Dipeptidyl Peptidase IV (CD 26) Gene Expression in Enterocyte-like Colon Cancer Cell Lines HT-29 and Caco-2

CLONING OF THE COMPLETE HUMAN CODING SEQUENCE AND CHANGES OF Dipeptidyl Peptidase IV mRNA LEVELS DURING CELL DIFFERENTIATION*

(Received for publication, August 23, 1991)

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A cDNA (DPCR1) specific for human intestinal dipeptidyl peptidase IV (DPP IV) has been isolated. This 1.7-kilobase cDNA, together with a previously published partial sequence, covers the entire open reading frame of human DPP IV plus 67 base pairs of the 3'-untranslated end. Human DPP IV is a 766-amino acid polypeptide with a high degree of homology with the rat liver protein. The characterization of this molecular probe allowed us to definitively confirm the identity of DPP IV with CD 26, a mouse thymocyte activation antigen, a conclusion strengthened by the fact that we observed identical patterns on Southern blot of human genomic DNA hybridized either with human DPP IV or mouse CD 26 cDNA probe. Using this new tool, we have investigated the expression of DPP IV during the onset of enterocytic differentiation of two cultured human colon cancer cell lines, HT-29 and Caco-2. Whatever the cell line and the culture conditions, DPP IV expression strictly correlates with the presence of a differentiated phenotype, as shown by enzyme activity and the steady state amount of the protein measured by indirect immunofluorescence and Western blot. Accordingly, DPP IV biosynthesis exclusively increases in cells that display an enterocytic differentiation. Neither the glycosylation nor the stability of the protein appear to be dependent on the state of enterocytic differentiation. The DPP IV mRNA level remains very low in undifferentiated cell populations and specifically increases in cells that undergo an enterocytic differentiation. These results strongly suggest that DPP IV gene expression is controlled at the transcriptional or posttranscriptional level during intestinal differentiation.

A particular feature of most of intestinal brush border-associated enzymes is the close relationship between their expression and the state of enterocytic differentiation. However, very little is known about the mechanisms whereby the level of hydrolase gene expression is controlled during enteroctytic differentiation. Conflicting results were reported for sucrase-isomaltase, aminopeptidase N, and lactase. Some results suggest that regulation occurs at the posttranslational level (1-3). In contrast, other results have demonstrated that the mRNA levels of sucrase-isomaltase and aminopeptidase N are very low in crypt as compared with villus cells of rat jejunum (4-6). Such discrepancies may be related to the fact that the expression of these hydrolases is not only differentiation-dependent but is also strongly controlled by nutritional and/or metabolic factors (2, 7, 8). To circumvent this difficulty, we chose to investigate the mechanisms involved in the differentiation-dependent expression of dipeptidyl peptidase IV (DPP IV), another brush border-associated hydrolase, for which no metabolic or nutritional modulation has been demonstrated (9).

Studies on the gene expression of intestinal hydrolases have been carried out in several biological systems, including isolated cells (10), intestinal explants (11, 12) and in vivo experiments (13). We and others have shown that some human colon cancer cell lines, namely LS174T, HT-29, Caco-2, HCT-EB, and HCT-Geo, may also be useful tools to study intestinal hydrolases, as these cell lines may express an enterocytic differentiated phenotype when grown in defined conditions (14-19). For example, the differentiation of Caco-2 and HT-29 cells is characterized by the presence of a well polarized monolayer endowed with a functional brush border membrane, as revealed by electron microscopy and enzyme activities (for a review see Ref. 20). In this paper, we focus on DPP IV gene expression in Caco-2 and HT-29 cells.

DPP IV is a serine protease associated with a variety of epithelial tissues, including kidney, liver, and intestine, through all stages of development from the fetus to the adult (21-27). This protein, which is not expressed in the human adult colon, has been shown to be reexpressed in some human colon cancers and in human colon cancer cell lines (18, 19, 28-30). Among the peptidase family, DPP IV is unique in that its cleavage site includes a proline or, less frequently, an

* This work was supported by institutional funding from the Institut National de la Santé et de la Recherche Médicale, by grants from the Association pour la Recherche sur le Cancer and BSN Co., and by a fellowship from the Fondation pour la Recherche Médicale (to D. D.). The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

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‡‡ The abbreviations used are: DPP IV, dipeptidyl peptidase IV; CD 26, cluster differentiation antigen 26; Ino, inosine; Gic+, glucose plus; Gic-, glucose minus; SDS, sodium dodecyl sulfate; PCR, polymerase chain reaction; kb, kilobase(s); bp, base pair(s).

