isomaltase may vary as a function of HT-29 cell differentiation.

The Newly Synthesized Sucrase-Isomaltase Is Rapidly Degraded in Undifferentiated Cells

Kinetic studies were undertaken in order to understand the subnormal biosynthesis of sucrase-isomaltase in undifferentiated cells. Both differentiated and undifferentiated cell populations were pulse-labeled for 3 h and then chased for 0 to 48 h. Fluorograms and scanning analysis of the corresponding gels are both presented in Figs. 4 and 5. It clearly appears that sucrase-isomaltase is synthesized to the same extent in both cell populations. However, the total amount of labeled enzyme decreases progressively in undifferentiated cells, whereas it remains constant in differentiated cells. These results clearly demonstrate that, in differentiated cells, the newly synthesized enzyme is stable during the 48 h of chase, whereas, in undifferentiated cells, it is rapidly degraded with an apparent half-life of 6 h.

Glycosylation of Sucrase-Isomaltase Is Impaired in Undifferentiated Cells

The processing of sucrase-isomaltase protein is severely impaired in undifferentiated cells as shown by the relative percentage of the high-mannose and the complex form derived from the fluorogram in Fig. 4. Quantitative scanning of this pulse-chase experiment (Fig. 6) shows that almost no further conversion from the high-mannose into the complex form of the enzyme could be observed in undifferentiated cells, suggesting that the extent of the processing is impaired in these cells. In contrast, differentiated HT-29 cells convert the high-mannose to the complex form with two unexpected results. (a) The conversion of the high-mannose form is never complete in contrast to the observation in Caco-2 cells, for example, in which a total conversion is observed after 4 h of chase (16; Rousset, M., I. Chantret, G. Trugnan, and A. Zweibaum, manuscript submitted for publication). This observation could explain why only 50–60% of the differentiated cells express sucrase-isomaltase on their apical membrane. This restricted expression is not related to a heterogeneity of the cell line, as it has also been observed in a cloned population of differentiated HT-29 cells developed in D. Louvard’s laboratory (HT-29-18; cited in reference 50). (b) Also unexpected is the decrease in the percentage of the complex form after 24 and 48 h of chase observed in two separate experiments. Whether this observation, which is so far unexplained, is particular to cultured cells has to be further documented.

Discussion

The results reported here show that the posttranslational processing, transport, and integration into the plasma membrane of sucrase-isomaltase depend on the differentiation state of the cells. In differentiated cells, the biosynthesis and posttranslational processing of the enzyme resemble that reported in pig small intestinal explants (6, 7) or in Caco-2 cells (16). In undifferentiated cells, in contrast, the enzyme is not transported to the membrane. This absence of membrane expression is not the result of an absence of biosynthesis, but rather that of an abnormal posttranslational processing of the enzyme characterized by (a) an impairment of the conversion of the high-mannose to the complex form of the enzyme; (b) an abnormal glycosylation of the complex form as suggested by its lower electrophoretic mobility; and (c) a rapid degradation of both the high-mannose and the complex form.

The same culture conditions (i.e., the presence or absence of glucose) that result in these variations of the posttransla-
tional processing of sucrase–isomaltase have also been shown to result in quantitative modifications of intracellular carbohydrates (30, 31, 47, 51) and changes in the energy metabolism of the cells, with undifferentiated cells grown on glucose exhibiting a high degree of glycolysis (9, 35, 51); differentiated cells grown in the absence of glucose, on the other hand, develop a neoglucogenic pathway (10). To determine how and to what extent these glucose-related metabolic modifications interfere with the posttranslational processing of sucrase–isomaltase in HT-29 cells we need to perform further experiments. These experiments should at least answer the following questions, all focused on the abnormal expression of sucrase–isomaltase in undifferentiated HT-29 cells.

(a) Are the appropriate transport pathways from the Golgi to the brush-border microvilli defective in these cells? Indeed it has been shown that glucose inhibits both the polarization of HT-29 cells and the formation of brush border microvilli (32, 50). More recently, it has been demonstrated that the level of expression and the intracellular distribution of villin strongly depend in HT-29 cells on the glucose content of the culture medium (24, 34). That the intracellular transport pathways of proteins to the plasma membrane is disorganized in these cells is consistent with these observed modifications of the cell morphology and cytoskeleton organization.

(b) Is the instability of sucrase–isomaltase the result of a glucose-dependent general increase of protein catabolism? Experiments from several laboratories (1–3, 17) have pointed out the close relationship between energy metabolism and intracellular protein breakdown. More particularly, it has been demonstrated in different systems that glucose deprivation prevents the lysosomal protein breakdown (17, 40). Furthermore, in the course of long-term pulse-chase experiments, we have observed that the overall protein-associated [1-35S]methionine radioactivity decreases much more rapidly in undifferentiated than in differentiated HT-29 cells reflecting most likely a high rate of protein breakdown (unpublished results).

(c) Is the abnormal glycosylation of the enzyme the consequence of a more general glucose-related alteration of protein glycosylation? It is well known that glucose is involved in the regulation of the processing of glycoproteins (8, 11, 14, 23, 37). In the particular case of HT-29 cells, previous studies have shown that compounds that play an important role in protein glycosylation (namely, UDP-N-acetylhexosamines [20, 38]) accumulate only in undifferentiated cells (47). Other studies have confirmed that several glycosylation steps are impaired in undifferentiated cells (Ogier-Denis, E., P. Codogno, I. Chantret, and G. Trugnan, manuscript in preparation).

(d) Is the protein structure of sucrase–isomaltase identical or not in differentiated and undifferentiated cells? Indeed, both the instability and the impaired glycosylation of the enzyme in undifferentiated cells could be explained by an abnormal protein structure. The recent isolation of cDNA clones coding for human (13) and for rabbit (Green, F., Y. Edwards, H. P. Hauri, S. Povey, M. W. Ho, M. Pinto, and D. Swallow, manuscript submitted for publication) intestinal sucrase–isomaltase will provide tools that should help to answer this question.

At present it is not possible to delineate the respective role of these mechanisms and their interactions in the regulation of the expression of differentiated functions in HT-29 cells. Moreover, it cannot be excluded that glucose may interfere differently and at different levels with the expression of different proteins. This is suggested by the results obtained with villin that have shown that, unlike sucrase–isomaltase, the protein, although expressed at a lower level in undifferentiated than in differentiated HT-29 cells (34), is equally stable in both conditions (24). In this case, the differentiation-related variations of the expression of villin have been shown to correlate with variations of the levels of the corresponding mRNAs (33).

As complex as the problem may be, the results reported here confirm that HT-29 cells can be used for studying the regulation of the cellular expression of sucrase–isomaltase. Furthermore, these cells also provide a relevant tool for studying, in long-term experiments, the regulation of the biosynthesis and degradation of endogeneous glycosylated proteins.

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