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Extracellular matrix components in intestinal development

P. Simon-Assmann, M. Kedinger, A. De Arcangelis, V. Rousseau and P. Simo

INSERM U.381, 3, avenue Molière, F-67200 Strasbourg (France), Fax +33 88 26 35 38

Abstract. Intestinal morphogenesis and differentiation are dependent on heterotypic cell interactions between embryonic epithelial cells (endoderm) and stromal cells (mesenchyme). Extracellular matrix molecules represent attractive candidates for regulators of these interactions. The structural and functional diversity of the extracellular matrix as intestinal development proceeds is demonstrated by 1) spatio-temporal specific expression of the classically described constituents, 2) the finding of laminin and collagen IV variants, 3) changes in the ratio of individual constituent chains, and 4) a stage-specific regulation of basement membrane molecule production, in particular by glucocorticoids. The orientation/assembly of these extracellular matrix molecules could direct precise cellular functions through interactions via integrin molecules.

The involvement of extracellular matrix, and in particular basement membrane molecules in heterotypic cell interactions leading to epithelial cell differentiation, has been highlighted by the use of experimental models such as cocultures, hybrid intestines and antisense approaches. These models allowed us to conclude that a correct elaboration and assembly of the basement membrane, following close contacts between epithelial and fibroblastic cells, is necessary for the expression of differentiation markers such as digestive enzymes.

Key words. Extracellular matrix; intestine; development; differentiation; interactions; integrins; hormones.

Introduction

Like many vertebrate organs, the digestive tract develops from heterogeneous embryonic anlagen. The epithelium derives from the endodermal germ layer, whereas the connective tissue and the musculature derive from the splanchnopleural mesenchyme.

The multistep process of intestinal morphogenesis and cell differentiation occurs in a precise species-specific temporal and spatial pattern. The sequences of structural and ultrastructural changes that take place in the epithelium during ontogenesis are closely similar in all species; only the timing of these processes is variable. For example, in rodents much of small intestinal development occurs late in the gestational period. This pattern differs from that of the human small intestine, which is well developed early in gestation. Whatever the species, morphogenetic and differentiation processes involved in small intestinal development include (see fig. 1) 1) formation of the primitive villi by folding of the embryonic endoderm, 2) progressive differentiation of endodermal cells into absorptive cells (also called enterocytes), goblet or mucus cells, and endocrine cells, 3) formation of crypts, at the base of villi, where dividing cells are then segregated at the perinatal period; a new specialized cell type appears, the Paneth cell which remains restricted to the bottom of the crypt, and 4) continuous maturation in the mature organ of the four main cell lineages from crypt to villus tip. During development as well as in the course of the crypt/villus maturation, differentiation of epithelial cells consists of morphological changes paralleled by the synthesis of specific proteins. The structural polarization of absorptive cells allows individualization of a specialized membrane domain, the apical brush border. This domain is composed of microvilli which are sustained by a highly organized cytoskeleton and endowed with functional proteins such as digestive enzymes (lactase, sucrase and peptidases) and transporters, implied in the terminal steps of carbohydrate/protein digestion and in absorption. Unlike the small intestine, the large intestine, or colon, is characterized by deep glands and a flat surface epithelium; its major function is to regulate fluid and electrolyte transport. A complete description of precise intestinal organogenesis is beyond the scope of this review and is well documented in detail in excellent reviews[62, 70, 83].

Ontogenic regulation by epithelium/mesenchymal interactions has been suggested for a number of organ systems. In the intestine, using several experimental models, it has been demonstrated that the mesenchyme plays a permissive role in the morphogenesis and cytodifferentiation of the digestive tract endoderm. This conclusion has been drawn by using, in particular, interspecies recombinants composed of chick and rat intestinal anlagen, in combination with species-specific morphological and biochemical analyses[65]. In addition, the potential involvement of reciprocal instructive interactions has been emphasized by the fact that, for example, intestinal mesenchyme was able to induce an intestinal cytodifferentiation of chick gizzard endoderm[48]. The presence of a sheath of fibroblasts underlying the crypt epithelium and migrating at least partly in

