Differentiation of HT-29 Human Colonic Adenocarcinoma Cells Correlates with Increased Expression of Mitochondrial RNA: Effects of Trehalose on Cell Growth and Maturation

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ABSTRACT

The HT-29 human adenocarcinoma cell line has been used extensively in the study of colonic cell differentiation and colon cancer. We report here that substitution of glucose with trehalose (α-D-glucopyranosyl-α-D-glucopyranoside) depresses growth and promotes mucin-producing, goblet-like maturation of HT-29. An initial characterization of this process was made by analyzing several cDNA clones whose RNA templates were differentially expressed at elevated levels in cells grown in trehalose-containing medium. Seven of the 9 clones examined corresponded to 6 mitochondrial genes whose expression levels, relative to those from glucose-grown cells, ranged from approximately 3-fold for 16S rRNA to 8–23-fold for NADH dehydrogenase subunit 4. On the other hand, levels of mitochondrial DNA copy, measured by using NADH dehydrogenase subunit 4 cDNA as probe, were shown to be unaffected by trehalose treatment. Elevation of cellular NADH dehydrogenase subunit 4 mRNA in HT-29 cultures grown in medium containing different components (sodium butyrate, galactose, no-sugar, glucose, cellobiose) generally correlated with depressed growth levels and specifically with increased numbers of mucin-producing cells present. Like butyrate, the sugar, trehalose, is an effective inducer of HT-29 differentiation, and may prove useful as a dietary therapeutic, and as a probe for elucidating mitochondrial involvement in colonic cell differentiation and transformation.

INTRODUCTION

Among the several adenocarcinoma cell lines derived from human colonic mucosa, HT-29 is one of the best described (1, 2). As a poorly differentiated, multipotent tumor cell type, HT-29 can be induced to mature into both enterocyte-like goblet and absorptive cell phenotypes by various substances such as sodium butyrate (3), suramin (4), 12-O-tetradecanoylphorbol-13-acetate (5), and galactose (6). Little is known about how these “inducers” work, and in particular how exogenous glucose levels (7, 8) directly modulate HT-29 cell growth and differentiation.

We are interested in the molecular genetic mechanisms that both trigger and facilitate carbohydrate-dependent differentiation and dedifferentiation responses in HT-29 cells for the purpose of obtaining a better understanding of how normal and transformed enterocytes evolve. Early investigations have shown that several sugars can substitute for glucose in supporting growth of normal and neoplastic cells (9). As a follow-up to this work, and related studies using insect cells in culture (10), we were interested in the effects that disaccharides such as trehalose might have on gene expression and regulation of colonic cells in general, and in their potential to mediate HT-29 differentiation and to attenuate the colonic tumor cell phenotype. Trehalose (α-D-glucopyranosyl-α-D-glucopyranoside) is a relatively nonlabile glucose derivative that occurs naturally, often in high abundance, in many lower organisms, plants, and insects, in which it has been suggested to play various roles in osmoregularity, regulation of sugar transport and metabolism, and thermotolerance (10–14). The functions of this sugar and its hydrolase, trehalase (EC 3.2.1.28) in mammals are not well understood; they may be involved in active transport of glucose and carbohydrate metabolism (14). Mammalian trehalase is associated with brush-border membranes of the small intestine and kidney (15); it is expressed at low levels in LLC-PK1 (pig kidney) epithelial cells after glucose limitation (16), and in HT-29 in the presence of glucose (17).

In this report, we analyzed cell growth, differentiation, and expression of some of the most abundant transcripts present in HT-29 cells treated with trehalose. Among cloned cDNAs detected by differential hybridization using cDNA probes made from total RNA of cells grown in medium containing either glucose or trehalose, the majority were found to be encoded by mtDNA. A comparison of mtRNA expression in HT-29 cells exposed to butyrate, galactose, no-sugar, and cellobiose further indicated that enhanced mt gene expression is linked generally to cultures exhibiting depressed levels of growth and increased numbers of differentiated cells. These observations extend the expression studies of cytochrome c oxidase in HT-29 (18, 19) using small chain fatty acids, and underscore the importance of understanding the differences in mitochondrial structure and expression so far observed for normal and abnormal colonic tissues (18–23).