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alanine in position 2 of the peptide NH\textsubscript{2} terminus (31). A role for DPP IV has been suggested in several biological processes, where X-Pro-containing peptides or proteins are involved. This includes cell-matrix interactions (32) and neurohormonal peptide inactivation (33). In the gut and in the kidney, where DPP IV is associated with the apical brush border (34), it has been suggested that this enzyme may be involved in the final steps of peptide absorption (35). More recently, several experiments strongly suggested that DPP IV and CD 26, a mouse thymocyte activation antigen, may be similar, since antibodies raised against DPP IV have been shown to recognize CD 26 and since CD 26 displays a typical DPP IV enzyme activity (36–40). However, until now, there was no molecular data to demonstrate this homology.

We describe here a 1.7-kb cDNA clone, DPCR1, that encodes most of the open reading frame of human intestinal DPP IV. This clone, together with DPP IV cDNA obtained from J. Fogh (Memorial Sloan Kettering Cancer Center, Rye, NY), covers the entire open reading frame of the protein. This allowed us to determine the complete amino acid sequence of human intestinal DPP IV and to definitively confirm its identity with CD 26. Our work provides human DPP IV-specific cDNA probes, making it feasible to study at the transcriptional level the expression of this particular protein during the onset of enterocytic differentiation of Caco-2 and HT-29 cells.

MATERIALS AND METHODS

Cell and Culture Conditions—HT-29 and Caco-2 cells were obtained from J. Fogh (Memorial Sloan Kettering Cancer Center, Rye, NY). HT-29 (HT-29 Glc–), passages 140–150, were maintained in Dulbecco’s modified Eagle’s essential medium (Eurobio, Les Ulis, France) supplemented with 10% heat-inactivated fetal calf serum (Boehringer Mannheim GmbH, Mannheim, Germany) and 100 IU/ml of penicillin and 100 pM of streptomycin at 37 °C. For experimental purposes, HT-29 cells were switched from this standard medium to either (i) the same medium devoid of glucose, supplemented with d-alanine [35] and d-alanine [36] fetal calf serum (Boehringer Mannheim GmbH, Mannheim, Germany), where DPP IV is associated with the apical brush border (34), where X-Pro-containing peptides or proteins are involved. Where DPP IV is associated with the apical brush border (34), where X-Pro-containing peptides or proteins are involved. Where DPP IV is associated with the apical brush border (34), where X-Pro-containing peptides or proteins are involved.

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To previously published techniques (29). Briefly, samples were fixed for 10 min at room temperature in 1% paraformaldehyde in Ca\textsuperscript{2+}- and Mg\textsuperscript{2+}-free phosphate-buffered saline. Alternatively, samples were fixed for 10 min in -20 °C methanol. These two fixation methods gave identical results. Three different antibodies specific for DPP IV were used: (i) A rabbit antiserum (L1650) raised against porcine DPP IV (Serva Fine Biochemicals, Heidelberg, Germany), which has been shown to precipitate DPP IV activity (47) and to give a quantitative and specific immunoprecipitation of rat intestinal DPP IV (47). (ii) A rat monoclonal antibody (4H3), provided by J. P. Gorvel (Centre de Biochimie et de Biologie Moléculaire, Marseille, France) was raised against immunoglobulins obtained from Institut Pasteur (Paris, France). Samples were mounted in glycerol solution containing 10% phosphate-buffered saline and 1% para-phenylendiamine.

Western Blot Analysis—Immunoblot analysis of DPP IV was performed as previously described (18). Briefly, 100 μg of protein from the indicated cells were separated on 7.5% SDS-polyacrylamide slab gels in the buffer system of Laemmli (50) and transferred to nitrocellulose sheets (Schleicher and Schuell, Dassel, Germany) by the method of Burnette (51). Rainbow protein molecular weight markers (Amersham International plc, Amersham, UK) were loaded on each gel. DPP IV was identified using L1650 or 4H3 antibodies. Antibody-antigen complexes were visualized using anti-rabbit or anti-rat immunoglobulins labeled with alkaline phosphatase (Promega Corp., Madison, WI).

Cell Labeling, Pulse-Chase Experiments, Immunoprecipitation, and Immunoperoxidase—Before 1\textsuperscript{125}I	extsuperscript{2}-Protein labeling, cells were preincubated for 2 x 10 min at 37 °C in a complete medium containing 10 mM unlabeled methionine (Institut J. Boy, Reims, France). Cells were then labeled for 3 h in the same methionine-free medium containing 250 μCi/ml of ~\textsuperscript{35}S	extsuperscript{2}-methionine (800 Ci/mmol; Amersham). At the end of the labeling period, 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 μM unlabeled methionine (pulse-chase experiments).

