Regulation of the gene for human dipeptidyl peptidase IV by hepatocyte nuclear factor 1 α

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Hepatocyte nuclear factor 1 was identified as the transcription factor binding to a 20 bp (–150 to –131) region of the gene for human dipeptidyl peptidase IV, which has been shown to be important for the expression of dipeptidyl peptidase IV in the human intestinal and hepatic epithelial cell lines Caco-2 and HepG2. Functional analysis of the hepatocyte nuclear factor 1 site was performed with two minimal dipeptidyl peptidase IV promoter constructs (–250 to –41, and –150 to –41) with and without a 3 bp mutation in the hepatocyte nuclear factor 1 sequence, and used in transient transfection experiments with Caco-2 cells. The results show that the mutated constructs were able to drive transcription at only 5–10% of the activity of the

non-mutated controls. Co-transfection of 3T3 cells with hepatocyte nuclear factor 1 (α or β) and dipeptidyl peptidase IV promoter constructs (–250 to –41 or –150 to –41) resulted in a 2.5–6-fold increase in transcription over controls with hepatocyte nuclear factor 1 α but not with hepatocyte nuclear factor 1 β . The results of this study show that hepatocyte nuclear factor 1 binds to the –150 to –131 region of the human dipeptidyl peptidase IV promoter and is necessary for transcriptional activation of the gene for dipeptidyl peptidase IV.

Key words: small intestine, peptidase, gene promoter, transcription factors.

INTRODUCTION

Previous studies from our laboratory have shown that dipeptidyl peptidase IV (DPPIV; EC 3.4.14.5) is of vital importance for the digestion and assimilation of prolyl peptides in the mammalian small intestine [1,2]. This enzyme has also been suggested to have important roles in a number of biological processes such as the activation of T lymphocytes [3], cell–substratum interactions [4,5], hormone regulation [6] and reclamation of urinary peptides in the kidney proximal tubule [7]. In the small intestine, DPPIV exhibits a characteristic pattern of distribution along the proximal–distal axis, with somewhat higher expression in the distal region [8,9]. DPPIV is also highly expressed in the differentiated enterocytes of the villus tip in comparison with crypt cells; whereas only very low levels of DPPIV are present in the normal colonic epithelium, it is sometimes aberrantly expressed in colonic tumours [10]. We have also previously shown that small-intestinal DPPIV activity, mRNA levels and gene transcription can be increased by diets rich in proline [11]. Taken together, these studies indicate that gut epithelium is an ideal tissue in which to study the expression of proteins such as DPPIV because not only does it show spatial and differentiation-dependent elements of control but it can also be responsive to ‘environmental changes’ in the intestine such as diet. At present, the study of the regulation and expression of intestinal proteins at the gene level is still in the early stages, although the excellent work by Gordon’s [12] and Traber’s [13,14] groups have provided significant insight into the regulation of the intestinal fatty-acid-binding protein and sucrase–isomaltase genes.

We recently described the isolation and partial characterization of an approx. 900 bp 5′-flanking region of the gene for human DPPIV [15] to begin an examination of the regulation of this important intestinal peptidase. It was observed that the first 500 bases of this portion of the 5′-flanking sequence consisted of an

unmethylated CpG island containing numerous potential transcription factor-binding sites but lacked a consensus TATA box. In addition, the 900 bp segment exhibited promoter activity in transient transfection experiments with HepG2 and Caco-2 cells [15]. Thus we identified a 30 bp region (–150 to –120) beginning 120 bp upstream of the start of translation that is important for relatively high levels of promoter activity in these cell lines. Computer analysis of this region revealed potential binding sites for the hepatocyte nuclear factor 1 (HNF-1) type of transcription factor proteins along with a ‘TATA-like’ element overlapping the HNF-1 site.

In the present study we have examined this region of the promoter in detail by using the human intestinal and hepatic epithelial cell lines Caco-2 and HepG2, both of which express DPPIV and mouse 3T3 cells, which have low levels of the enzyme. The results of this study show that the HNF-1 α transcription factor binds to the –150 to –120 region of the DPPIV promoter in Caco-2 and HepG2 cells but not in 3T3 cells. Furthermore the results indicate that this site is very important for basal expression of DPPIV because its removal or mutation results in a marked decrease in promoter activity in Caco-2 cells. Co-transfection of the –150 to –41 region of the DPPIV promoter and a HNF-1 α expression vector into mouse 3T3 fibroblasts stimulated reporter gene expression.