MATERIALS AND METHODS

Cell Culture and Treatment. HT-29 cells (American Type Culture Collection 8616, cell passage 128) were initially grown in McCoy's 5A medium, and maintained thereafter in this laboratory on glucose-free Dulbecco's modified Eagle's medium (Gibco BRL; Grand Island Biological Co., Grand Island, NY) supplemented with 25 mM glucose, 10% fetal bovine serum (v/v), and 40 μg/ml gentamicin at 37°C and 8.0% CO₂. Cells (passage 130) were subsequently adapted to Dulbecco's modified Eagle's medium containing one of the following: 25 mM cellobiose (β-1,4-glucosyl-D-glucose; Sigma Chemical Co., St. Louis, MO), 5 mM galactose, 25 mM glucose, 2 mM sodium butyrate, or 25 mM trehalose (α-D-glucopyranosyl-α-D-glucopyranoside; Sigma). Since HT-29 rapidly consumes glucose, glucose-containing medium was replaced daily to minimize spontaneous differentiation. Medium in all other culture regimes was replaced every 3 days. For the nutrient shift experiments, approximately 10⁶ cells grown in glucose-containing medium were incubated subsequently for different time intervals in medium containing either trehalose or trehalose for 6 days and then shifted to glucose. Cells were harvested following trypsin-EDTA treatment by pelleting in a clinical centrifuge and frozen at −75°C until use. A Neubauer hemocytometer was used to count cells. Monolayers of cells, fixed in buffered 10% formalin, were stained for acid mucopolysaccharide.

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3The abbreviations used are: mt, mitochondrial; pfu, plaque-forming units; SSC, standard saline-citrate; SDS, sodium dodecyl sulfate; ND4, NADH dehydrogenase subunit 4.

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rides (mucin) according to the method of Thompson (24), using 1% alcian blue-3% acetic acid, pH 2.5 (30 min), followed by counterstaining in 0.1% nuclear fast red solution (10 min) and mounting in Hydramount (Gurr's, United Kingdom) for photography with a Reichert Zetopan/Nikon UFX assembly.

Construction and Screening of cDNA Libraries. cDNA was prepared according to the method of Gubler and Hoffman (25) with modifications (26). First-strand synthesis was carried out in a reaction volume of 50 µl containing 70 µg of RNA, 5 µg of oligo-dT, and 3 units of reverse transcriptase. cDNA was methylated with 100 units of EcoRI methylase and ligated to phosphorylated EcoRI linker (5'-CCCGAATTCCGGG-3'; Pharmacia) at a molar ratio of 100:1. After excessive digestion with EcoRI, the cDNA was passed through a 1-ml Bio-Gel A-50 m (Bio-Rad, Ontario, Canada) column to remove extraneous EcoRI linker. The fractions containing the cDNA were ligated with EcoRI digested X-galO and packaged into phage according to commercial protocols (Stratagene, La Jolla, CA; see also Ref. 27). For differential screening of 2 cDNA libraries (2 x 10^6 pfu), double stranded cDNA (80 to 100 ng) was prepared as described above and labeled using random hexamers in a reaction containing 100 µCi of [a-32P] dATP to yield approximately 2 x 10^6 cpm (28). Use of this method was necessary since 32P-labeling of first strand cDNA during synthesis led to poor hybridization to specific probes.

