Involvement of activator protein 1 complexes in the epithelium-specific activation of the laminin γ2-chain gene promoter by hepatocyte growth factor (scatter factor)

Jørgen OLSEN*†[1], Olivier LEFEBVRE*, Christine FRITSCH*, Jesper T. TROELSEN†, Veronique ORIAN-ROUSSEAU*, Michèle KEDINGER* and Patricia SIMON-ASSMANN*†

*INSERM U.381, 3 avenue Molière, 67200 Strasbourg, France, and †Department of Medical Biochemistry and Genetics, Biochemistry Laboratory C, The Panum Institute, Building 6.4, University of Copenhagen, Blegdamsvej 3, DK-2200 Copenhagen N, Denmark

Laminin-5 is a trimer of laminin α3, β3 and γ2 chains that is found in the intestinal basement membrane. Deposition of the laminin γ2 chain at the basement membrane is of great interest because it undergoes a developmental shift in its cellular expression. Here we study the regulatory elements that control basal and cytokine-activated transcriptional expression of the *LAMC2* gene, which encodes the laminin γ2 chain. By using transient transfection experiments we demonstrated the presence of constitutive and cytokine-responsive *cis*-elements. Comparison of the transcriptional activity of the *LAMC2* promoter in the epithelial HT29mtx cells with that in small-intestinal fibroblastic cells (C20 cells) led us to conclude that two regions with constitutive epithelium-specific activity are present between positions −1.2 and −0.12 kb. This was further validated by transfections of primary foetal intestinal endoderm and mesenchyme. A 2.5 kb portion of the *LAMC2* 5′ flanking region was equally responsive to PMA and hepatocyte growth factor (HGF), whereas it was less responsive to transforming growth factor β1. A minimal promoter limited to the initial 120 bp upstream of the transcriptional start site maintained inducibility by PMA and HGF. This short promoter fragment contains two activator protein 1 (AP-1) elements and the 5′-most of these is a composite AP-1/Sp1 element. The 5′AP-1 element is crucial to the HGF-mediated activity of the promoter; analysis of interacting nuclear proteins demonstrated that AP-1 proteins containing JunD mediate the response to HGF.

Key words: extracellular matrix, Fos, gene regulation, intestine, Jun.

INTRODUCTION

The intestine develops from a hollow tube consisting of an inner stratified endoderm surrounded by mesenchyme. By way of complex morphogenetic events this structure is converted into the adult-type intestinal mucosa, submucosa and muscular layers (reviewed in [1,2]). The adult small-intestinal epithelium is a continuum of protruding villi with a central stromal core and of crypts penetrating into the underlying stroma. Stem cells populate the crypts, daughter cells migrate along the crypt–villus axis and enter their terminal differentiation programme during this migration (reviewed in [3–5]).

As in other organs, the development of the intestine depends on interactions between the mesenchymal and epithelial cells [6,7] that are in part mediated by cytokines secreted locally in the intestine. Examples of such cytokines are hepatocyte growth factor (HGF; scatter factor) and transforming growth factor β1 (TGF-β1). A high level of HGF is produced by the intestinal mesenchymal cells at the time of villus formation; this is thought to facilitate the invasion of the multilayered epithelium by the mesenchymal cells [8]. In addition, HGF has been suggested to stimulate the resealing of wounded areas in intestinal cell cultures [9,10]. TGF-β1 is produced at high levels by the epithelial cells at all stages of intestinal development [11–13]. The physiological effects of TGF-β proteins are diverse and include the regulation of cellular proliferation and the differentiation and deposition of the extracellular matrix (ECM) (reviewed in [14]). The HGF receptors (the product of the c-*met* proto-oncogene) are found predominantly on intestinal epithelial cells [8,15,16], whereas the type II TGF-β1 receptors are located on intestinal mesenchymal cells [17,18].

The basement membrane, a specialization of ECM, also provides an important framework for epithelial and mesenchymal interactions because it imposes important signals on the intestinal cells via specific receptors. This structure that separates epithelial and stromal compartments has a dual origin in the intestine. Some components such as collagen IV are produced mainly by the mesenchymally derived stromal fibroblasts, whereas perlecan is produced exclusively by the epithelial cells. The laminins, however, are produced by both the epithelial cells and mesenchymal cells, with variations during development (reviewed in [19,20]). The laminins have been found to be of particular importance for ECM-mediated signalling in various systems [21] and in the intestine; laminin-1 (a trimer of the α1, β1 and γ1 laminin chains) has been shown to induce and maintain the differentation of intestinal epithelial cells [19,22–24]. Laminin-5 (a trimer of the α3, β3 and γ2 laminin chains) is another laminin variant found in the intestine [25,26]. The precise function of laminin-5 in the intestine is not yet clear. However, the fact that this molecule has been shown (1) to be localized in the villus and not in the crypt basement membrane in the adult intestine [25] and (2) to facilitate the migration of intestinal epithelial cells into wounded epithelial areas [9], and the fact that its γ2 chain is expressed in colon carcinoma cells positioned at the invasive front of malignant tumours [27,28], suggests that laminin-5 regulates the migration of normal and neoplastic intestinal

Abbreviations used: AP-1, activator protein 1; CMV, cytomegalovirus; ECM, extracellular matrix; EMSA, electrophoretic mobility-shift assay; HGF, hepatocyte growth factor; *LAMC2*, the gene encoding the laminin γ2 chain; RLU, relative light units; RT–PCR, reverse-transcriptase-mediated PCR; TGF-β1, transforming growth factor β1.

[1] To whom correspondence should be addressed at the Panum Institute (e-mail jolsen@biobase.dk).

epithelial cells. The pattern of deposition of the laminin $\gamma 2$ chain at the subepithelial level during intestinal development is of interest because it is produced by the intestinal epithelial cells at early stages of development, whereas the intestinal fibroblasts express this molecule later on [25]. Moreover, the laminin $\gamma 2$ chain mRNA is stimulated by a variety of growth factors in human keratinocytes and cancer cell lines [29,30].

Cross-talk between ECM-mediated and cytokine-mediated signalling occurs constantly. The two pathways share several cytoplasmic signal transduction molecules (reviewed in [31–34]) and they can influence the expression of each other's signalling and receptor molecules. Thus the effects of cytokines produced locally, such as HGF and TGF-β1, might translate into long-term effects by inducing changes in the composition of the intestinal basement membrane. Here we have focused on the regulation of the *LAMC2* gene encoding the $\gamma 2$ chain of the laminin-5 basement membrane protein [35,36]. The purpose of this work was to identify *cis*-elements and interacting transcription factors that are involved in the regulation of *LAMC2*, in unstimulated and in TGF-β1-stimulated and HGF-stimulated intestinal epithelial cells. The tissue specificity of the basal and cytokine-stimulated transcriptional activity was also addressed.

MATERIALS AND METHODS

Materials

Oligonucleotides were acquired from Life Technologies and Pharmacia Biotech. The *LAMC2*-containing PAC clone (clone ID PAC-0127-E18) was obtained from Genome Systems. The pGL3-basic vector was purchased from Promega and the pBluescript SK+ and KS+ vectors were purchased from Stratagene. The β-galactosidase pCMVb expression vector was obtained from Clontech. The RiboProbe T7 *in vitro* transcription system was from Promega Biotech and the restriction enzymes were from Promega or Eurogentec. The T4 polynucleotide kinase and the *Pwo* DNA polymerase were purchased from Eurogentec. The RNase protection kit RPA-II was obtained from Ambion. The Titan TM One Tube RT–PCR system was from Boehringer Mannheim. MicroSpin G-25 columns were obtained from Pharmacia Biotech. The Exgen500 transfection agent was obtained from Euromedex. Cell culture media were purchased from Life Technologies. Radiochemicals were from ICN Pharmaceuticals. The human recombinant HGF, TGF-β1 and PMA were obtained from Sigma Chemical Co. The antibodies against activator protein 1 (AP-1) proteins, Sp1, Sp3 and Ets-1/2 were purchased from Santa Cruz. The AlfExpress sequencer was from Pharmacia Biotech and the Lumistar luminometer was from BMG LabTechnologies SARL.