EXPERIMENTAL

Cell culture and transfection

Caco-2, 3T3 and HepG2 cells were obtained from the American Type Culture Collection. They were maintained in Dulbecco’s modified Eagle’s medium supplemented with fetal calf serum (FCS), 100 i.u./ml penicillin and 100 i.u./ml streptomycin under air/CO₂ (19:1) at 37 °C. Caco-2 cell cultures were maintained

Abbreviations used: CAT, chloramphenicol acetyltransferase; DPPIV, dipeptidyl peptidase IV; EMSA, electrophoretic mobility-shift assay; FCS, fetal calf serum; HNF-1, hepatocyte nuclear factor 1.

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with 20% (v/v) FCS, whereas 3T3 and HepG2 cells were maintained in 10% (v/v) FCS. For transient transfection, adherent confluent cells were seeded in 35 mm dishes at 10^6 cells per dish and used for transfection 24 h later. Plasmids for transfection were prepared by alkaline lysis, CsCl banding and purification over columns of Sepharose CL-4B. Liposome-mediated transfection was performed with Lipofectamine reagent (Gibco BRL). Lipofectamine (30 μ g) was mixed with luciferase reporter plasmid (pGL2 vector; Promega) (10 μ g) and a plasmid (5 μ g) containing chloramphenicol acetyltransferase (CAT) as a transfection efficiency control in serum- and antibiotic-free medium (200 μ l). After incubation at room temperature for 15 min, additional medium (800 μ l) was added and the entire solution was carefully layered on the cells. After 24 h, the transfection medium was removed and replaced with normal growth medium. Cells were washed with PBS and harvested 48 h after the start of transfection. Soluble lysates were prepared by sonication in 500 μ l of Reporter Lysis Buffer (Promega) followed by centrifugation for 2 min in a Microfuge. In other experiments, expression vectors for HNF-1 α and HNF-1 β (5 μ g) were co-transfected with the DPPIV (10 μ g) and CAT (5 μ g) plasmids. The pBJ5 expression vector harbouring murine HNF-1 α or HNF-1 β has been described previously [16] and was a gift from Dr. Gerald Crabtree (Stanford, CA, U.S.A.).

Supernatants were assayed for luciferase activity by using a luciferase assay reagent (Promega); the photons released were measured for 30 s in a Monolight 2010 luminometer (Analytical Luminescent Laboratory). CAT activity was measured for 1 h with [3 H]chloramphenicol and butyryl-CoA by following a previously described procedure [17]. The resulting luciferase activities were divided by the respective CAT activity to correct for transfection efficiency and preparation of the cell extracts. These results were then normalized to a percentage of the value obtained with a positive control vector (pGL2-control; Promega) that directs the synthesis of luciferase. Under the conditions described above, pGL2-control-transfected 3T3 and Caco-2 cells typically had approx. 750 000 luciferase units.

Analysis of DNA-binding proteins

Nuclear extracts were prepared from confluent cells [18] and 5 μ g of protein was used in electrophoretic mobility-shift assays (EMSAs). Oligonucleotide probes were synthesized by the UCSF Biomolecular Resource Center and end-labelled with T4 polynucleotide kinase and [γ - 32 P]ATP to a specific radioactivity of approx. 7.5×10^5 c.p.m./ng. EMSA binding reactions (20 μ l) contained 1 ng of double-stranded 32 P-labelled oligonucleotide and reagents from a BandShift kit (Pharmacia Biotech) consisting of 40 mM Tris/HCl, pH 7.5, 200 mM NaCl, 5 mM MgCl₂, 10% (v/v) glycerol, 2 mM dithiothreitol, 0.05% Nonidet P40 and 1 μ g of poly(dI-dC). In competition studies, a 100-fold molar excess of the indicated unlabelled oligonucleotide was used. The HNF-1 antibody used in supershift experiments was a gift from Dr. Marco Pontoglio (Pasteur Institute, Paris, France). Antibody against the MAX transcription factor (catalogue no. sc-765X) was purchased from Santa Cruz Biotechnology (Santa Cruz, CA, U.S.A.). A 'QuikChange' site-directed mutagenesis kit (Stratagene, La Jolla, CA, U.S.A.) was used to make a 3 bp mutation at the HNF-1-binding site.