Table 1. Characterization of HT-29 cells grown in different carbon sources

<table>
<thead>
<tr>
<th>Carbon source</th>
<th>% Mucin-containing cells</th>
<th>Total cell no. x 10^6</th>
</tr>
</thead>
<tbody>
<tr>
<td>Butyrate</td>
<td>10.0</td>
<td>0.3-0.4</td>
</tr>
<tr>
<td>Cellohiose</td>
<td>1.7</td>
<td>0.3-0.4</td>
</tr>
<tr>
<td>Galactose</td>
<td>5.2</td>
<td>0.3-0.4</td>
</tr>
<tr>
<td>Glucose</td>
<td>3.1</td>
<td>1.0-2.0</td>
</tr>
<tr>
<td>No sugar</td>
<td>4.2</td>
<td>0.3-0.4</td>
</tr>
<tr>
<td>Trehalose</td>
<td>6.6</td>
<td>0.3-0.4</td>
</tr>
</tbody>
</table>

* Culture conditions are described in "Materials and Methods."

† Based on 10^7 cells counted from 8 and 14 days of culture stained with alcian blue dye (see "Materials and Methods").

‡ Cells counted after 8 and 14 days of culture.
Fig. 2. Analysis of cloned cDNA inserts by use of polymerase chain reaction and differential hybridization. A, ethidium bromide-stained agarose gel showing lanes with 75-200 ng of insert from clones DP1.2 (Lane a), DP2.2 (Lane b), DP4.2 (Lane c), DP7.2 (Lane e), DP6.2 (Lane f), and DP11.2 (Lane h) amplified by polymerase chain reaction using Taq polymerase (Gibco BRL) as described initially by absorbance measurement at 260 nm and by the amount of ethidium bromide staining of 185 and 285 rRNA per gel lane. Relative RNA concentration was further determined by using DNA probes (see Table 2) for the abundant RNA species, elongation factor EF1a and 28S rRNA. The murine EF1a cDNA probe has a homology of 92% at the nucleic acid level to the human sequence (32). Total RNA (12 μg) for each sample was dissolved in 20 nL of 44% formamide, 20 mM 3-(N-morpholino)propanesulfonic acid, 5 mM sodium acetate, 1 mM EDTA, 6% formaldehyde, 0.25% bromophenol blue, 0.25% xylene cyanol FF, pH 7.0, and heated 10 min at 70°C. The 1% agarose gel was dialyzed against TE buffer for 3 h, and SDS was added (1% final) before extraction (once with phenol and once with chloroform/isoamyl alcohol, 24:1) and precipitation with ethanol. For Southern transfers, DNA was digested with EcoRI and electrophoresed in agarose gels (1%) with TBE buffer for 18 h at 2 V/cm before capillary blotting (Nytran membrane; Schleicher & Schuell, Dassel, Germany) in 25 mM sodium phosphate buffer, pH 7.2, cross-linking by exposure to short UV and vacuum-drying at 80°C. Recombinant λ-gt10 DNA was isolated using a miniscule protocol (30). Sizes of inserts from the λ-gt10 vector were measured following EcoRI digestion and verified by amplification (polymerase chain reaction) using commercially available flanking primers and Taq polymerase (Gibco BRL) as described elsewhere (30). Inserts were subcloned into a plasmid vector for sequencing. DNA sequencing was carried out (30) with Sequenase (USB Corp.) using the manufacturer’s protocol. Nucleotide sequences of individual clones were compared with those in the EMBL/GenBank data base by the FASTA method in the GCG package (Version 6) from the Wisconsin University (31).

RNA Analysis. Total RNA was extracted from HT-29 cells (approximately 10^8 cells/sample) by addition of 1.5 ml of 4 M guanidium isothiocyanate containing buffer and purified by CsCl centrifugation (30). Purified RNA was dissolved in H2O and extracted once with phenol. RNA concentration for comparative analysis was estimated initially by absorbance measurement at 260 nm and by the amount of ethidium bromide staining of 18S and 28S rRNA per gel lane. Relative RNA concentration was further determined by using DNA probes (see Table 2) for the abundant RNA species, elongation factor EF1a and 28S rRNA. The murine EF1a cDNA probe has a homology of 92% at the nucleic acid level to the human sequence (32). Total RNA (12 μg) for each sample was dissolved in 20 μl of 44% formamide, 20 mM 3-(N-morpholino)propanesulfonic acid, 5 mM sodium acetate, 1 mM EDTA, 6% formaldehyde, 0.25% bromophenol blue, 0.25% xylene cyanol FF, pH 7.0, and heated 10 min at 70°C. The 1% agarose gel containing running buffer [20 mM 3-(N-morpholino)propanesulfonic acid, 5 mM sodium acetate, and 1 mM EDTA, pH 7.0] and 2% formaldehyde was developed for 18 h at 2 V/cm. RNA was transferred to Nytran membranes in 40 mM Tris-acetate, 1 mM EDTA at 4°C for 1 h at 15 V, followed by 3 h at 45 V using an electrotransfer unit (Hoeffer Scientific Instrument, San Francisco, CA).