Plasmid construction

An oligonucleotide probe corresponding to positions −60 to −36 of *LAMC2* [36] was used to probe a Southern blot of DNA prepared from a human genomic PAC clone (clone ID PAC-0127-E18) containing the 5′ end of *LAMC2* [36]. This revealed the presence of 2.5 kb *Acy*I and 1.2 kb *Nhe*I/*Acy*I fragments (Figure 1), both with 3′ ends corresponding to the *Acy*I site at position +35 of *LAMC2*. The 2.5 kb *Acy*I fragment was cloned into the *Cla*I site of pBluescript SK+ to generate lamprosk+. A −2.5 kb *LAMC2*/luciferase construct was produced by cloning this *LAMC2* fragment from lamprosk+ as a *Sac*I/*Sal*I fragment into pGL3-Basic digested with *Xho*I and *Sac*I. A −1.2 kb *LAMC2*/luciferase construct was produced by cloning the corresponding *Sac*I/*Nhe*I fragment from lamprosk+ into pGL3-Basic. The sequence from positions −339 to +35 was determined

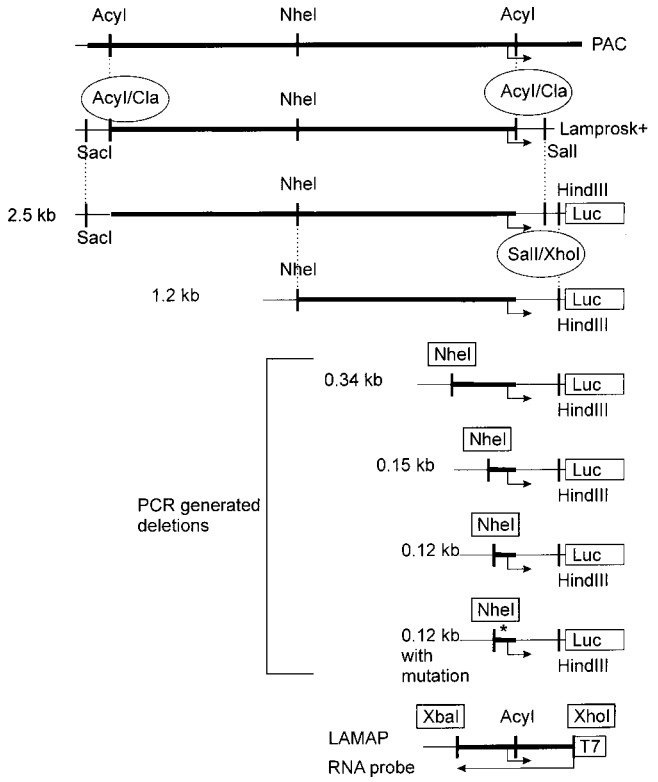

Figure 1 Plasmid constructs

A description of the constructs used is given and the route for their construction (for details see the Materials and methods section) is indicated. All *LAMC2* fragments (heavy horizontal lines) originate from the 2.5 kb *Acy*I fragment present in the human genomic PAC clone. The 2.5 and 1.2 kb *LAMC2*–luciferase constructs were generated by subcloning of fragments from the genomic PAC clone as indicated by vertical dotted lines. In this process the restriction sites were destroyed (restriction sites in ellipses). The shorter *LAMC2*–luciferase constructs were generated by PCR and in these cases a 5′ *Nhe*I site was included (*Nhe*I sites in boxes) in the primers. The 0.12 kb constructs carrying mutations (only one construct is shown) were generated by including the mutation (indicated by an asterisk) in the 5′ primer. The LAMAP construct used for the generation of the *LAMC2* anti-sense riboprobe (Figure 2) was made by PCR with primers directed against the region surrounding the *LAMC2* transcriptional initiation site (rightward arrow). Heavy vertical lines indicate restriction sites, thin horizontal lines represent vector sequences and all restriction sites placed in boxes mean that they originate from PCR primers.

on both strands with the ThermoSequenase system and an AlfExpress sequencer. From the deduced sequence, PCR-based *LAMC2*/luciferase constructs were made corresponding to positions −339 (0.34 kb), −149 (0.15 kb) and −115 (0.12 kb). All 5′ primers had the same 5′ extension containing a *Nhe*I restriction site. For all constructs a primer complementary to the 5′ end of the luciferase gene was used as 3′ primer and the −1.2 kb/luciferase construct was used as template. PCR reactions were performed with the *Pwo* thermostable DNA polymerase in accordance with the manufacturer's instructions. Products were subsequently cloned as *Nhe*I/*Hin*dIII fragments into pGL3-Basic.

Similarly, specific mutations in the 0.12 kb *LAMC2* construct were introduced by the use of PCR (except mutation 4). The 5′ primers had (except for their 5′ extensions with a *Nhe*I site) a 5′ end corresponding to position −115. The 3′ ends were at least 18 nt downstream of the mutation included in the primer sequence. Mutations 4 and 5 contained a mutation in the 3′AP-1 site. This mutation was produced by filling in the *Tth*111I site located in

the 3′AP-1 site. A construct (LAMAP) for the generation of the RNA anti-sense probe used for RNase mapping was made by PCR with a 5′ primer corresponding to nt −329 to −310 in *LAMC2* and a 3′ primer complementary to nt 145 to 163 in the laminin γ2 chain mRNA [37]. The PCR fragment was generated by the use of *Pwo* DNA polymerase with the PAC clone as template; it was cloned into pBluescript SK+ digested with *Xho*I and *Xba*I. The integrity of all constructs was verified by sequencing of both strands.

Reverse-transcriptase-mediated PCR (RT–PCR) and RNase mapping

RNA was extracted from cells by the guanidium-isothiocyanate/phenol method [38]. RT–PCR was performed with 1 μg of total RNA prepared from either HT29mtx cells or C20 small-intestinal fibroblasts with the Titan™ One Tube RT–PCR system, in accordance with the manufacturer's instructions. Primer pairs for the laminin γ2 chain mRNA (5′-AATGGGAAGTCCAGGCAGTGTATC-3′ and 5′-ACAGCGTTCTCCAGTAACAGCTG-3′) and human glyceraldehyde-3-phosphate dehydrogenase mRNA (5′-GGCTGAGAACGGGAAGCTTGTGATCAATGG-3′ and 5′-TGTCGCTGTTGAAGTCAGAGGAGACCACCT-3′) were used and generated PCR fragments of 407 and 663 bp respectively. A total of 35 PCR cycles were performed and the resulting products analysed on an ethidium bromide-stained 3% (w/v) agarose gel, with *Hae*III-digested pBluescript SK+ DNA as markers.

The LAMAP plasmid was linearized with *Not*I and transcribed with T7 RNA polymerase in the presence of 50 μCi of [α-^{32}P]UTP (800 Ci/mmol) in accordance with the manufacturer's instructions. Template DNA was removed by digestion with 20 units of RNase-free DNase I followed by extraction with phenol/chloroform and purification on a MicroSpin G-25 column. A 1 μl portion of the flow-through (50 μl total volume) was used for each hybridization. Hybridization and RNase digestion were performed with the RPA-II kit in accordance with the manufacturer's instructions. In essence, 30 μg of total RNA was evaporated to dryness and dissolved in 30 μl of hybridization buffer. Hybridization was done at 45 °C and RNase digestion was performed with RNase A (2.5 units/ml) and RNase T1 (100 units/ml) at 37 °C for 45 min. Protected RNA fragments were separated by electrophoresis through a 7 M urea/6% (w/v) polyacrylamide sequencing gel and detected by autoradiography. ^{32}P-labelled DNA fragments derived from pBluescript KS+ digested with *Hae*III were used as markers.

Intestinal cell cultures

Epithelial and mesenchymal cell lines

Human colon carcinoma HT29mtx cells [39] were cultured in DMEM (Dulbecco's modified Eagle's medium) supplemented with 10% (v/v) heat-inactivated foetal calf serum and antibiotics (100 i.u./ml penicillin, 100 i.u./ml streptomycin and 40 μg/ml gentamycin). The HT29mtx cells had been selected from the parental HT29 colon carcinoma cell line by adaptation to growth in the presence of 10 μM methotrexate and subsequently cultured without the drug; under these conditions the cell line consisted of a homogeneous population of columnar mucus-secreting epithelial cells [39].

The C20 fibroblast cell line is derived from a duodenal biopsy [40] and was cultured in RPMI 1640 medium supplemented with 10% (v/v) foetal calf serum and antibiotics as indicated above.