DNase I footprint analysis was performed with the -251 to -41 segment of the DPPIV promoter in the Bluescript plasmid. To label the coding strand, the plasmid was cut with *Hin*-dIII/*Sma*I and the fragment was isolated by preparative electrophoresis followed by a QIAquick Gel Extraction Kit (Qiagen). The overlapping end was filled with 32 P-labelled dCTP and

Klenow enzyme followed by additional purification with a QIAquick PCR Purification Kit (Qiagen). Nuclear proteins were incubated with 32 P-labelled fragment for 30 min and subjected to digestion for 1 min with DNase I (4 units) at room temperature as described [19]. After extraction with phenol/chloroform and precipitation with ethanol, the DNA was separated on a denaturing polyacrylamide gel and autoradiographed. The gel was calibrated with fragments from a known sequencing reaction.

RESULTS

DNase I footprint analysis

The -250 to -41 region of the DPPIV promoter was used in footprint analysis with nuclear extracts isolated from Caco-2 cells. Previous studies of this region with the use of a series of deletion constructs in cell transfection studies had indicated that the -150 to -119 region was important for promoter activity in both Caco-2 and HepG2 cells. The analysis shown in Figure 1 revealed a single strong footprint with the labelled coding strand spanning bases -150 to -126. When the non-coding strand was labelled (results not shown), a broader but less distinct footprint was observed encompassing bases -195 to -126. Figure 2 shows these two regions with potential nuclear-protein-binding sites as determined by computer analysis.

EMSA

On the basis of these observations and the earlier transfection studies, two oligonucleotides covering this region (-195 to -120) were synthesized for use in EMSAs to examine patterns of nuclear protein binding to the DPPIV promoter. One was a

Figure 1 DNase I footprint of the DPPIV promoter (-250 to -41 bp region)

Increasing amounts of nuclear protein isolated from Caco-2 cells were incubated with labelled coding strand before digestion with DNase I. Lanes 1 and 5, BSA control (no nuclear protein); lanes 2, 3 and 4, Caco-2 cell nuclear protein (10, 20 and 40 μ g respectively). The footprint was calibrated with fragments from a known sequencing reaction; lengths are indicated in bases.

Figure 2 Sequence of potential transcription factor-binding sites identified by footprint analysis of the -250 to -41 promoter region

(A) Region -195 to -156; (B) HNF-1/TATA (-155 to -108) region. Potential binding sites were determined by computer, with the use of MacVector 6.0 software.

35 bp oligonucleotide spanning regions -155 to -120 shown in earlier studies to be necessary for promoter activity in transfected Caco-2 cells. The second covered bases -195 to -156. Figure 3(A) shows an EMSA with the -155 to -120 region of the promoter and nuclear extracts prepared from 3T3, HepG2 and Caco-2 cells. A single, major prominent band was observed in Caco-2 cells, whereas at least two less intense EMSA bands were observed in extracts from HepG2 cells. No corresponding nuclear binding proteins were apparent in extracts from 3T3 cells. The addition of excess (100-fold) unlabelled oligonucleotide eliminated the major bands in Caco-2 and HepG2 cells. It was subsequently discovered that identical banding patterns were observed when a shorter oligonucleotide spanning bases -150 to -131 was used. This oligonucleotide was then used in further studies of this region. EMSA patterns of the extended region (-195 to -156 oligonucleotide) (Figure 3B) revealed a single band in extracts from Caco-2 and 3T3 cells; each seemed to have a slightly different mobility from the other. No corresponding bands were evident in HepG2 nuclear extracts.