DPP IV was immunoprecipitated using one of the above-mentioned antibodies and a protein A-Sepharose assay for differential affinity purification (18, 28). All these antibodies gave similar results. Immunopurified DPP IV was analyzed on 7.5% polyacrylamide slab gels. For each gel, the same amount of cell protein was loaded on each lane in order to allow quantitative comparisons. \textsuperscript{35}S-Labeled protein standard molecular weight markers (Amersham) were also loaded on each gel. After electrophoresis, gels were impregnated with Amplify (Amersham) and analyzed by fluorography. Quantitation of DPP IV labeling was achieved by scanning the fluorograms using a densitometer (model Mark IIICS, Joyce, Loebel, and Co., Gatehead, United Kingdom). Control immunoprecipitations were done in the absence of antibody. In this case, no DPP IV band was identified.

Isolation and Characterization of a New Human DPP IV cDNA—Sequence analysis of the partial cDNA clone DPP 101 (41) that corresponds to the COOH-terminal region of human DPP IV (molecules 1833–2075 in Fig. 9) revealed a strong homology with rat DPP IV cdna (49). We hypothesized that this should also be true for the NH\textsubscript{2}-terminal coding sequence. Therefore, we synthesized an oligonucleotide starting at the initiator methionine codon of rat cDNA in order to isolate the remaining part of the coding sequence by the polymerase chain reaction (PCR) protocol (see below).

Total RNA was isolated from the human duodenal biopsy (Promega Corp., Madison, WI). We selected by oligo(dT)-cellulose chromatography, and 0.8 μg was used to synthesize single-stranded cDNA using Moloney murine leukemia virus reverse transcriptase (Boehringer Mannheim GmbH, Mannheim, Germany) and random hexanucleotide primers. After DNase treatment the RNA, one-tenth of the purified cDNA product was used in the PCR

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**Isolation and Characterization of a New Human DPP IV cDNA—** Sequence analysis of the partial cDNA clone DPP 101 (41) that corresponds to the COOH-terminal region of human DPP IV (molecules 1833–2075 in Fig. 9) revealed a strong homology with rat DPP IV cdna (49). We hypothesized that this should also be true for the NH\textsubscript{2}-terminal coding sequence. Therefore, we synthesized an oligonucleotide starting at the initiator methionine codon of rat cDNA in order to isolate the remaining part of the coding sequence by the polymerase chain reaction (PCR) protocol (see below).
reaction. In addition to the cDNA, the PCR reaction contained 50 
µM dNTP, Taq polymerase buffer, 50 pmoles of each primer, and 5 
units of Taq polymerase (Cetus Corp.) in a 50-µl final volume. The 
sequence of the 5′ primer (ATGAGACAGCTGGAGGGTTCTCT- 
CCTG) corresponds to nucleotides 1–24 in the published rat DPP IV 
sequence (41). This 5′ primer sequence (TAGATGTGCTTGAGACAGCTGGAGGGTTCTCTCCTG) 
was derived from that of DPl 101 (41) (see Fig. 9). After 10 min at 95 °C, Taq polymerase was added, and 
then the following program was used: (i) 95 °C, 10 s; 45 °C, 30 s; 
50 °C, 15 s; 60 °C, 15 s; 72 °C, 2 min (one cycle); (ii) 95 °C, 10 s; 
45 °C, 30 s; 50 °C, 15 s; 60 °C, 15 s; 72 °C, 5 min (10 cycles); (iii) 
95 °C, 10 s; 50 °C, 15 s; 72 °C, 5 min (30 cycles and terminated by a 
20-min period at 72 °C). Analysis of the amplified products on a 1% 
agarose gel revealed a clearly amplified fragment of the expected 
size (1700 bp) within a smear of larger and smaller products. The 1700-
bp fragment hybridized to a 32P-labeled rat DPP IV cDNA probe and 
was purified by electrophoresis through a 1% low melting point 
agarose gel. Following purification, the probe was inserted in the unique Smal site of PT1Z18R vector (Pharmacia-
LKB, Uppsala, Sweden). The complete sequence of the insert from 
one positive clone was established after subcloning of the SmaI-
 or Real-generated fragments (see Fig. 9) in the same vector by the 
dideoxynucleotide strategy, using the double-stranded plasmids as 
templates (53). It is well known that Taq polymerase may induce 
mutations, with a rate of between 2 × 10⁻⁴ and 2 × 10⁻⁵ (54). This 
means that in the present work, 0.35 mutation would have been 
introduced. Although this is a relatively low probability, we cannot 
totaIly rule out this possibility, and therefore, the reported sequence 
has to be considered as a tentative sequence. Sequence data were 
analyzed with the PC-Gene software package from IntelliGenetics 
and the nucleic acids and protein analysis program from CITI 2 (55). 