Prehybridizations (2 h) were done in 6X SSC, 5X Denhardt’s solution, 0.5% SDS, and 100 μg/ml denatured salmon sperm DNA (29). Hybridizations were carried out for 18 h in 6X SSC, 0.5% SDS, and 100 μg/ml denatured salmon sperm DNA containing 5 x 10^4-5 x 10^5 cpm/μg probe. Hybridized membranes were washed for 15 min at 23°C using 2X SSC, 0.2% SDS, and twice for 30 min at 65°C in 0.2X SSC, 0.2% SDS. Filters were exposed to Kodak X-Omat films. Hybridized blots were reused for control experiments by washing each blot for 30 min at 95°C in 0.2X SSC, 0.2% SDS.

Scanning and Quantitation. Autoradiographs of hybridizations were analyzed using a computing scanning densitometer (Model 300A; Molecular Dynamics, CA) with software (ImageQuant version 3.0). Intensities of hybridization signals were compared using the “volume integration” option of the software program.
Table 2 Characterization of cDNA clones and corresponding transcripts from trehalose-grown HT-29 cells

<table>
<thead>
<tr>
<th>cDNA clone</th>
<th>cDNA size (kilobase pairs)</th>
<th>RNA size (kilobase pairs)</th>
<th>Relative RNA concentration</th>
<th>Gene designation (EMBL/GenBank)</th>
</tr>
</thead>
<tbody>
<tr>
<td>DP2.2</td>
<td>1.6</td>
<td>1.6</td>
<td>3.0</td>
<td>ATPase 6, ATPase 8, cytochrome oxidase II</td>
</tr>
<tr>
<td>DP4.2</td>
<td>1.2</td>
<td>1.2</td>
<td>7.0</td>
<td>Nuclear, unknown</td>
</tr>
<tr>
<td>DP5.2</td>
<td>1.4 + 1.6</td>
<td>3.0</td>
<td>3.6</td>
<td>NADH dehydrogenase 4/4L</td>
</tr>
<tr>
<td>DP6.2</td>
<td>1.5</td>
<td>1.6</td>
<td>8-23</td>
<td>NADH dehydrogenase 4/4L</td>
</tr>
<tr>
<td>DP7.2</td>
<td>1.2</td>
<td>1.6</td>
<td>8-23</td>
<td>Nuclear, unknown</td>
</tr>
<tr>
<td>DP8.2</td>
<td>2.4</td>
<td>&gt;10</td>
<td>3.1</td>
<td>NADH dehydrogenase 4/4L</td>
</tr>
<tr>
<td>DP9.2</td>
<td>1.2</td>
<td>1.8</td>
<td>8-23</td>
<td>ND4, NADH dehydrogenase 4/4L</td>
</tr>
<tr>
<td>DP10.2</td>
<td>0.2</td>
<td>1.6</td>
<td>7.0</td>
<td>165S rRNA</td>
</tr>
<tr>
<td>DP11.2</td>
<td>1.2</td>
<td>1.4</td>
<td>7.0</td>
<td>Cytochrome b</td>
</tr>
<tr>
<td>DP12</td>
<td>1.7</td>
<td>1.7</td>
<td>1.20</td>
<td>EF1α</td>
</tr>
<tr>
<td>PCD29</td>
<td>1.7</td>
<td>1.7</td>
<td>1.10</td>
<td>EF1α (murine)</td>
</tr>
<tr>
<td>PCD22</td>
<td>1.1</td>
<td>5.1</td>
<td>1.15</td>
<td>28S rRNA (murine)</td>
</tr>
</tbody>
</table>

- Determined by agarose gel electrophoresis after digestion of cloned DNA with EcoRI.
- Determined from hybridization analysis using RNA from HT-29 cells grown in glucose or trehalose for 6 days.
- Quantitation of hybridizations to RNA from trehalose-grown cells (relative to glucose) using computing densitometer (see "Materials and Methods" and Fig. 4).