The identity of the nuclear protein(s) binding to the -150 to -131 region was further investigated by using a series of oligonucleotide competitors containing binding sites based on HNF-1 and TATA-binding protein. These included a 13 bp HNF-1 consensus sequence (5'-GTTAATGATTAAC-3') [20,21], a 22 bp HNF-1 sequence from the α 1-antitrypsin inhibitor promoter (5'-CCTTGGTTAATATTCACCAGCA-3') [22] and a 25 bp consensus sequence for the TATA-binding protein TFIID (5'-GCAGAGCATATAAAATGAGGTAGGA-3') (Santa Cruz Biotechnology). As seen in Figure 4 with Caco-2 cell extracts, a 100-fold excess of unlabelled oligonucleotide (-150 to -131) competed, as did the two oligonucleotide competitors containing HNF-1-binding sites. Interestingly, even though the 13 bp HNF-1 consensus sequence was an effective competitor, the cor-

Figure 3 EMSA of the HNF-1/TATA sites and extended region

(A) Labelled HNF-1/TATA oligonucleotide (-155 to -120); (B) labelled -195 to -156 oligonucleotide. The indicated unlabelled oligonucleotide competitors from different regions of the DPPIV promoter were included in the binding reaction at 100-fold the concentration of the labelled oligonucleotide. Lane O, control containing no nuclear protein.

responding 13 bp sequence from the DPPIV HNF-1 site (-147 to -135) itself was not. No binding of nuclear protein was observed when the -147 to -135 oligonucleotide was labelled and used as a probe in EMSA (results not shown), confirming the observation on competition. In addition, oligonucleotides containing a consensus TATA site and one spanning the DPPIV 'TATA-like' element (-141 to -131) did not compete. Serving as controls, oligonucleotides (-195 to -156 and -130 to -121) that spanned regions immediately adjacent to the -150 to -131 region of the DPPIV promoter were ineffective as competitors, as was an unrelated oligonucleotide from the intestinal mucin gene promoter (MUC2, -214 to -185) [23]. Identical patterns of competition were observed with nuclear extracts from HepG2 cells (Figure 4).

An antibody against HNF-1 was used in EMSA supershift assays as shown in Figure 5. As can be seen, inclusion of this antibody in the Caco-2 binding reactions caused a shift of the HNF-1 complex to a lower electrophoretic mobility than was observed in its absence. This was also true of HepG2 extracts, although the supershifted species were less obvious. The inclusion of an antibody recognizing the MAX transcription factor had no effect on any of the protein bands binding to this oligonucleotide.

We next looked to see if we could perturb the binding of the nuclear factors observed in Caco-2 cell extracts by mutating the HNF-1 site (Figure 2). For these studies we used a 3 bp mutation

Figure 4 Effect of oligonucleotide competitors on nuclear protein binding to the HNF-1/TATA element

Nuclear proteins from HepG2 cells were incubated with labelled oligonucleotide (—150 to —131) in the presence of 100-fold excess of unlabelled competitor oligonucleotide. Numbered competitors refer to regions of the DPPIV promoter that were used. HNF-1C is a 13 bp HNF-1 consensus oligonucleotide (5'-GTAAATGATTAAC-3'); antitrypsin is a 22 bp region from the human α 1-antitrypsin gene promoter containing an HNF-1 site (5'-CCTGGTAAATATTCACAGCA-3'); TFIID is a 25 bp oligonucleotide containing a TATA element (5'-GCAGAGCATATAAATGAGGTAGGA-3'). MUC2 is a 30 bp oligonucleotide from the human mucin 2 gene promoter (—214 to —185) [23]. Lane 0, control containing no nuclear protein.

[21] in the HNF-1 site (G⁻¹⁴¹TTTAAC⁻¹³⁵ to GTGGGAC) of the —150 to —131 oligonucleotide used previously. When the mutated sequence was used in EMSA, no bands were observed in nuclear extracts of HepG2 and HepG2 cells (results not shown). This observation is corroborated by the results in Figure 6, in which normal labelled oligonucleotide had a banding pattern typical of HNF-1 proteins in nuclear extracts of HepG2 and HepG2 cells. As Figure 6 shows, these bands are effectively competed for by a 100-fold excess of unlabelled normal oligonucleotide but not by an equivalent amount of oligonucleotide containing the mutated HNF-1 site.