**RESULTS**

**Differentiation of Human Colon Carcinoma Cells in Cultu-
re—** In a first set of experiments, we defined the conditions 
in which cultured cells undergo an enterocyte differentiation. 
For Caco-2 cells, the differentiation process was simply fol-
lowed during the cell growth by comparing exponentially 
growing (undifferentiated) and confluent (differentiated) 
cells, since it is well established that the onset of Caco-2 cell 
differentiation is a growth-related event (16, 20). For HT-29 
cells, we used several distinct culture conditions, as described 
under “Materials and Methods.” In the two conditions where 
HT-29 cells were grown in a glucose-containing Dulbecco’s 
mixed Eagle’s essential medium (HT-29 Glc⁺ or HT-29 
Ino-Glc⁺), they remain essentially undifferentiated (15, 17, 
18). In contrast, in the two conditions where HT-29 cells were 
grown in a glucose-deprived medium (HT-29 Glc⁻ or HT-29 
Ino) they display an enterocytc-like differentiation. In the 
case of HT-29 Glc⁻, the emergence of a differentiated pheno-
type is probably due to a selection/adaptation process, as 
suggested by the fact that more than 90% of the cells die at 
the first passage in the glucose-deprived medium (42, 59). In 
contrast, HT-29 grown in a glucose-deprived inosine-supple-
mented medium does not follow this selection/adaptation 
pathway. This acquisition of a differentiated phenotype ap-
pers to be progressive, as the number of passages increases, 
with no cell mortality. This is assessed by the progressive 
increase of sucrase-isomaltase and DPP IV enzyme activities 
(Fig. 1) in both the whole cell homogenate and a brush border-
enriched fraction. Electron microscopy (Fig. 2) and immuno-
fluorescence studies (see below) confirm that HT-29 Ino cells 
are well differentiated.

**DPP IV Activity Depends on the State of Enterocytic Dif-
ferentiation of Caco-2 and HT-29 Cells—** The activity of DPP 
IV was measured as a function of the differentiation of Caco-

**FIG. 1. Sucrase-isomaltase (SI)- and dipeptidyl peptidase 
IV-specific enzyme activities as a function of cell passages in 
differentiated HT-29 cells.** Standard HT-29 cells (Glc⁺) were 
passed in glucose-free medium containing 2.5 mM inosine. Cells 
were then passaged each week in the same medium. At day 30 of each 
passage, cells were harvested and enzyme activities were measured 
on whole cell homogenate (H) and on brush border-enriched fraction 
(P2). Each point represents the mean ± S.D. of three independent 
experiments. Note the constant increase of the two enzyme activities 
that correspond to the emergence of a differentiated phenotype. The 
same experiments done on HT-29 Ino-Glc⁻ cells have shown that 
neither sucrase-isomaltase nor DPP IV enzyme activities increase as 
the number of passages increases (not shown). MU, millilitrons.
2 and HT-29 cells. As shown in Fig. 3A, DPP IV enzyme activity increases as the differentiation takes place in Caco-2 cells. The specific activity is approximately 10 times higher in differentiated Caco-2 cells (20 days) than in undifferentiated cells (5 days). Fig. 3B shows that in the two culture conditions where HT-29 cells do not differentiate, DPP IV enzyme activity remains low in confluent cells, whereas this activity increases 4–7 times in the two culture conditions in which HT-29 cells acquire a differentiated phenotype.

The Amount of DPP IV Protein Depends on the State of Enterocytic Differentiation of Caco-2 and HT-29 Cells—Whether the changes in DPP IV activities in Caco-2 and HT-29 cells are related to the steady state amount of protein was investigated by indirect immunofluorescence and Western blot analysis. Immunofluorescence studies were performed using one of the three antibodies described above. The three antibodies gave the same labeling pattern in all the conditions tested. Fig. 4 shows typical results obtained with undifferentiated and differentiated HT-29 and Caco-2 cells. Undifferentiated cells are either negative or display a low plasma membrane positivity. In contrast, differentiated cells show a strong fluorescence that appears to be apical. It should be pointed out that differentiated Caco-2 and HT-29 cells are not similarly labeled; 80–100% of Caco-2 cells are apically labeled, whereas only 50–70% of differentiated HT-29 cells display such an apical labeling. This is probably due to the heterogeneity of this cell line (59).

Results from Western blot analysis are shown in Fig. 5 and clearly confirm that DPP IV enzyme activity is directly related to the amount of DPP IV protein. The quantity of immunodetectable DPP IV increases in Caco-2 cells as the differentiation takes place, and accordingly, this quantity is higher in differentiated HT-29 Ino cells than in undifferentiated HT-29 Ino-Glc+ cells. In addition there is no change in the apparent molecular weight of DPP IV in undifferentiated as compared with differentiated cells, thus suggesting that the

![Western blot analysis of DPP IV in Caco-2 and HT-29 cells.](image)

Fig. 5. Western blot analysis of DPP IV in Caco-2 and HT-29 cells. One hundred micrograms of protein from whole cell homogenates of Caco-2 on day 5 (J5), 10 (J10), or 15 (J15) or HT-29 cells Ino or Ino-Glc+ (JG) on day 20 were loaded on 7.5% polyacrylamide slab gels. DPP IV was stained with 4H3, a rat monoclonal antibody, and anti-rat immunoglobulin coupled to alkaline phosphatase. Note the faint band in nondifferentiated cells (Caco-2 cells on day 5 and confluent HT-29 Ino-Glc+ cells). Similar results were obtained using L1650, the DPP IV-specific polyclonal antibody. Arrows indicate the migration of standard molecular weight markers.