RESULTS

Effect of Trehalose on Cell Growth. When glucose in Dulbecco's medium was replaced by trehalose, differences in growth rate and morphology of HT-29 were observed. As shown in Fig. 1A, cell density after 7 days in medium containing trehalose was approximately 4- to 5-fold lower than that observed for cells grown in glucose for the same period of time. At the microscopic level, cells grown in glucose-containing medium were seen to form densely packed, disorganized multilayers of undifferentiated cells, while cells in trehalose-containing medium formed uniform monolayers that were often polarized, polygonal in shape, and delimited by large extracellular spaces (Fig. 1, B and C). Staining of both types of cell population with alcian blue to detect mucin-producing cells (Fig. 1, D and E) revealed that approximately 10-fold more goblet-like enterocytes were produced with trehalose than with glucose in the medium. Cells shifted back from 6 days of treatment with trehalose to glucose resulted in recovery of the glucose-culture cell phenotype after several days of incubation. Similar assays carried out using 8- and 14-day cultures of HT-29 adapted to alternate carbon sources (Table 1) indicated that in comparison to cells grown in trehalose, cultures in butyrate produced more mucin-producing cells, and cultures in galactose, no-sugar, glucose, and cellobiose (relative order) produced less. However, except for glucose, total cell production was about the same using any of the other treatment regimes.

Selection of Differentially Expressed Genes. Considering the morphological and histochemical changes noted for HT-29 after shifting from glucose to trehalose-containing medium, we expected differences in gene expression to occur. As a start in the characterization of these genetic events, we constructed 2 recombinant λ-gt10 cDNA libraries from total RNA of trehalose-grown cells and screened them for cDNA clones that encoded RNA sequences whose abundance changed the most during HT-29 cell adaptation to trehalose (see "Materials and Methods" for details). From an initial screening of approximately 6 x 10⁴ clones (pfu) with radiolabeled cDNA made from RNA of cells cultured with either glucose or trehalose, we...
obtained 9 candidate clones. The selectivity of this hybridization screening procedure is illustrated in Fig. 2, A-C (Lanes b, c, e, f, and h) using the in vitro amplified (polymerase chain reaction) cDNA inserts from some of these clones. Hybridization of these cDNA inserts and HT-29 mt DNA (Fig. 2, A-C, Lanes j and k) was about 10-fold higher with \([\text{P}^3]cDNA\) made from RNA of trehalose-grown cells (Fig. 2C) than with \([\text{P}^3]cDNA\) made from RNA of glucose-grown cells (Fig. 2B). This was in contrast to results obtained for cDNA of clone DP1.2 (Fig. 2B, Lane a), which was initially selected on the basis of equivalent hybridization to \([\text{P}^3]cDNA\) probe made from either source of RNA. This cDNA contains sequence coding for elongation factor EF1\(\alpha\), a highly expressed gene that is homologous to the mouse EF1\(\alpha\) sequence also used as a standard in this experiment (Fig. 2B, Lane i) and in subsequent analyses (see Figs. 4 and 5).