Foetal intestinal endodermal and mesenchymal primary cultures

Intestinal endoderms and mesenchymes were isolated from 12-day mouse foetuses by dissection of collagenase-treated intestines, as described previously [41]. The tissue segments were cut into approx. 1 mm pieces and transferred to 24-well culture dishes coated with collagen I prepared from rat tail. Material corresponding to 1.5 endoderm or 1 mesenchyme was added to each well containing DMEM (1 ml) supplemented with heat-inactivated foetal calf serum (2.5%, v/v), human transferrin (10 μg/ml), human insulin (0.2 i.u./ml), human epidermal growth factor (10 ng/ml) and antibiotics as indicated above. The cells were cultured for 48 h before transfection.

Transfection experiments

For transfections, exponentially growing HT29mtx cells (1.2×10^5 cells per well) or C20 cells (0.5×10^5 cells per well) were seeded in 24-well culture dishes and cultured for 24 h before transfection. The poly(ethyleneimine)-based transfection agent Exgen500 was used in accordance with the manufacturer's instructions. In brief, Exgen500 (11 μl per transfection of HT29mtx cells and 4 μl per transfection of fibroblasts or primary embryonic cells) was mixed with DNA (2 μg per transfection) and added to the cells, followed by centrifugation (280 ***g***, 5 min). After 2 h the medium was changed. Each transfection contained 2.67 pmol of *LAMC2*/luciferase construct and 0.2 μg of a cytomegalovirus (CMV) IE promoter-driven LacZ gene construct (pCMVβ). In some cases 0.1 μg of an expression vector for JunD (a gift from Dr P. Sassone-Corsi) was included as indicated in the Table legends. Transfected cells were lysed after 48 h (deletion and mutation analysis in HT29mtx cells and C20 fibroblasts) or 20 h (primary cells, and JunD co-transfection) in 150 μl of Dual Light lysis buffer. In some experiments, HGF (50 ng/ml final concentration), TGF-β1 (10 ng/ml final concentration) or PMA (50 μM final concentration) was added 24 h after transfection and the cells were harvested 24 h later.

Luciferase and β-galactosidase activities were measured with the Dual Light system and a Lumistar luminometer. The luminometer was set to measure luminescence every 0.2 s. The 0.4 s measuring point corresponded to the peak in luminescence and was in all cases recorded as relative light units (RLU). To permit comparisons between the various experimental situations, the luciferase activities were normalized to the protein content in the extracts. The protein concentration was measured by the Bradford method [42], with BSA as standard. Normalization to protein content gave standard deviations similar to those obtained by normalization to β-galactosidase activity. The results from the transfected primary cells were normalized to β-galactosidase activity. This was decided because the tissue culture wells were coated with collagen I and blocked with BSA, which might have interfered with the protein determination.

Preparation of nuclear extracts, DNase I footprinting and electrophoretic mobility-shift assay (EMSA)

Nuclear extracts were prepared from untreated and PMA- and HGF-treated (4 h) HT29mtx cells by the procedure described in [43], with the modification that proteins in the cleared lysate were precipitated by the addition of $(NH_4)_2SO_4$ (0.3 g/ml). Precipitated proteins were pelleted (20000 ***g***, 30 min), resuspended in 25 mM Hepes (pH 7.6)/40 mM KCl/0.1 mM EDTA/10% (v/v) glycerol/1 mM dithiothreitol/10 μg/ml leupeptin/10 μg/ml aprotinin and dialysed twice against the same buffer. The dialysate was cleared (20000 ***g***, 30 min) and the protein concentration was

determined as described above. For DNase I footprinting, the −0.34 kb *LAMC2*/luciferase construct was linearized by either *Nhe*I (the 5′ end of the promoter fragment) or *Hin*dIII (in the polylinker between the 3′ end of the *LAMC2* promoter and the luciferase gene) and labelled with [γ-^{32}P]ATP (4000 Ci/mmol) by the use of T4 polynucleotide kinase. End-labelled fragments were released by digestion with either *Hin*dIII or *Nhe*I and purified by agarose-gel electrophoresis. The DNA fragments were incubated with 40 μg of nuclear proteins and 2 μg of poly(dI-dC) followed by treatment with DNase I as described previously [44,45].

EMSA was performed as described previously [45] with 500 ng of poly(dI-dC) and 100 ng of sonicated salmon-sperm DNA as non-specific competitors in each binding reaction. In some cases, antibodies (1 μg) against AP-1 proteins (c-Jun, sc-45; JunB, sc-46; JunD, sc-74; c-Fos, sc-52; FosB, sc-48; Fra-1, sc183; Fra-2, sc-604), Sp1 (sc-59), Sp3 (sc-644) or Ets-1/2 (sc-112) were added to the binding reactions and incubated for 50 min at 16 °C before the addition of the probe. The 5′AP-1/Sp1 probe, its mutated versions (mutants 2, 3 and 7), the truncated 5′AP-1 probe and the Sp1-con probe were double-stranded oligonucleotides; the sequences shown in Figure 7(A) (see Figure 6 for the sequences of the mutations) were prepared by annealing complementary oligonucleotides as described [45]. Labelling with [γ-^{32}P]ATP (4000 Ci/mmol) was performed by the use of T4 polynucleotide kinase followed by purification in a non-denaturing polyacrylamide gel (10 %, w/v).

RESULTS

Characterization of the basal transcriptional activity of the *LAMC2* promoter

For the characterization of the 2.5 kb 5′ flanking region of *LAMC2*, the colon carcinoma cell line HT29mtx was chosen as a model for intestinal epithelial cells expressing laminin-5; indeed, immunoprecipitation with an antibody against the laminin-5 trimer revealed the expression of the three constituent chains of laminin-5 in these cells [46]. We confirmed that HT29mtx cells express the *LAMC2* mRNA encoding the laminin-5 γ2 chain (Figure 2A).

To evaluate the role of the various upstream elements in controlling the basal expression of *LAMC2*, a series of 5′ deletions of the *LAMC2* 5′ flanking region were generated and placed in front of the luciferase gene (Figure 1). After transfection into the epithelial HT29mtx cells, cytoplasmic extracts were prepared and the luciferase activities were determined (Table 1). The 2.5 kb *LAMC2*/luciferase construct led to a significant luciferase activity that was approx. 40 % of the activity observed with equimolar concentrations of the simian virus 40 early-promoter-driven luciferase gene. Deletion from position −1.2 kb to position −0.34 kb resulted in a halving of reporter gene activity; deletion from position −0.15 kb to −0.12 kb reduced the luciferase activity further to 28 %. Determination of the promoter sequence (Figure 3) to position −0.34 kb followed by computer-mediated sequence analysis resulted in the identification of two consensus AP-1-binding sites (the 5′AP-1 site from positions −92 to −86 and the 3′AP-1 site from positions −51 to −45) and a putative TATA box from positions −32 to −28. This TATA box has previously been reported and shown to be functional [36]. Inspection of the region surrounding the 5′AP-1 site led to the identification of a sequence (positions −87 to −80; lower strand) with some similarity to the consensus binding sequence for members of the Sp1 transcription factor family. An identical *LAMC2* promoter sequence was reported recently, although the atypical Sp1 site was not mentioned [47].

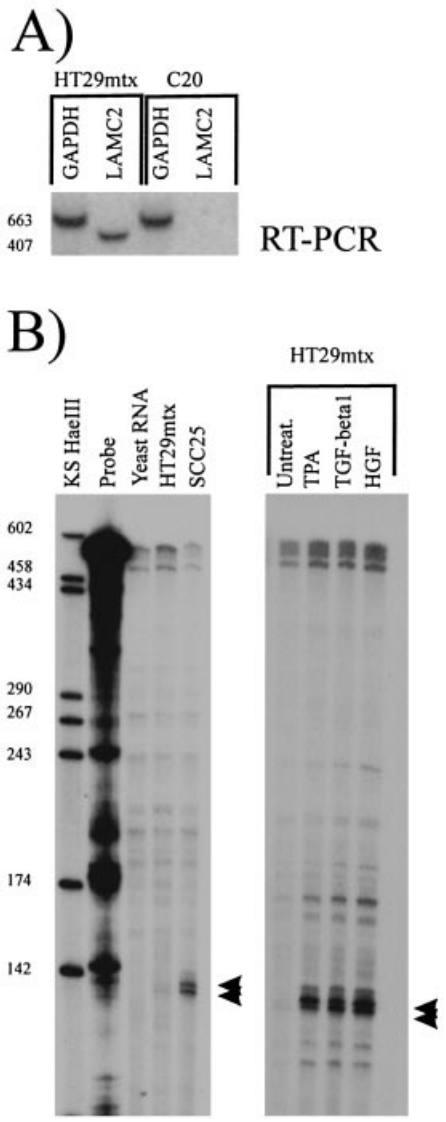

Figure 2 Expression of the laminin γ2 chain mRNA

(**A**) RT–PCR analysis of total RNA prepared from HT29mtx cells or C20 small-intestinal fibroblasts was performed for 35 cycles, with primer pairs for either the laminin γ2 chain (*LAMC2*) mRNA or the human glyceraldehyde-3-phosphate dehydrogenase (GAPDH) mRNA. Specific RT–PCR fragments for *LAMC2* (407 bp) or GAPDH (663 bp) were detected after electrophoresis through an 3 % (w/v) agarose gel. (**B**) RNase mapping was performed with an anti-sense RNA probe that spanned the transcriptional initiation site in the *LAMC2* promoter. Unstimulated HT29mtx cells express low levels of the laminin γ2 chain transcript compared with that in the squamous carcinoma cell line SCC25. After treatment with PMA (TPA), TGF-β1 or HGF, the level of laminin γ2 chain mRNA was increased in the HT29mtx cells to a similar extent. The sizes of the DNA marker bands are indicated and the double arrowhead indicates the bands that were protected owing to the presence of laminin γ2 chain mRNA.