![DPP IV biosynthesis in Caco-2 and HT-29 cells.](image)

Fig. 6. DPP IV biosynthesis in Caco-2 and HT-29 cells. Caco-2 (on day 5, 10, or 15) or HT-29 cells (Ino or Ino-Glc+, day 20) were labeled for 3 h with [35S]methionine. Labeled DPP IV was immunoprecipitated using HBB/3/775/42, a DPP IV-specific monoclonal antibody and analyzed on 7.5% polyacrylamide slab gels followed by fluorography. Panel A, DPP IV biosynthesis in Caco-2 cells (left panel) and densitometric analysis of the fluorogram (right panel). Note that DPP IV biosynthesis increases from day 5 to day 10 and then remains stable. Panel B, DPP IV biosynthesis in differentiated (HT-29 Ino) and undifferentiated (HT-29 Ino-Glc+) cells. Gels were overloaded in order to visualize the high mannose forms. Endo-β-N-acetylglucosaminidase H and F (Endo H and Endo F) digestions were incomplete. In the case of Endo H digestion in differentiated HT-29 cells, this produces several intermediate forms that probably correspond to different N-glycosylation sites.
FIG. 7. DPP IV pulse-chase experiments in Caco-2 cells. Caco-2 cells were labeled for 1 h with [35S]methionine and then chased for the indicated periods in the presence of excess unlabeled methionine. Immunoisolated DPP IV was analyzed on 7.5% polyacrylamide slab gels followed by fluorography. Since the amount of newly synthesized protein was 10 times lower in Caco-2 cells on day 5 than on day 10 or 15 (as in Fig. 6A), the amount of radioactivity loaded was adjusted in order to start with a comparable signal at the zero time of each chase experiment. The lower panel displays a densitometric analysis of the gels and shows that DPP IV's half-life is long (more than 48 h) and is comparable at each day.

FIG. 8. DPP IV pulse-chase experiment in HT-29 cells. HT-29 Ino (differentiated) or Ino-Glc+ (undifferentiated) cells were labeled on day 20 for 1 h with [35S]methionine and then chased for the indicated period in the presence of excess unlabeled methionine. Immunoisolated DPP IV was analyzed on 7.5% polyacrylamide slab gels followed by fluorography. The lower panel displays a densitometric analysis of the gels and shows that DPP IV's half-life is long (more than 48 h) whatever the cell population considered. Open square, differentiated cells; open circle, undifferentiated cells.

Observed differences in enzyme expression do not depend on posttranslational modifications. Since Western blot analysis only reflects a steady state level of protein, these results do not allow us to distinguish between a true increase of the rate of DPP IV biosynthesis and/or a decreased turnover rate of the enzyme protein in differentiated cells or an increased turnover in nondifferentiated cells.

The Rate of DPP IV Biosynthesis Depends on the State of Differentiation of Caco-2 and HT-29 Cells—Radiolabeling experiments were performed on undifferentiated and differentiated Caco-2 and HT-29 cells. Cells were pulse-labeled for 3 h using [35S]methionine. Radiolabeled DPP IV was immunoisolated and analyzed by SDS-polyacrylamide gel electrophoresis. As shown in Fig. 6A, the synthesis of DPP IV increases from day 5 to day 15 in Caco-2 cells, i.e., as the differentiation process takes place. These data correlate very well with the enzyme activities and Western blot results. Comparison of DPP IV biosynthesis in differentiated and undifferentiated HT-29 cells is shown in Fig. 6B. The biosynthesis is lower undifferentiated HT-29 Ino-Glc+ cells than in differentiated HT-29 Ino cells. Scanning of the fluorograms obtained from three independent experiments indicates that differentiated HT-29 Ino cells synthesize 4–5 times more DPP IV than undifferentiated HT-29 Ino-Glc+ cells (not shown). Similar experiments done with the other HT-29 cell populations indicate that whatever the culture conditions used, the biosynthesis of DPP IV is always higher in differentiated than in undifferentiated HT-29 cells (not shown). DPP IV immunopurified from both HT-29 cell populations was subjected to enzymatic digestion using endo-β-N-acetylglucosaminidases H and F. The former hydrolyzes only the high mannose chains of glycoproteins, whereas the latter is also active on complex type chains. As shown in Fig. 6B, there is no significant difference in DPP IV glycosylation pattern between differentiated and undifferentiated cells.