To reveal the identity of the 9 cDNA clones, cross-hybridization of the cDNA inserts and partial nucleotide sequencing were carried out. These results are summarized in Table 2 and Fig. 3. The cDNA of DP5.2 and DP6.2, which are unrelated to any of the other clones, also did not show any significant sequence analogues in the EMBL/GenBank database. However, sequences of the remaining 7 clones indicated them to be derived from mt DNA-encoded transcripts. The homologies shown in Fig. 3 between HeLa mt DNA sequence (33) and the cDNA sequences were: 99.6% in 227 base pairs of DP2.2 with 16S rRNA; 99.5% in 199 base pairs of DP4.2 with DNA spanning the coding region for ATPase 6, ATPase 8, and cytochrome oxidase II (referred to here as ATP6/ATP8/COII); 100% in 113 base pairs of DP6.2 with DNA of NADH dehydrogenase subunit 4/4L (referred to as ND4); 95.2% in 188 base pairs of DP11.2 with cytochrome b (referred to here as Cyto b).

Inserts from all 4 clones were sequenced from both ends, and sequence integrity of these clones was further checked by restriction site mapping using enzymes HincII, Kpnl, and SacII for DP2.2 (16S rRNA); AccI, Apal, and XbaI for DP4.2 (ATP6/ATP8/COII); HindIII and SpeI for DP6.2 (ND4); and AccI for DP11.2 (Cyto b). All clones gave the expected diagnostic restriction cuts (data not shown). DP4.2 (ATP6/ATP8/COII) is unusual and appears to have been generated from an unprocessed, polycistronic RNA. The one nucleotide mismatch noted in ATP6/ATP8/COII (A to G at nucleotide position 8, 860) would result in an amino acid change from threonine (polar uncharged) to an alanine (nonpolar). A significant sequence mismatch of 8 nucleotides was found in Cyto b at nucleotide positions 15,545 to 15,567, resulting in a change in sequence from HIRAWC to HIKPEWY (single letter amino acid code). Since the Cyto b was sequenced from the noncoding strand, the mismatches were not the beginning of the analyzed sequence and therefore not a result of incorrect reading.

Quantitation of Elevated Transcript Levels. Data in Fig. 4, A-D, show that cDNA of clones DP2.2, DP4.2, DP6.2, and DP11.2 all hybridize to RNA, whose expression levels were highest in cells grown in trehalose rather than in glucose. This confirms the results obtained in Fig. 2 and indicates that specific cDNA synthesis and abundance generally reflected template RNA copy levels and not RNA template preference. Equality in concentration of total RNA in samples (see “Materials and Methods”) was confirmed on the Northern blots using cDNA probes for 28S rRNA (data not shown) and EF1\(\alpha\) (Fig. 4, E-H). This is also supported by data presented in Fig. 2 using both murine and HT-29 EF1\(\alpha\) cDNA as standards.

Since the cDNA sequences were identified, most of the RNA sizes revealed by Northern analysis in Fig. 4 could be interpreted. For example, the 2 major bands in Fig. 4B are consistent with their being ATPase 6/ATPase 8 and cytochrome c oxidase II mRNA (33, 34), and the single bands in Fig. 4, C and D, are consistent with their being NADH dehydrogenase subunit 4 and cytochrome b, respectively. However, the faint 2.4-kilobase band near the 18S rRNA in Fig. 4B could not be identified.

The intensities of the hybridizations to the different RNA species were further quantified using a computing densitometer. While all mt RNAs showed an elevated expression, the magnitude of their increase varied from 3-fold for 16S rRNA to 8- to 23-fold for ND4 (Table 2). The elevation of transcript for each mt gene is considered real since the same Northern blots when stripped and hybridized to probes for commonly expressed RNA species (28S rRNA and EF1\(\alpha\)) gave comparable results (±1.20).