Response of the *LAMC2* promoter to PMA, HGF and TGF-β1

The potential effect of two cytokines, HGF and TGF-β1, and of a phorbol ester, PMA, was analysed with the RNAse assay. When a RNA anti-sense probe spanning the previously reported transcriptional initiation site of the *LAMC2* promoter [36] was hybridized to RNA from HT29mtx cells, two faint protected bands migrating just below the 142 nt marker band were observed (Figure 2B). This is in agreement with the expected sizes of 136 nt and 134 nt if the major initiation sites previously described for

Table 1 Deletion analysis of the LAMC2 5′ flanking region in HT29mtx cells

Various deletions ranging from positions −2.5 to −0.12 kb of the LAMC2 5′ flanking region were placed in front of the luciferase reporter gene and transfected into the human colon carcinoma cell line HT29mtx. Luciferase activity was determined in cytoplasmic extracts and expressed relative to the protein content; results are means ± S.D. and the numbers of experiments are indicated in parentheses. As a control the simian virus 40 early-promoter-driven luciferase (SV40-luc) gene was used in separate experiments. The regularity of the transfection efficiency was checked by co-transfection of the β-galactosidase-expressing CMV–LacZ construct in each experiment.

Construct	RLU/μg of protein
2.5 kb	1767 ± 191 (8)
1.2 kb	1426 ± 139 (4)
0.34 kb	861 ± 164 (4)
0.15 kb	774 ± 133 (9)
0.12 kb	220 ± 91 (8)
SV40-luc	4339 (2)

Figure 3 Sequence of the *LAMC2* promoter

The sequence of the first 339 bp upstream of the transcriptional initiation site is shown. It corresponds to the sequence of the −0.34 kb deletion construct. The positions of the −0.15 kb (Δ149) and −0.12 kb (Δ115) deletion constructs are indicated. The positions of the 5′AP-1-binding site, the 3′AP-1-binding site and the Sp1-binding site are written above the sequence. The TATA box is also indicated above the sequence. The regions downstream of position −0.15 kb that were protected by nuclear proteins from unstimulated or PMA-stimulated HT29mtx cells during DNase I footprinting (Figure 5) are shown: dark grey boxes represent protection by the extract from PMA-stimulated cells only, the untinted box represents protection by the extract from unstimulated cells only, and the light grey boxes represent protections that were observed with both extracts. The arrows (in the same colour codes) indicate the presence of hypersensitive sites.

the *LAMC2* promoter [36] are also used in HT29mtx cells. Moreover, the level of γ2 chain mRNA was substantially lower in HT29mtx cells than in the squamous carcinoma cell line SCC25 used as control (Figure 2B), which expresses laminin-5 at high levels [48]. Stimulation of the HT29mtx cells for 24 h with PMA, TGF-β1 or HGF resulted in a clear and comparable increase in the level of γ2 chain mRNA (Figure 2B). Thus the expression of *LAMC2* was stimulated to a similar extent by both TGF-β1 and HGF in HT29mtx cells. It has previously been reported [49] that the treatment of subconfluent HT29mtx cells with PMA results in their scattering, i.e. in the dispersion of cell colonies and in changes in the morphology of the individual

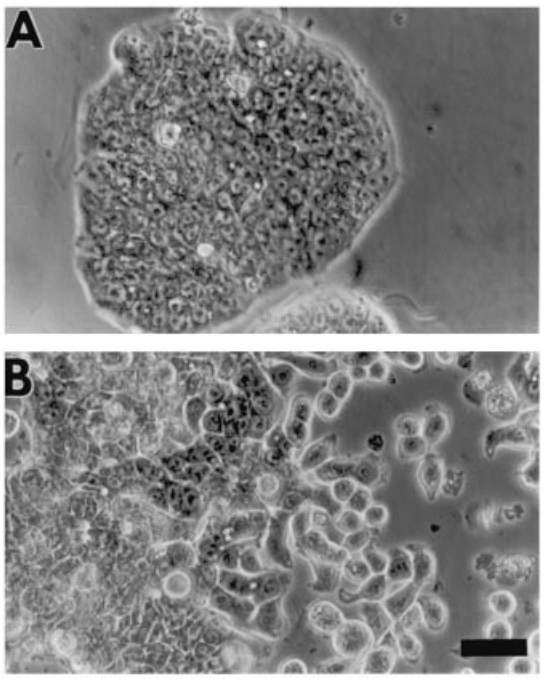

Figure 4 HGF-induced scattering activity on HT29mtx cells

Phase-contrast microscopy reveals that after 24 h of culture in HGF-containing medium (**B**), the edges of the colonies were no longer delineated and cells had lost their intercellular contacts in comparison with control cells (**A**). Scale bar, 30 μm.

cells to a fibroblast-like appearance. The observation of the HGF-treated HT29mtx cells shows (Figure 4) that HGF had a similar scattering activity on these cells. After 24 h of HGF treatment, the cells at the periphery of the HT29mtx cell colonies had moved away from one another and cell-to-cell contacts diminished. No morphological difference was noted when TGF-β1 was added to the cells (results not shown).

To define the promoter region implicated in the response to PMA and cytokines, the 2.5 and 0.12 kb *LAMC2*/luciferase constructs were transfected into HT29mtx cells and treated by PMA, HGF or TGF-β1 for 24 h. The results (Table 2) show that the 2.5 kb construct was strongly stimulated by both PMA and HGF (6.7-fold and 8.9-fold respectively). The 0.12 kb construct was also responsive to PMA and HGF, although HGF led only to a 6.1-fold stimulation. Similar results were obtained for the −0.15 kb construct in HT29mtx cells (results not shown). TGF-β1 stimulated only the activity of the 2.5 kb construct and to a smaller extent (2.3-fold). The control promoter (CMV–lacZ) analysed in these experiments revealed that the β-galactosidase reporter gene activity was also increased after treatment with PMA (2.3-fold), HGF (1.3-fold) or TGF-β1 (1.3-fold); this could have been due either to a stimulation of this promoter by stress-activated kinases such as the Jun N-terminal kinase [50] or to a general small increase in transfection efficiency.

Importance of the 5′AP-1 site for the epithelial response to PMA and HGF

To dissect further the critical *cis*-elements involved in the positive response, DNase footprinting and mutation analysis were performed.

Nuclear extracts were prepared from unstimulated and PMA-stimulated HT29mtx cells and used to protect a DNA fragment

Table 2 Stimulation of the LAMC2 promoter by PMA, HGF and TGF-β1 in HT29mtx cells

The −2.5 and −0.12 kb LAMC2–luciferase constructs were transfected into HT29mtx cells, followed by treatment with PMA, HGF or TGF-β1. The β-galactosidase activities resulting from the co-transfected CMV–lacZ construct have been pooled for each experimental condition. Results are means ± S.D.; the numbers of experiments are indicated in parentheses. A Student's t test was performed for each promoter construct to determine the significance of the observed stimulation. $^{*}P < 0.05$, $^{**}P < 0.01$.

		RLU/μg of protein			
Construct	Treatment ...	None	PMA	HGF	TGF-β1
−2.50 kb		1767 ± 191 (8)	11888 ± 1164 (5)**	15715 ± 6860 (5)**	4101 ± 776 (5)**
−0.12 kb		220 ± 91 (8)	2419 ± 386 (8)**	1352 ± 341 (8)**	379 (2)[1]
CMV–lacZ		5442 ± 902 (22)	12412 ± 2499 (18)**	6837 ± 1802 (6)*	6822 ± 1459 (5)*

[1] No t test was performed because of the small number of experiments.