The Turnover Rate of DPP IV Is Independent of the Differentiation State of HT-29 and Caco-2 Cells—Pulse-chase experiments were performed on Caco-2 and HT-29 cells. Cells were pulse-labeled for 1 h and chased for 0–48 h. As shown in Fig. 7 there is no major change in the turnover rate of DPP IV, measured in exponentially growing or confluent Caco-2 cells. In these conditions, the protein appears to be stable over the 48-h chase period. Comparison of pulse-chase experiments carried out in differentiated and undifferentiated HT-29 cells leads to an identical conclusion, i.e., there is no detectable modification of the stability of neosynthesized DPP IV as a function of the differentiation state of HT-29 cells (Fig. 8). This very slow turnover rate associated with an increased biosynthesis contributes to the accumulation of DPP IV protein and enzymatic activity as the cells differentiate.
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FIG. 9. Tentative nucleotide sequence of the entire open reading frame and a portion of the 3'-untranslated region of human intestinal DPP IV. Panel A, complete nucleotide sequence of DPCR1 plus DPI 101. DPCR1 was obtained by PCR using two 27-mer oligonucleotides (underlined). The 5'-oligonucleotide was derived from the corresponding 5'-sequence of rat DPP IV cDNA (49) and the 3'-oligonucleotide was derived from the 5'-part of DPI 101 (41). DPCR1 stops exactly at the end of the 3' oligonucleotide (1655) and DPI 101 starts at nucleotide 1631. The stop codon TAG is also underlined. Note the presence of a 14-unit-long 3' poly(A) tail and of an ATTAAA sequence (starting at 2332) that may serve as a polyadenylation signal. Panel B, strategy for DPCR1 cloning and sequencing. Purified DPCR1 was inserted into the unique SmaI site of pTZ18R vector. DPCR1 restriction fragments were obtained by using either RsaI or PstI. These fragments were inserted into the same vector. Insert sequences were determined using the dideoxynucleotide strategy.

These results strongly suggest that DPP IV expression is directly controlled by its biosynthetic rate. This could be explained either by transcriptional, posttranscriptional, or translational mechanisms. To discriminate between these different possibilities we first needed to isolate and characterize a full-length human DPP IV cDNA. Therefore, we isolated a cDNA coding for the entire open reading frame (see below). Using this probe, we have performed Northern blot analysis on mRNAs from undifferentiated and differentiated HT-29 and Caco-2 cells.

Characterization of a cDNA Encoding the Whole Open Reading Frame of Human Intestinal DPP IV—We previously published the sequence of a partial cDNA clone, DPI 101, coding for the COOH-terminal part of human intestinal DPP IV (41). Using data from this sequence and from that of rat liver DPP IV (49), we have obtained, by the use of PCR, a new cDNA clone, DPCR1. DPCR1 and DPI 101 cover the entire open reading frame of human intestinal DPP IV as shown by sequence analysis (Fig. 9). Nucleotide sequence analysis allowed us to identify a 2298-bp-long open reading frame starting at nucleotide 1 with an ATG codon. Comparison of the size of this open reading frame (2300 bp) with the estimated size of DPP IV mRNA obtained by Northern blot analysis (4000 bp, see Fig. 11) indicates that the human DPP IV RNA has a 1700-nucleotide untranslated sequence.

Translation of the open reading frame predicts a primary translation product consisting of 766 amino acids (Fig. 10). Comparison of this sequence to the one obtained in the rat (49) reveals 86.4% identity, suggesting a relatively high degree of interspecies conservation. Additionally, we noted an 89% identity between human DPP IV and mouse thymocyte activation antigen, CD 26 (Fig. 10). This result confirms previous reports that have suggested that DPP IV and CD 26 are related proteins (38, 40, 60). In addition, the hybridization of human genomic DNA fragments with either the human DPP IV probe or the mouse CD 26 cDNA probes gives identical patterns (not shown), thus allowing us to conclude the identity of the two gene products.

We looked for the presence of consensus sequences for potential sites for N-glycosylation, phosphorylation, and sulfation. Results shown on Fig. 10 indicate that there are nine potential N-glycosylation sites, six potential sulfation sites.

and 28 potential phosphorylation sites (12 for protein kinase, 15 for casein kinase II, and one for tyrosine kinase). There is no "RGD" cell attachment sequence, although some authors have suggested that DPP IV may be involved in cell-matrix interactions (61). Finally, we do not find a typical serine protease active site consensus sequence, suggesting that DPP IV may be part of a new serine protease family.