Mitochondrial DNA and ND4 RNA Concentration. To distinguish whether the observed increases in mt RNA were due to an increase in stable transcription or an increase in mt DNA copy during induced cell differentiation, RNA and DNA were isolated from the HT-29 cells, 1 and 3 h, and 1, 2, 4, and 6
MITOCHONDRIA! GENE EXPRESSION AND COLON CANCER CELL DIFFERENTIATION

Fig. 5. Expression of ND4 and mitochondria DNA copy. Top insert, autoradiogram of Northern blot of total RNA hybridized with ND4 cDNA. RNA (approximately 12 µg/lane) was isolated from HT-29 cells cultured for 0, 1, and 3 h, and 1, 2, 4, and 6 days in 25 mM trehalose. Bottom insert, corresponding Southern blot of DNA hybridized to ND4 cDNA. The DNA (10 µg/lane) was isolated from the same cells as the RNA described above, and cut with EcoRI. Dotted bars, quantitation of hybridization signals of HT-29 RNA to ND4 cDNA; solid bars, quantitation of HT-29 DNA hybridized to ND4 cDNA; hatched bars, quantitation of HT-29 DNA hybridized to EF1α cDNA. Numerical values correspond to the integrated volume (see "Materials and Methods").

Fig. 6. Changes in level of ND4 mRNA with cell culture conditions. Left to right, HT-29 cells cultured for 6 days in trehalose, 6 days after transfer from trehalose to glucose, and 6 days in glucose. Insert, autoradiogram of corresponding Northern blot of RNA (12 µg/lane) from cultured cells. Hybridization to ND4 cDNA probe was quantitated as described in Fig. 5 and "Materials and Methods." days after transferring from glucose to trehalose. To monitor mt DNA copy, total DNA was digested with EcoRI to generate the 6.5-kilobase mt DNA fragment containing ND4 (see Fig. 3, map). For quantitation, both Southern and Northern blots were hybridized to the ND4 cDNA probe. Fig. 5 shows that while the ND4-related mt DNA content remained constant, an increase in the amount of ND4 mRNA could be observed as early as a few hours after shift from glucose to trehalose. mt DNA content in total DNA isolated from cells at 0 and 6 days after transfer to trehalose were also the same when probed with total mt DNA.

The quantitation presented in Fig. 5 further shows that maximal relative increases in ND4 mRNA took place during the first 2 to 4 days, after which time levels remained constant. Confirmation of the elevated expression level of ND4 in cells exposed to trehalose was established by comparing specific RNA and DNA levels of cultured cells maintained in medium containing either glucose or trehalose with levels in cells transferred from trehalose back to glucose. As shown in Fig. 6, the expression of the ND4 RNA remained high as long as HT-29 cells were maintained in trehalose medium.

ND4 Expression in Cells Exposed to Other Sugars or Butyrate. We used the ND4 cDNA as a probe to measure ND4 RNA levels in RNA isolated from HT-29 cells cultured in medium containing different carbon regimes as described in Table 1. The hybridization analysis summarized in Fig. 7 indicates that except for cellobiose, expression levels of cellular ND4 RNA correlated approximately with the amount of cell differentiation that had taken place as estimated by measurement of mucin-producing cells (Table 1).

DISCUSSION

When glucose was replaced by trehalose in the culture medium, HT-29 cells were found to undergo phenotypic changes that are more similar to those observed using either sodium butyrate or galactose than to those using no-sugar or glucose. Among the genes identified so far whose expression changed the most, 7 were mt DNA encoded and 2 others were nuclear DNA encoded but unrelated to any gene sequence presently in the EMBL/GenBank databases. The corresponding transcript of one of these clones (DP8.2) is unusually large and is currently being analyzed. Use of internal standards such as EF1α and 28S rRNA for quantitation of mt RNA in total RNA extracted from cells further revealed probable differences in relative amounts of mt RNA ranging in magnitude from approximately 3-fold for 16S RNA to 8–23-fold for ND4. The level of ND4 RNA expression in total RNA from cells exposed to sodium butyrate, galactose, no-sugar, and cellobiose (order of diminished expression) correlates favorably with decreased cell growth and particularly with increased numbers of mucin-producing cells present. With refinement, levels of mt RNA such as ND4 may serve as a generic indicator of the state of
HT-29 differentiation. Because of the relatively large changes in ND4 (to COIII for example), the ND4 gene might also be useful as a probe in the assessment of colon biopsies.