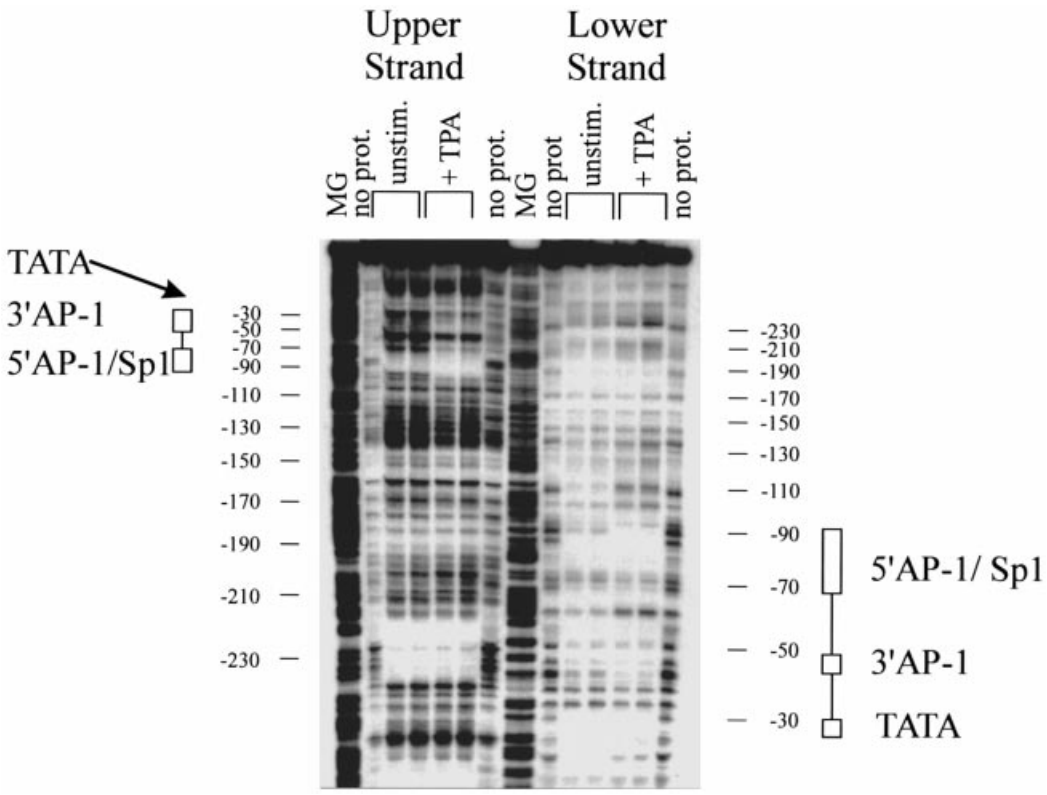

Figure 5 DNase I footprinting of the *LAMC2* promoter

Nuclear extracts were prepared from unstimulated or PMA (TPA)-stimulated (24 h) HT29mtx cells and used to protect the fragment shown in Figure 1 from digestion by DNase I. The footprinting was performed on both the upper and the lower strands. MG indicates Maxam/Gilbert G/A sequencing reactions on each strand. The positions (in bp) relative to the transcripional start site are indicated. The positions of the 5′AP-1 and the 3′AP-1 sites on both the upper and lower strands are indicated by boxes. The TATA box region is indicated by a box on the lower strand and by an arrow on the upper strand.

spanning the *LAMC2* transcriptional initiation site from digestion by DNase I (Figure 5). Protected regions were detected both upstream and downstream of position −0.15 kb but as the region between positions −0.34 and −0.15 kb was without functional significance in the transfection experiments (see Table 1), only the footprints downstream of position −0.15 kb are described in detail. The footprint experiment was performed on both the upper and lower DNA strands and the precise limits of protected regions and the positions of hypersensitive sites are indicated on Figure 3. Downstream of position −0.15 kb, only a region surrounding the 5′AP-1 site was protected on both strands. With the unstimulated HT29mtx nuclear extract, the protection of the 5′AP-1 site covered the region from positions −91 to −70 on the upper strand and positions −77 to −92 on the lower strand. After stimulation with PMA, the footprint was extended and covered the region from positions −70 to −95 on the upper strand and from positions −77 to −97 on the lower strand. No clear protection was observed over the 3′AP-1 site but the bands that appeared in the region were less intense with the PMA-stimulated extract than with unstimulated HT29mtx extract on both strands; moreover, a site hypersensitive to DNase I was observed on the upper strand with both extracts just

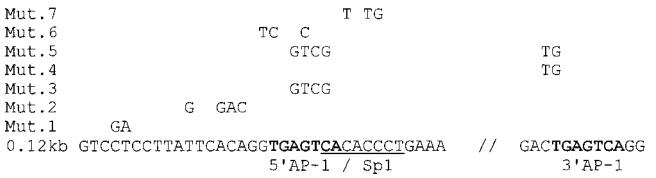

Figure 6 Mutations introduced in the *LAMC2* promoter

Seven LAMC2 promoter fragments (Mut. 1–7) carrying the indicated mutations were generated by PCR using the −0.12 kb *LAMC2* promoter construct as template. The fragments were subsequently placed in front of the luciferase reporter gene and used to transfect HT29mtx cells (see Table 3). Characters in **bold** represent the AP-1 consensus binding sequences and the characters underlined represent the potential Sp1-binding site.

Table 3 Mutational analysis of the *LAMC2* promoter

Constructs (mutants 1–7) consisting of the −0.12 kb *LAMC2*–luciferase construct carrying the mutations shown in Figure 6 were transfected into HT29mtx cells. After transfection, the cells were either left untreated or treated with PMA or HGF. Cytoplasmic extracts were prepared 24 h after stimulation (48 h after transfection) and luciferase activity was determined. The measured luciferase activities were normalized to the protein content in the extracts. Numbers of experiments are indicated in parentheses. For each experimental condition (no treatment, PMA treatment and HGF treatment) the one-way ANOVA test was performed and led to the definition of groups with high (H), intermediate (I) or low (L) reporter gene activity, as indicated by superscripts ($P < 0.01$).

		RLU/μg of protein		
Construct	Treatment ...	None	PMA	HGF
Mutant 7		249 (2)	3422 (2)	1495 (2)
Mutant 6		39 (2)	330 (2)	135 (2)
Mutant 5		20 ± 3 (4)^{L}	137 ± 16 (4)^{L}	46 ± 3 (4)^{L}
Mutant 4		158 ± 26 (4)^{H}	1840 ± 223 (4)^{I}	720 ± 101 (4)^{I}
Mutant 3		19 ± 3 (6)^{L}	92 ± 21 (6)^{L}	33 ± 10 (6)^{L}
Mutant 2		184 ± 15 (3)^{H}	2432 ± 738 (3)^{H}	1294 ± 175 (3)^{H}
Mutant 1		168 ± 44 (3)^{H}	2586 ± 221 (3)^{H}	1324 ± 114 (3)^{H}
−0.12 kb		220 ± 91 (8)^{H}	2419 ± 386 (8)^{H}	1352 ± 341 (8)^{H}

upstream (position −55) of the 3′AP-1 site. On the lower strand there was additional protection over the TATA box. This protection covered the region from positions −17 to −33 with the unstimulated extract and from positions −26 to −33 with the PMA-stimulated extract. On the upper strand this region was close to the top of the gel but it is clearly seen that no region with a size corresponding to the protection on the lower strand was protected in this area.