**DPP IV mRNA Levels Increase in Differentiated Caco-2 and HT-29 Cells**—Northern blot analysis was performed on mRNA purified from Caco-2 cells on days 5, 10, and 15 of culture, from several undifferentiated and differentiated HT-29 cell populations, and from normal human jejunum. DPP IV mRNA appears as a single 4-kb mRNA in all conditions tested (Fig. 11). It should be noted that human jejunal DPP IV mRNA also migrates as a unique 4-kb band. The level of DPP IV mRNA increases in Caco-2 cells from day 5 (undifferentiated cells) to day 15 (fully differentiated cells). In undifferentiated HT-29 cells (HT-29 Glc), the DPP IV mRNA level is very low, whereas this level progressively appears as a function of the number of passages (this paper), or in HT-29 Glc−, where a selection/adaptation mechanism has been described (42, 59), In addition, it has been shown that DPP IV expression increases in all the differentiated state of two human colon cancer cell lines. We showed that DPP IV expression increases in all the differentiated cell populations analyzed. This is observed in Caco-2 cells where a spontaneous differentiation occurs (18), as well as in HT-29 Ino cells, where a differentiated phenotype progressively appears as a function of the number of passages (data not shown).

**DISCUSSION**

In the present work, we studied the different steps that could regulate the expression of DPP IV as a function of the differentiation state of two human colon cancer cell lines. We showed that DPP IV expression increases in all the differentiated cell populations analyzed. This is observed in Caco-2 cells where a spontaneous differentiation occurs (18), as well as in HT-29 Ino cells, where a differentiated phenotype progressively appears as a function of the number of passages (this paper), or in HT-29 Glc−, where a selection/adaptation mechanism has been described (42, 59). In addition, it has been shown that other human colon cancer cell lines that may also undergo an enterocytic differentiation frequently display an increased DPP IV enzyme activity (19). Therefore, it can be concluded that DPP IV expression in human colon cancer cell lines strictly correlates with the acquisition of a differentiated phenotype. In contrast, this phenomenon is independent of the colon cancer cell line used and is unrelated to the mechanisms underlying the emergence of a differentiated phenotype ("spontaneous" or simple adaptation or selection/adaptation). It should be noted that the level of DPP IV enzyme activity varies from one differentiated cell population to another (Fig. 3). Accordingly, immunofluorescence studies show that differentiated Caco-2 and HT-29 cells are not
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Fig. 11. Northern blot analysis of DPP IV mRNA in Caco-2 and HT-29 cells and in normal human jejunum. Panel A, messenger RNAs, prepared either from Caco-2 cells after 5, 10, or 15 days in culture or undifferentiated (Glc+ and Ino-Glc+ (IG)) and differentiated (Ino and Glc−) HT-29 cells or from a biopsy of normal adult human jejunum. Note that the sizes of DPP IV mRNA from Caco-2, HT-29, and the jejunum are identical. IG, Ino-Glc+; G+, Glc+; G−, Glc−.

Fig. 12. Southern blot analysis of the human DPP IV gene. Total DNA was extracted from differentiated (Ino) and undifferentiated (Ino-Glc+) (IG) HT-29 cells. The same amount (5 µg) was treated either with EcoRI or HindIII. DNA restriction fragments were separated on 1% agarose gel and visualized after DNA blotting by hybridization with 32P-labeled DPCR1, as described under “Materials and Methods”. Ten micrograms of total cellular RNA were loaded in each lane. Panel B, the densitometric analysis of the fluorogram indicates that the DPP IV mRNA level is low in undifferentiated HT-29 and Caco-2 cells and high in differentiated cells, as well as in the jejunum. Note that the sizes of DPP IV mRNA from Caco-2, HT-29, and the jejunum are identical. IG, Ino-Glc+; G+, Glc+; G−, Glc−.

Restriction Enzyme | EcoRI | HindIII
--- | --- | ---
Culture conditions | IG Ino | IG Ino

23.1 | 4.3 | 2.3
9.4 | 6.6 | 4.3
6.6 | 4.3 | 2.3

Equally labeled (Fig. 4). Since it has been established that these two cell lines do not display the same level of enterocytic differentiation (62), our observations again confirm that DPP IV expression accurately reflects the differentiation level. Interestingly, other authors have shown that DPP IV expression may also increase when additional epithelial (63–65) or nonepithelial (36, 60) cell types differentiate, raising the possibility that there is a general significance of DPP IV expression as a function of cell differentiation.