The effects of trehalose on HT-29 production of mt RNA and goblet-like cells are not equivalent to those obtained by removal of glucose (6–8) or by substitution with other carbohydrates such as galactose or cellobiose, a β-1,4-linked dimer of glucose. Also, unlike butyrate (19), it appears that trehalose is not metabolized to any major extent by HT-29 cells, and unlike insect cells in culture that exhibit 4- to 8-fold increases in trehalase activity when exposed to trehalose (10), HT-29 cells in the presence of trehalose or glucose (17) express only low levels of trehalase activity. HT-29 cells are also in apparent contrast to LLC-PK₁, pig kidney epithelial cells that exhibit glucose-dependent changes in trehalase expression (16). It is possible that removing or substituting glucose with another sugar stimulates the cells to respire. However, in view of trehalose’s purported role in osmoregulation, thermotolerance, and carbohydrate metabolism in various organisms (10–14), trehalose may also affect HT-29 membrane permeability and general uptake of nutrients. It will be interesting to determine whether the changes in ND4 RNA levels after treatment with HT-29 cells with the various carbon regimes described here are due to a general stress response involving modulated expression of glucose/oxygen/heat shock-regulated proteins (35, 36).

The finding that expression levels of several mt genes, including COII and cytochrome c oxidase subunit II, are elevated when HT-29 cells are induced to differentiate by culturing with sugars other than glucose or no-sugar extends observations showing elevation of COI and COIII RNA in HT-29 cells cultured with small chain fatty acids (19), and the depression of COIII RNA in tissue biopsies of colon adenomas and carcinomas (18). At this time, levels of expression of ND4 RNA relative to COIII RNA have not been measured in HT-29 to determine which is the highest. However, the increased levels of expression of these RNAs in trehalose-induced (this study) and butyrate-induced (18) HT-29 cells are not accounted for by any demonstrable amplification or deletion in mt DNA that may have occurred during cell adaptation to these carbon sources. mt DNA levels from colonic tumors and polyps are also similar to levels in normal colonic tissue (22, 23, 37). The finding of elevated levels of RNA encoded by 4 mt genes, including subunits COI and COII, in biopsies from familial polyposis coli patients (22), would appear to be inconsistent with a similar analysis using COIII cDNA as probe (18). Age and origin of cell mass in such samples are likely important factors that will have to be qualified further (38). We have noted that trehalose does not promote elevated expression of mt genes in the monocyte line HL-60. Recent studies on noncolonic cells clearly demonstrate that neoplastic transformation of either rat hepatoma cells (39) or human fibroblasts (40) is associated with increased levels of mt RNA.

Differences in morphology, rhodamine-123 staining, and enzyme properties of mitochondria from human colon carcinoma cell lines have been noted previously (20, 21). At this time, we do not know whether the increases in the levels of RNA encoded by the different mt genes corresponds to similar increases in activity of the enzyme products. Treatment of HT-29 cells with small chain fatty acids enhances cytochrome c oxidase activity (19). It is uncertain whether the nucleotide sequence mismatches we detected between Hela mt genes (33) and the mt cDNA clones have any functional significance in HT-29. The cDNA clones we identified code for 6 mt genes that are distributed rather uniformly over the mt genome and are themselves products of polycistronic RNA corresponding to almost the total length of the mt genome (33, 34). The differences in specific mt RNA levels, which ranges from 3-fold to 23-fold, may be a result of different rates of turnover or processing. The unusually high level of ND4 transcript expression has no apparent relationship to location of ND4 coding region relative to the 2 major mt DNA promoters. Interestingly, Corral et al. (39) noted that during rat hepatocarcinogenesis 16S rRNA, ND1, ND6, COII, and Cyto b mt RNA levels were moderately increased by 2- to 3-fold over control levels and that NDS mt RNA was increased by about 10-fold. In this case, mt DNA heteroplasmy and/or mutations may explain the differences in RNA expression (39). Further elucidation of mt genome structure, and the molecular events involved in coordinating expression of nuclear and mt genes in HT-29 cells, would appear to be important for understanding the metabolic relationships between growth and differentiation phases of these cells.

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