The importance of the 5′AP-1 site was investigated by introducing mutations in this area (Figure 6 and Table 3) in the −0.12 kb construct. The effects of these mutations on the response to PMA and HGF were analysed by transfection into HT29mtx cells (Figure 6 and Table 3). The effects of mutations 1 to 5 were evaluated with a one-way ANOVA (analysis of variance). In the untreated HT29mtx cells, two groups with significantly different ($P < 0.01$) reporter gene activities were identified. The first group retained most of the activity obtained with the wild-type 0.12 kb construct: this group contained mutants 1, 2 and 4; mutant 4 possessed a mutated 3′AP-1 site. The second group of constructs, displaying severely decreased reporter gene activity, consisted of mutants 3 (mutation of the 5′AP-1 site) and 5 (the 5′AP-1/3′AP-1 double mutation). The same differences were observed after stimulation with PMA and HGF, except for the mutant 4 construct, with a mutation in the 3′AP-1 site, which displayed a reporter gene activity intermediate between the high-activity group (the wild-type construct and mutants 1 and 2) and the group with low reporter gene activity (mutants 3 and 5). Thus it can be concluded that the 5′AP-1 site was essential for both basal and HGF-stimulated *LAMC2* promoter activity. The 3′AP-1 site, in contrast, was not important for basal activity of the promoter in HT29mtx cells but made a minor contribution to promoter activity during stimulation with HGF (assessed by mutant 4). Mutation in the potential Sp1 site (mutant 7) did not change the basal or HGF-stimulated activity. Thus the HGF response was not influenced by the ability of putative Sp1 proteins to bind downstream of the 5′AP-1 site.

HGF and PMA treatments change the patterns of proteins binding to the 5′AP-1/Sp1 region

EMSA was performed with a probe spanning the protected region at the 5′AP-1 site (this region is referred to below as the 5′AP-1/Sp1 region) together with nuclear extracts prepared from untreated, HGF-treated or PMA-treated HT29mtx cells (Figure 7). An intense (I) and a faint (II) complex were generated with the 5′AP-1/Sp1 probe (Figure 7B, lane 1) in the HT29mtx extract. After treatment with HGF or PMA, the intensity of complex II was increased (Figure 7B, lanes 2 and 3). The shorter 5′AP-1 probe, lacking most of the sequence with similarity to the Sp1 consensus binding sequence, generated only one complex (Figure 7B, lanes 4–6) migrating with a mobility intermediate between that of complexes I and II. The intensity of this 5′AP-1 probe complex was increased after treatment with PMA and HGF. The Sp1 consensus oligonucleotide (Sp1-con) generated one major complex and three additional complexes of lower intensity (Figure 7B, lanes 7–9). The intensity of the major Sp1-con protein–DNA complex was not affected by treatment with either HGF or PMA. A non-specific band (marked with an asterisk) that could not be competed out with unlabelled specific or unrelated oligonucleotides (results not shown) was also observed. All other complexes represented specific protein–DNA interactions as judged by competition experiments with specific or unrelated oligonucleotides (results not shown).

AP-1 and Sp1 proteins bind to overlapping sequences in the 5′AP-1/Sp1 region

The consequences of the introduced mutations (Figure 6 and Table 3) for the binding of proteins to the 5′AP-1/Sp1 region were investigated by EMSA (Figure 8). With the nuclear extract prepared from untreated HT29mtx cells together with either the 5′AP-1/Sp1 probe or the mutant 2 probe, almost identical patterns with an intense complex I and a faint complex II were seen (Figure 8, lanes 1 and 2). EMSA performed with the mutant 3 oligonucleotide (with a mutation in the 5′AP-1 site) or the mutant 7 oligonucleotide (with a mutation in the putative Sp1-binding site in the 5′AP-1/Sp1 region) resulted respectively in the disappearance of complex II (Figure 8, lane 3) or of complex I (lane 4). This suggests that distinct proteins bind to the AP-1 and the Sp1 parts of the 5′AP-1/Sp1 region.

After treatment with HGF the pattern of the complexes was modified. The intensities of both complexes I and II, which were now equal, were decreased with the mutant 3 oligonucleotide (Figure 8, lane 7), whereas there were no differences with the mutants 2 and 7 oligonucleotides (lanes 6 and 8). This suggests that the amounts of AP-1 proteins were increased and that both complexes I and II now mainly contained AP-1 proteins. Treatment with PMA led to similar observations (results not shown).

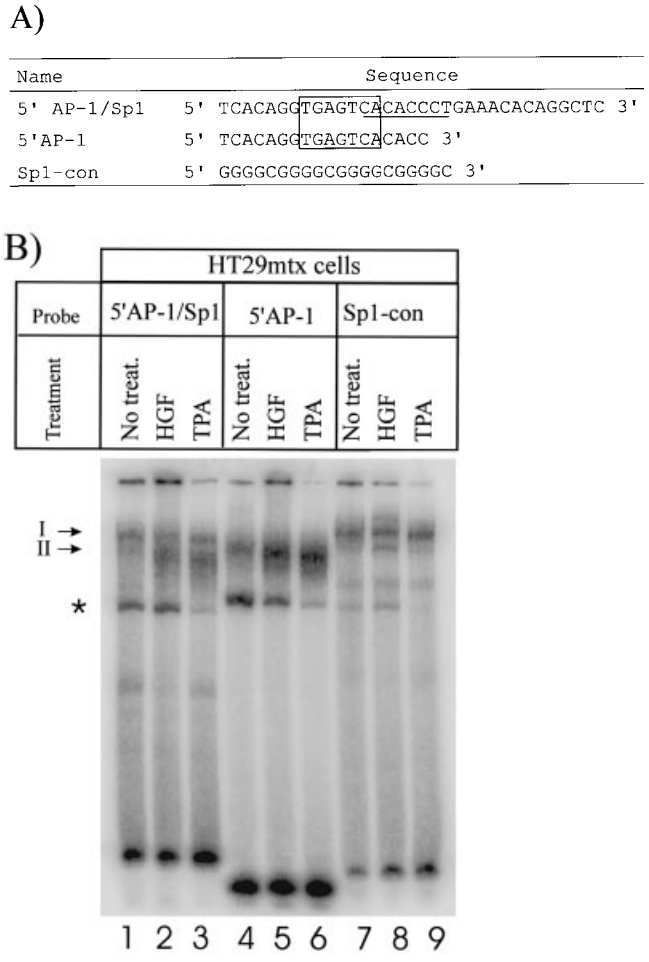

Figure 7 Changes in the binding pattern of proteins to the 5′AP-1/Sp1 region after treatment with HGF or PMA

(**A**) The double-stranded regions of the oligonucleotides used as probes and competitors in this study. The 5′AP-1/Sp1 oligonucleotide contained the overlapping AP-1 and Sp1 sites. The 5′AP-1 oligonucleotide was a 3′ truncated version of the 5′AP-1/Sp1 probe. The Sp1-con oligonucleotide contained a consensus binding site for Sp1. The AP-1 (boxed) and the Sp1 (underlined) binding sites are indicated in the *LAMC2*-promoter-derived sequences. (**B**) EMSA was performed with the probes indicated and nuclear extract (3 μg) prepared from untreated, HGF-treated or PMA (TPA)-treated HT29mtx cells. The slowly migrating complexes (I and II) obtained with the 5′AP-1/Sp1 probe are indicated with arrows. The asterisk marks a non-specific band.

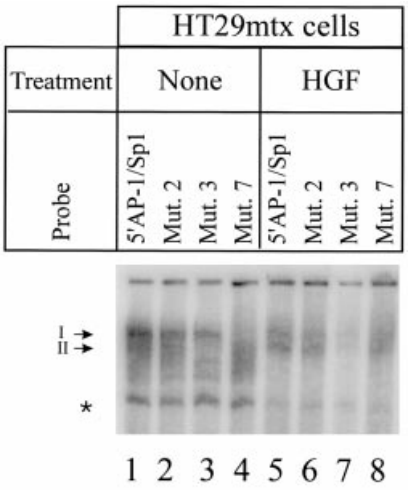

Figure 8 Protein binding to the AP-1 and Sp1 parts of the 5′AP-1/Sp1 region

EMSA was performed with the 5′AP-1/Sp1 probe or with mutated versions carrying the mutations (mutants 2, 3 and 7) shown in Figure 6. The nuclear extracts were prepared from untreated or HGF-treated HT29mtx cells. With the extract from untreated HT29mtx cells, the mutant 3 probe affected the complex II band and the mutant 7 probe affected the complex I band. With extracts from HGF-treated HT29mtx cells, the mutant 3 probe affected the bands of both complexes I and II equally, whereas the mutants 2 and 7 probes were without effect. The asterisk marks a non-specific band.