Functional analysis of the HNF-1 region

The promoter activity of the HNF-1 region was analysed with the use of two deletion constructs (—250 to —41 and —150 to —41) of the DPPIV promoter. Two equivalent constructs containing the 3 bp mutation described above in the putative HNF-1-binding site were also examined. For these studies HepG2 cells were used. As shown in Table 1, luciferase activity was expressed when the two normal constructs were used; approx. 2–3-fold higher activity was observed with the —150 to —41 construct.

Figure 5 EMSA supershift experiments

Binding reactions were performed in the presence (+) or absence (—) of antibody (1 μ g) against HNF-1 and MAX transcription factors for 30 min before electrophoresis. Labelled —150 to —131 oligonucleotide was used in the reactions. Lane 0, control containing no nuclear protein.

Figure 6 Effect of a mutated HNF-1 site on binding of nuclear protein

Labelled —150 to —131 oligonucleotide was incubated in the presence of a 100-fold excess of either unlabelled normal (—150 to —131) oligonucleotide or one containing a 3 bp mutation to the HNF-1 site (—150 to —131 Mut). As a reference, a known non-competitor (—195 to —156) from the DPPIV promoter was included. Lane 0, control containing no nuclear protein.

However, when constructs containing the mutated HNF-1 site were used for transfection, promoter activity was decreased by 90–95%.

Table 1 Promoter activity of DPPIV reporter constructs containing normal and mutated HNF-1 sites

Two DPPIV promoter constructs (–150 to –41 and –250 to –41) in the pGL2 vector were used in transfection studies with Caco-2 cells. The mutated constructs (–150 to –41 mut and –250 to –41 mut) contained a 3 bp mutation in the HNF-1 site. Luciferase was measured in cell extracts and CAT activity was used as a control for transfection efficiency. Normalized luciferase activity was calculated as described in the Experimental section. Results are means $\pm$ S.D. for at least eight individual experiments.

Construct	Luciferase activity (% positive control)
–150 to –41	6.5 $\pm$ 1.8
–150 to –41 mut	0.6 $\pm$ 0.4
–250 to –41	2.4 $\pm$ 0.6
–250 to –41 mut	0.2 $\pm$ 0.1

Table 2 Activation of the DPPIV promoter by HNF-1 α in mouse 3T3 cells

Mouse 3T3 cells were co-transfected with the indicated region of the DPPIV promoter (–150 to –41 or –250 to –41 in the pGL2 vector) and either the pBJ5 expression vector alone or vectors containing the coding region for murine HNF-1 α or HNF-1 β . Luciferase activity was measured in cell extracts and CAT activity was used as a control for transfection efficiency. Normalized luciferase was calculated as described in the Experimental section. Results are means $\pm$ S.D. for four individual experiments.

Plasmid	Addition	Luciferase activity (% positive control)
–150 to –41	pBJ5	3.6 $\pm$ 0.5
	HNF-1 α	21.0 $\pm$ 4.2
	HNF-1 β	5.2 $\pm$ 1.7
–250 to –41	pBJ5	4.2 $\pm$ 0.6
	HNF-1 α	10.2 $\pm$ 2.9
	HNF-1 β	4.1 $\pm$ 2.2

Figure 7 Expression of HNF-1 α and HNF-1 β in mouse 3T3 cells

Mouse 3T3 cells were transfected with the pBJ5 expression vector alone or with vectors containing the coding sequence for either murine HNF-1 α or murine HNF-1 β . The standard transient transfection and incubation protocols described in the Experimental section were used in these studies. Nuclear proteins were isolated from 35 mm dishes (two of each) containing either transfected or untreated cells, then used in EMSA with labelled –150 to –131 oligonucleotide. Caco-2 cell nuclear proteins were included as a reference. Oligonucleotide competitors (100-fold excess) included the –150 to –131 sequence itself or a 22 bp sequence from the human α 1-antitrypsin promoter containing a HNF-1 site. The arrowhead indicates the position of HNF-1. Lane O, control containing no nuclear protein.