In this work, we showed that there is no variation in the apparent molecular weight of DPP IV either by Western blot or by pulse or pulse-chase labeling and immunoprecipitation techniques. This is in good agreement with data recently presented by the group of Y. S. Kim (30). Our results demonstrate that two major posttranslational processes, namely protein glycosylation and stability, are not involved in the differentiation-dependent expression of DPP IV. It cannot be ruled out, however, that other posttranslational modifications of DPP IV may occur, since sequence analysis of the DPP IV cDNA sequence indicates that there are a number of potential phosphorylation or sulfation sites (Fig. 10). Whether these potential sites are involved in the differentiation-dependent expression of DPP IV remains to be determined. Our biochemical data indicate that if such protein modifications exist they do not induce any detectable change in the apparent molecular weight of the protein.

The results presented here address the question of the mechanism(s) that control(s) the differentiation-dependent expression of intestinal brush border hydrolases. Previous studies from our laboratory have demonstrated that sucrase-isomaltase, another intestinal brush border hydrolase, is controlled in a totally different way when HT-29 cells differentiate (18). The biosynthesis of sucrase-isomaltase is only slightly decreased in undifferentiated, as compared with differentiated HT-29 cells. In contrast, we have shown that the N-glycosylation of this protein and its turnover rate are dramatically altered in undifferentiated HT-29 cells. Studies on sucrase-isomaltase in the rat intestinal crypt-villus axis have also shown that this protein is still synthesized in the poorly differentiated crypt enterocytes but that this protein is not processed to its fully glycosylated complex form (1). These results are in good agreement with our data, which show that the N-glycan processing and the stability of [3H]mannose-labeled glycoproteins are severely impaired in undifferentiated HT-29 cells (66–68). Other studies on lactase-phlorizin-hydrolase expression along the rat intestinal crypt-villus axis have shown that this protein is not controlled by its mRNA level (69, 70). The above observations argue for the involvement of posttranslational events in the control of brush border hydrolase expression as a function of cell differentiation.

In addition to posttranslational modification of intestinal brush border hydrolases, we show here that the differentiation-dependent expression of DPP IV is mostly if not exclusively controlled by its mRNA level (Fig. 11). Other recent data are in line with our results. Studies of alkaline phosphatase demonstrate that the mRNA level of this protein also rises during the onset of Caco-2 cell differentiation (71). In situ hybridization of aminopeptidase N mRNA in rabbit jejunum has shown that the mRNA levels for this microvillar hydrolase are very low in poorly differentiated crypt enterocytes, whereas the level of its mRNA greatly increases in well differentiated villus enterocytes (5). Northern blot analysis of
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sucrease-isomaltase mRNA in rat jejunum leads to a similar conclusion that sucrease-isomaltase mRNA levels change during intestinal differentiation along the rat intestinal crypt-villus axis (4, 6). In a recent study, we have also shown that the level of DPP IV mRNA is 7 times higher in rat jejunal villus cells than in crypt cells (47). Recent experiments with transgenic mice have demonstrated that it is possible to promote in the intestine the expression of a reporter peptide in a differentiation-dependent manner by using fragments of the promoter region of fatty acid-binding protein, a protein whose expression is differentiation-dependent in the gut (72). Therefore, both posttranslational and transcriptional processes appear to coexist. Whether the two processes are simultaneously active or are alternatively used, depending on a particular protein, remains to be elucidated.

Northern blot analysis demonstrates that DPP IV mRNA levels increase in all situations where the cells differentiate. In addition, we show here that the DPP IV gene does not appear to be modified or amplified as a function of cell differentiation. The mechanisms responsible for such an mRNA level increase remain unknown. The low amount of mRNA observed in undifferentiated Caco-2 and HT-29 cells may reflect a decreased mRNA stability. This type of control has been demonstrated in other cell systems, where the stability of a specific set of mRNA could be increased severalfold when cells enter the differentiation process (73–75). It has been suggested that untranslated sequences of mRNA may be involved in the control of mRNA stability (76). It is possible that part of the 1700-bp untranslated region of DPP IV mRNA could be implicated in the control of its stabilization/degradation equilibrium. A good candidate for this function may be the ATTAAA unit (present at the end of DPP 101) that has been shown capable of initiating polyadenylation.

However, this point must await the sequencing of the full-length human DPP IV cDNA and construction of chimeric genes to be answered. Alternatively, changes in the rate of transcription of the DPP IV gene could also explain our results. This implies the presence of differentiation-dependent regulatory sequences on the human DPP IV gene, as previously shown for some other intestinal genes (72, 77, 78). Run-on experiments using nuclei prepared from differentiated and undifferentiated cells and analysis of the promoter region of this gene are in progress in our laboratory and should help us to determine whether DPP IV mRNA stability or transcription is regulated in these cells.

Acknowledgments—We are grateful to Dr. D. Swallow for the gift of oligonucleotides and for helpful discussion, G. Chevallier for the electron microscopy work, A. Richard for artwork, Drs. M. Laburthe and P. Codogno for continuous support and helpful discussion, and to B. Wic for critical reading of the manuscript.

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