The protein composition of the protein–DNA complexes was analysed by EMSA in combination with antibodies against AP-1 and Sp1 proteins (Figure 9). With the use of nuclear extracts from untreated HT29mtx cells together with the 5′AP-1/Sp1 probe, strong supershifts were observed with antibodies against the Sp1 family proteins Sp1 and Sp3 (Figure 9A, lanes 10 and 11). Moreover, the intensity of the complex I band was strongly decreased on the addition of the Sp1 antibody, confirming that this complex contained Sp1. With antibodies against AP-1 proteins, only very faint supershifted bands were observed with JunD, Fra-1 and Fra-2 antibodies (Figure 9A, lanes 4, 7 and 8). With the use of the truncated 5′AP-1 probe, which lacked most of the Sp1-binding site, a strong supershift was observed with the JunD antibody (Figure 9B, lane 4), whereas only very little effect of the Sp1 antibody was now detected (Figure 9B, lane 10). The simultaneous addition of both JunD and SP1 antibodies did not result in supershifted bands with lower mobility in comparison with the addition of the antibodies individually (compare Figures 9A and 9B, lanes 12, with Figures 9A and 9B, lanes 4 and 10), indicating that the binding of JunD and Sp1 to the 5′AP-1/Sp1 region was mutually exclusive. The fact that only a faint JunD supershift was observed with the 5′AP-1/Sp1 probe in comparison with the results with the truncated 5′AP-1 probe might suggest that the Sp1 proteins prevented the binding of AP-1 proteins to the probe. This contrasts somewhat with the findings obtained with the mutated probes (Figure 8); a more likely explanation is that the antibody decreased the affinity of the AP-1 dimer for the site, allowing Sp1 to bind instead. Thus such an inhibitory effect of the antibody on the AP-1 dimer was not seen with the truncated probe because it lacked the Sp1-binding site. Such an effect of the antibody might have been due to a destabilization of the AP-1 dimer.

JunD-containing AP-1 dimers mediate the HGF response

In EMSA with nuclear extracts prepared from HGF-treated HT29mtx cells together with the 5′AP-1/Sp1 probe and antibodies against AP-1 and Sp1 proteins (Figure 9A), a clear supershift was generated with the JunD antibody; moreover, the intensities of both complexes I and II were decreased after the addition of the antibody (Figure 9A, lane 4, HGF panel). With this nuclear extract the binding of Sp1 proteins was also decreased (Figure 9A, lane 10, HGF panel). This finding supports the conclusion drawn from the EMSA experiment with mutated probes (Figure 8) that AP-1 proteins are the dominating proteins binding to the 5′AP-1/Sp1 probe after treatment with HGF. With the use of the truncated 5′AP-1 probe and the extract from HGF-treated cells, a strong supershift with the JunD antibody

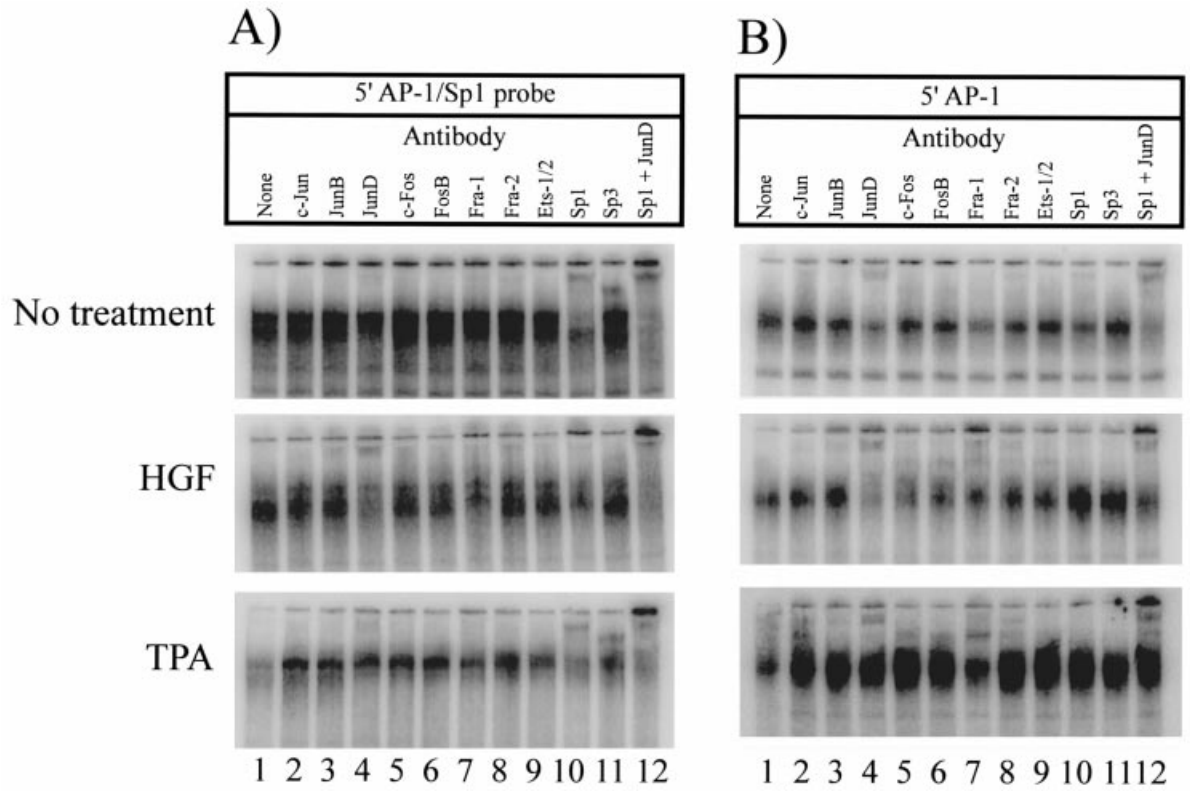

Figure 9 JunD, Sp1 and Sp3 bind to the 5′AP-1/Sp1 probe in HGF-stimulated nuclear extracts

EMSA was performed with the 5′AP-1/Sp1 probe (**A**) or the 5′AP-1 probe (**B**) and nuclear extracts from untreated, HGF-treated or PMA (TPA)-treated HT29mtx cells. Antibodies against AP-1 proteins (c-Jun, JunB, JunD, c-Fos, Fra-1, Fra-2) and Sp1 proteins (Sp1 and Sp3) were added as indicated to the binding reactions. An antibody against the conserved Ets domain of Ets transcription factors was used as a negative control (lanes 9).

Table 4 Overexpression of JunD stimulates the LAMC2 promoter following HGF addition

The −0.12 kb LAMC2 promoter–luciferase construct was co-transfected into HT29mtx cells together with an expression vector for JunD with or without the addition of HGF. The luciferase activities were normalized with respect to the activity obtained in Expt 1. The differences observed between Expts 1 and 2 and between Expts 3 and 4 respectively were evaluated with Student's *t* test. The stimulations of the promoter activity as a result of the addition of HGF are indicated in the last column. JunD overexpression resulted in a statistically significant (*, $P < 0.001$) stimulation of the promoter activity only when HGF was present. Results are means ± S.D.; the numbers of experiments (*n*) are indicated in parentheses.

Expt	Co-transfection	Addition	Normalized activity	Stimulation by HGF (fold)
1	None	None	1.0 ± 0.4 (3)	
2	JunD	None	2.2 ± 0.6 (3)	
3	None	HGF	8.6 ± 1.4 (3)	8.6
4	JunD	HGF	26.6 ± 0.4 (3)*	12.1

was also seen (Figure 9B, lane 4, HGF panel). With both probes, fainter supershifted bands were also observed with antibodies against JunB and against the Fos family proteins c-Fos, FosB, Fra-1 and Fra-2 (Figures 9A and 9B, lanes 3 and 5–8). With the use of nuclear extracts from PMA-treated HT29mtx cells, similar results were obtained with both probes (Figures 9A and 9B, PMA panel), again clearly demonstrating the involvement of JunD (Figures 9A and 9B, lane 4, PMA panel). In conclusion, our results show that the 5′AP-1/Sp1 region is occupied by JunD-containing AP-1 dimers and that the levels of these are increased after treatment with either HGF or PMA. To evaluate the functional significance of this observation an expression vector for JunD was co-transfected with the −0.12 kb *LAMC2* promoter–luciferase construct with and without the addition of HGF (Table 4). Overexpression of JunD led to a slight (but not statistically significant, $P > 0.05$) increase in *LAMC2* promoter activity. The addition of HGF alone led to the the above-described stimulation of the *LAMC2* promoter; the concomitant overexpression of JunD resulted in a further increase in the promoter activity that was 12.1-fold higher than that obtained under the basal conditions. This experiment thus demonstrated the ability of JunD-containing AP-1 dimers to influence the response of the *LAMC2* promoter to HGF.