The importance of the HNF-1 transcription factor in regulating the expression of the gene for DPPIV was next examined by co-transfecting expression vectors containing murine HNF-1 α and HNF-1 β into mouse 3T3 fibroblasts. This cell line normally expresses very low levels of DPPIV, and as we have observed in this study it has undetectable amounts of HNF-1 (Figure 3A). When plasmid containing either HNF-1 α or HNF-1 β in the pBJ5 expression vector was transfected into 3T3 cells, newly expressed HNF-1 transcription factor could be detected by EMSA in nuclear extracts from these cells. This is shown in Figure 7. As can be seen, cells transfected with the pBJ5 vector alone had no proteins that bound to the –150 to –131 oligonucleotide. However, cells transfected with plasmid containing the HNF-1 α insert expressed a nuclear protein that

bound to the –150 to –131 oligonucleotide and had approximately the same electrophoretic mobility as the protein–oligonucleotide complex observed in Caco-2 cells. The protein expressed in 3T3 cells was also competed for either by excess unlabelled DPPIV HNF-1 oligonucleotide (–150 to –131) or by the α 1-antitrypsin oligonucleotide containing an HNF-1 site (described above). Cells transfected with the pBJ5/HNF-1 β plasmid expressed a more diffuse band of slightly greater mobility.

On the basis of these results, we next co-transfected the pBJ5/HNF-1 α or β expression plasmids into 3T3 cells along with the luciferase expression plasmid (PGL2) containing either the –250 to –41 or –150 to –41 region of the DPP IV promoter. As shown in Table 2, the combination of the HNF-1 α expression vector and the –150 to –41 region of the DPP IV promoter resulted in levels of transcription that were approx. 5.5-fold higher than when the –150 to –41 construct was transfected alone. Interestingly, HNF-1 β was not effective in increasing levels of transcription above control values. A similar pattern was observed with the –250 to –41 construct but activation with HNF-1 α was lower (2.5-fold).

DISCUSSION

A number of important dietary proteins such as collagen, gliadin and α -casein contain relatively high amounts of proline and hydroxyproline. Previous studies from our laboratory have shown that prolyl peptides are hydrolysed primarily in the small intestine by a number of brush border membrane-associated enzymes that are unique in their ability to hydrolyse the prolyl peptide bond [24]. Foremost is DPPIV, which is a major constituent of the small-intestinal brush border membrane [1,2]. In the intestine, DPPIV displays elements of spatial and cell lineage patterns of control and is also regulated by the amount and type of protein in the diet [11]. Thus we believe that an examination of the regulation of this important peptidase can yield important information about the DNA elements and transcription factors that direct the expression of the gene for DPPIV in the intestine.

In the present study we show that a 20-bp region encompassing bases –150 to –131 of the DPPIV promoter is important for directing the basal activity of the gene. In previous work we identified this region as being important by a deletional analysis of an approx. 900 bp region immediately 5' of the DPPIV coding sequence [15]. Although the overall sequence of the mouse DPPIV promoter [25] is significantly different from its human

counterpart, it contains an identical 15 bp region (−28 to −13) corresponding to the human −147 to −133 sequence (Figure 2), suggesting that this region is conserved and might be important for transcription of the mouse gene as well. Thus the goal of this study was to identify any transcription factors that might be binding to this area and assess their functional role in controlling the expression of the gene for DPPIV. Initially DNase I footprinting revealed an area encompassing bases −150 to −128 showing that Caco-2 cell nuclear protein(s) bind to this region of the promoter. These observations were confirmed by mobility-shift assays which show that nuclear proteins from both Caco-2 and HepG2 cells bind to the −150 to −131 oligonucleotide containing the overlapping HNF-1 site and TATA-like element. The oligonucleotide competition analysis and use of specific antibody in EMSA demonstrate that the HNF-1 transcription factor binds to the −150 to −131 region. The 13 bp HNF-1 DPPIV sequence (−147 to −135) itself was not an effective competitor and did not bind nuclear protein when used in EMSA assays. However, the 13 bp HNF-1 consensus sequence was an effective EMSA competitor. This might be due to the 3 bp sequence difference between the two and to the fact that the HNF-1 recognition sequence might extend to 15 or 17 bases [22,26]. Although the EMSA banding patterns for Caco-2 and HepG2 nuclear proteins were clearly different, the major bands were supershifted by HNF-1 antibody in each cell line. Because the various bands are indicative of HNF-1 homodimers and heterodimers [16], they most probably reflect differences in the amounts of the HNF-1 α and HNF-1 β variants in the two cell types.