Tissue specificity of the transcriptional activity of the *LAMC2* promoter

To analyse whether the *LAMC2* 5′ flanking region contains *cis*-elements that provide epithelium-specific transcriptional activity, we performed complementary experiments with a small-intestinal fibroblast cell line (C20), which was established from a duodenal mucosal biopsy [40]. RT–PCR with primers for the laminin γ2 chain mRNA failed to detect the expression of *LAMC2* in these cells (Figure 2A). Transfection of the 1.2 kb

LAMC2 promoter–luciferase construct in the C20 cells led to a luciferase activity that was one-sixth of that in HT29mtx cells [235 ± 45 RLU/μg of protein ($n = 3$) and 1426 ± 139 RLU/μg of protein ($n = 4$) respectively]. For the short 0.12 kb construct this difference was decreased to 2.3-fold [97 ± 18 RLU/μg of protein ($n = 3$) and 220 ± 91 RLU/μg of protein ($n = 8$) respectively]. Because this study was performed in epithelial and fibroblast cell lines, we decided to analyse whether the epithelium-specific *cis*-elements upstream of position −0.12 kb also worked in freshly isolated tissues. Embryonic endoderm and the surrounding sheet of mesenchyme were separated by the dissection of collagenase-treated 12-day foetal mouse intestines and were treated separately. Co-transfection of the 1.2 kb promoter–luciferase construct with the CMV–LacZ construct into the primary cells yielded significantly higher ratios of luciferase to β-galactosidase [18.3 ± 6.7 and 1.05 ± 0.4 ($n = 6$) respectively] in the endoderm than in the mesenchyme. These results show that the 1.2 kb *LAMC2* fragment upstream of the transcriptional initiation site can direct a cell-type-specific transcription in foetal intestinal endoderm in a way that mimics the expression of endogenous *LAMC2*.

DISCUSSION

Taken together, the different approaches used in this study led to the conclusions that (1) at least two *cis*-element regions, between positions −2.5 and −0.12 kb of the promoter, are important in the epithelium-specific expression of *LAMC2*, (2) the 120 bp region immediately upstream of the transcription start site allows a basal transcription of *LAMC2* in cells of both epithelial and mesenchymal origins, and (3) this region comprises regulatory elements responsible for the cytokine stimulation.

Although elements necessary for driving the cell-specific expression of the *LAMC2* promoter are present in the 1.2 kb segment of the promoter, a recent study indicates that elements outside −5900 and +55 might be needed for the normal expression of *LAMC2 in vivo* [47]. Here we have concentrated our attention mainly on the AP-1 sites of the 120 bp region. The mutation analysis shows that the AP-1 part of the 5′AP-1/Sp1 region is essential for both basal transcription and the HGF or PMA response of the *LAMC2* promoter; in contrast, the 3′AP-1 region has only a limited importance. Furthermore, the supershift analysis demonstrates that JunD-containing AP-1 dimers are the major proteins that bind to the 5′AP-1/Sp1 region during stimulation with HGF and PMA. It could also be demonstrated that treatments with HGF and PMA both result in a clear increase in AP-1 dimers binding to the probe. In these conditions two protein–DNA complexes (I and II) are generated with the full 5′AP-1/Sp1 probe and the intensities of both complexes are decreased by mutations in the AP-1-binding site. The 5′AP-1 site overlaps with a non-consensus binding site for the ubiquitously distributed Sp1 transcription factor [51,52]. Sp1 and Sp3 proteins are able to bind to this site. However, mutations in the Sp1 region do not change the transcriptional activity of the 120 bp promoter construct, indicating that the binding of the Sp1 and Sp3 proteins to this site is not of prime importance in the transcription of *LAMC2*. In addition, the binding of Sp1 proteins to the 5′AP-1/Sp1 region does not interfere with AP-1 protein binding and functionality, suggesting that Sp1 and Sp3 bind with a lower affinity than AP-1 dimers to the same region. This conclusion is based on the finding that mutations in the Sp1-binding site do not lead to an increased binding of AP-1 proteins in EMSA or to an increased reporter gene transcription in transfection experiments. However, the mutation does lead to a marked decrease in the intensity of the complex I band generated with extracts from untreated HT29mtx cells, this complex also being supershifted by antibodies against Sp1. It is therefore possible that the Sp1 site might have a role in *LAMC2* transcription *in vivo* when the levels of AP-1 proteins are low, for example in other cell types.

The comparison of the *LAMC2* promoter with those of other ECM-coding genes reveals some interesting peculiarities. Among the laminin constituent chains, the laminin γ1 chain (the product of the *LAMC1* gene) is found in at least 10 laminin variants [53]. The promoter for *LAMC1* is GC-rich; proteins binding to CTC- and GC-rich elements, including Sp1, are important for its activity [54,55]. The *LAMC1* promoter shares these characteristics with several other promoters for genes encoding proteins present in the basement membrane, such as collagen IV [56,57], perlecan [58] and the laminin β1 chain [59]. Therefore the strict dependence on an AP-1 element makes the *LAMC2* promoter fundamentally different from these other promoters. The promoter of the *LAMA3A* gene encoding the laminin α3 chain, another chain constituent of laminin-5, comprises three functional AP-1 elements [60]. One of these is essential for basal activity of the promoter, whereas the two others are important for the promoter response to TGF-β in keratinocytes [61]. This could therefore be a common feature of the genes encoding the laminin chains present in laminin-5. Many keratinocyte-specific genes are found to have functionally important AP-1 elements in their promoters (reviewed in [62]) and the presence of two AP-1 elements in the *LAMC2* promoter is very likely to be the basis for the efficient expression of this gene also in keratinocytes.

Cytokine signalling might be of importance in the cell-specific transcription of *LAMC2* during foetal intestinal development. In the early stages of mouse intestinal development, including the stage of villous outgrowth, the mesenchyme surrounds the stratified layer of endodermal cells. In this situation the laminin γ2 chain is found in the basement membrane separating the inner layer of endodermal cells from the mesenchyme; it is produced exclusively by the endodermal cells [25]. At the same developmental stages, the HGF mRNA species are expressed by a narrow layer of mesenchymal cells adjacent to the basement membrane, whereas transcripts for the HGF receptor (the product of the c-*met* gene) are found in the endodermal cells [8]. These observations are in agreement with our transfection experiments of primary cultures, clearly showing that the *LAMC2* promoter activity is higher in endodermal cells than in the mesenchymal population, as in HT29mtx epithelial cells compared with the C20 fibroblastic cell line. In addition we report here that the *LAMC2* promoter is strongly stimulated by HGF in the epithelial HT29mtx cells. In contrast, HGF had no effect on the *LAMC2* promoter in the C20 intestinal fibroblast cell line (results not shown). This differential response pattern could be due at least partly to a differential expression of the HGF receptor. Indeed we detected, by the use of RT–PCR, higher levels of the c-*met* transcripts in the HT29mtx cells than in the C20 cells (results not shown); however, these latter cells synthesize HGF mRNA species [40]. Thus our results suggest a simple model to explain how the expression pattern for the laminin γ2 chain is stimulated and maintained during early intestinal development: HGF produced by the mesenchyme might induce a strong positive signal for *LAMC2* transcription via its binding to the c-*met* receptor present in the endodermal cell. These observations are of particular interest linked to the results reported by Salo et al. [47], who describe, for transgenic mice expressing *LAMC2* promoter–LacZ constructs, that the low basal transcriptional activity of the constructs in the skin is strikingly stimulated in wounding conditions when the keratinocytes are induced to migrate. Indeed, in similar wounding conditions it has

been shown, by studies *in vitro* with intestinal epithelial cells, that HGF on the one hand and laminin-5 on the other hand are involved in a compensatory migration process of this kind [9,10]. It remains to be established what is the nature of the signals emanating from the epithelial cells *in vivo* that allow the synthesis of the γ2 chain to be turned on by the mesenchymal cells, as observed during late development [25]. In future the use of co-cultures of endodermal cells and fibroblastic C20 cells stably transfected with the promoter constructs should allow us to answer this question.

In conclusion, the results presented here emphasize that a reciprocal cytokine-mediated cross-talk between epithelial and mesenchymal cells regulates the expression of basement membrane molecules. The finding that the promoter of the gene for laminin γ2 chain is up-regulated by HGF is of particular interest because it indicates a potential role for laminin-5 in intestinal morphogenesis; in addition, our results corroborate previous observations that suggest that this molecule is involved in intestinal epithelial cell migration and even invasion.

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