Mutation of the HNF-1 site in the DPPIV promoter eliminated the binding of the major EMSA bands observed in Caco-2 and HepG2 cells. The 3 bp mutation used in this study was based on previous work with β -fibrinogen, in which a similar mutation was used to alter the inverted palindromic nature of the HNF-1 site [21]. Transient transfection of Caco-2 cells with the −250 to −41 and −150 to −41 constructs containing the 3 bp mutation decreased the level of transcription to 5–10% of that observed with non-mutated controls, indicating that HNF-1 is important for transcriptional activation of the DPPIV gene.

We observed that mouse 3T3 fibroblasts do not normally express HNF-1 α or HNF-1 β under the growth conditions used in our laboratory. Other investigators have also reported that this cell line does not express mRNA for these two proteins [27]. Thus when either HNF-1 α or HNF-1 β was expressed in mouse 3T3 fibroblasts, we found that they had different effects on the DPPIV promoter. HNF-1 α was very effective in activating transcription when co-transfected with the −150 to −41 region of the DPPIV promoter, whereas HNF-1 β was not. Other researchers have also observed that HNF-1 β tends to be a weak or ineffective inducer of transcription with the albumin [28], β -fibrinogen [16] and sucrase-isomaltase [29] promoters. HNF-1 α had a smaller effect with the longer DPPIV promoter construct (−250 to −41). This was also true of Caco-2 cells, in which the −250 to −41 construct was less efficient in driving transcription in comparison with the −150 to −41 segment. Thus there might be an inhibitory element in the immediate region downstream of the HNF-1 site, a possibility suggested by the cell nuclear protein's binding to the −195 to −156 region of the promoter in 3T3 and Caco-2 cells (Figure 3).

The HNF-1 transcription factor has been shown to be involved in the regulation of the genes for several hydrolases associated with the small intestinal brush border membrane such as aminopeptidase N [30] and sucrase-isomaltase [28,31]. In addition, genes such as those for α -fetoprotein [32], α 1-antitrypsin [22] and guanylin [27] have HNF-1-binding sites in their promoters and

are expressed in gastrointestinal tissues. Originally identified as a liver-specific factor, HNF-1 is now known to be expressed more widely, being found in the kidney, intestine, pancreas, thymus, ovary and lung [16,20,22,26]. HNF-1 consists of two variants, α and β , which are the products of different genes but are highly similar homeodomain-containing proteins [26]. They bind to the same consensus sequence in a number of genes as either homodimers or heterodimers [16,20] and might be phosphorylated [33]. Multimerization of the HNF-1 subunits also involves the interaction of a cytoplasmic protein (DCoH) that has enzymic activity and is involved in the metabolism of phenylalanine [34]. Recent evidence also suggests that HNF-1 α has a role in the demethylation and remodelling of chromatin structure [35]. Although both HNF-1 variants are present in similar tissues such as the liver, kidney, intestine and pancreas, their levels have been found to differ significantly [16,20,26]. In the mouse intestine the two types of HNF-1 have been shown to be localized primarily in the differentiating crypt cells [36], although mRNA transcripts for both types have been noted in the more differentiated villus cells [33]. They also display different levels of mRNA expression along the proximal–distal axis of the small intestine: the highest levels are found distally [36]. In this regard it has been suggested that different ratios of the α and β variants might have a role in controlling the levels of expression of intestinal proteins along the longitudinal gradient [29]. Taken together, these characteristics of HNF-1 give it the potential for a high degree of complexity in the regulation of genes such as that for DPPIV.

Although there is limited information about the regulation of HNF-1, a mouse knock-out model for HNF-1 (α) has been described [37]. These animals had renal and liver dysfunction but, somewhat surprisingly, not all genes thought to be regulated by HNF-1 were affected. It has not been reported whether these animals have decreased levels of DPPIV in their various tissues.

In summary, we have shown that HNF-1 α is necessary for the transcriptional activation of the gene for DPPIV. This study and those by other investigators indicate that HNF-1 has an important and until recently unsuspected role in the regulation of a number of intestinal genes. Additional studies will be required to delineate the interaction of HNF-1 with other transcription factors that modulate and direct the expression of DPPIV in the enterocyte.

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