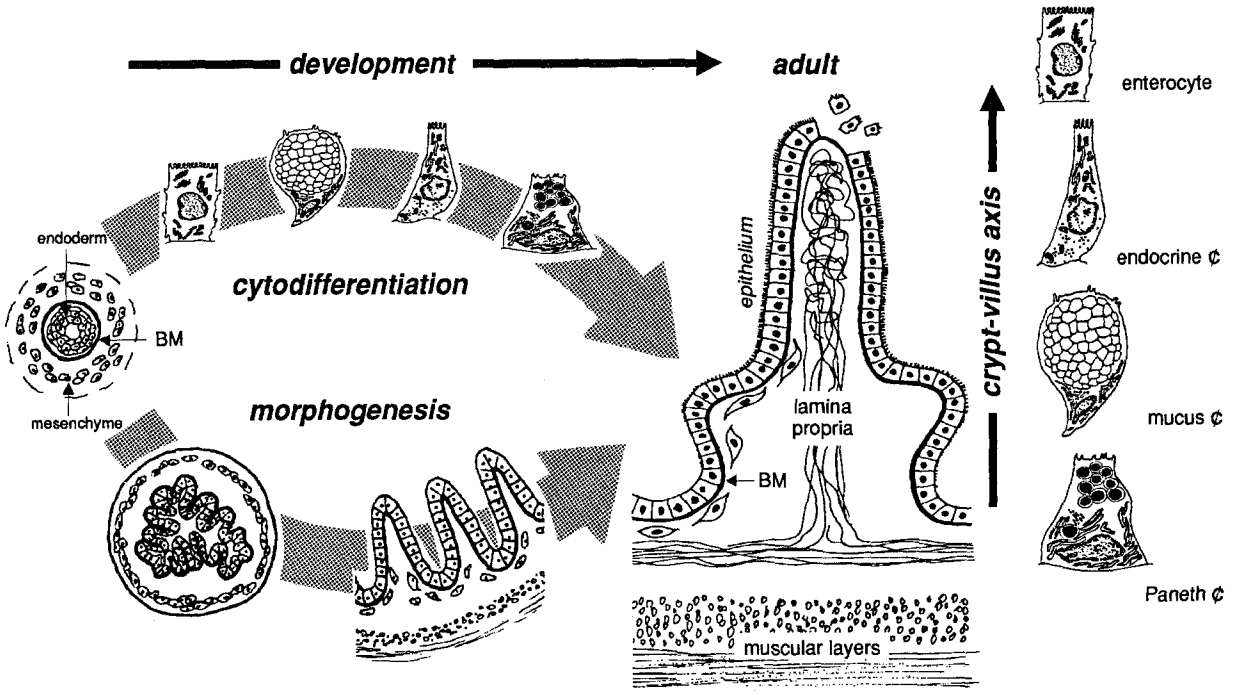

Figure 1. Schematic representation of the main morphological features and onset of the major epithelial cell types during intestinal morphogenesis and crypt to villus migration. The endoderm progressively differentiates into the villus epithelium; in the adult, cell diversification arises from the crypt compartment located at the base of the villi. The mesenchyme gives rise to the connective tissue – the lamina propria – and to the muscular layers. The subepithelial basement membrane (BM) is located at the epithelial/mesenchymal interface from early stages of development up to the adult stage. ¢: cell.

synchrony with epithelial cells strongly suggested that epithelial/mesenchymal interactions were still operating in the adult mature organ. This hypothesis has been demonstrated experimentally in recombination experiments showing that neonatal crypt cells as well as fibroblasts from the neonatal lamina propria retain properties similar to those of their embryonic forms[48,68].

It is well established that in multicellular organisms extracellular matrix (ECM) molecules control cell growth and differentiation. This ECM network includes the interstitial matrix and basement membranes (BM). Interstitial matrix surrounds cells in the stromal connective tissue compartment, while BM are specialized sheet-like ECM that separate the connective tissue from epithelia, muscle fibers, blood vessels and nerves. Little is known about the functional role of ECM components in intestinal tissue although numerous sporadic data on location/expression of ECM molecules and receptors are available. The aim of the present review is 1) to integrate these descriptive data in order to understand how ECM could contribute to cell movements, and lead to the establishment and maintenance of polarized epithelial cells, and 2) to describe in vitro models that allow the study of the regulation of cell-ECM interactions.

Changes in ECM organization accompany morphogenesis of the developing intestine

ECM is formed by a complex set of collagens, non-collagenous glycoproteins and proteoglycans that has a unique composition in each organ. Expression of ECM molecules is tightly regulated; some are transiently expressed at particular times in development, whereas others are continually expressed up to adulthood.

At the electron-microscopic level, the subepithelial basement membrane corresponds to a continuous sheet of amorphous electron-dense material, also referred to as the 'basal lamina'. Electron-microscopic immunostaining of rat duodenum performed by Laurie et al.[77] showed for the first time that type IV collagen, laminin, and heparan sulfate proteoglycan were not layered, but were integrated together in the basal lamina. The distribution of known matrix components within the entire human and murine developing intestinal mucosa has been reported in comprehensive immunohistological studies[10,49,125,127]. The location of the major BM components in the adult intestine is schematically summarized on figure 2. All together, the data revealed that BM components are present at the intestinal epithelial/mesenchymal interface early in embryonic development and that changes in the spatial distribution of some ECM proteins are associated with morphogenetic processes.

Laminin is a glycoprotein having an approximate molecular weight of 900 kDa. This major component of the basement membrane is a cross-shaped heterotrimeric molecule. Laminin isolated from the Engelbreth-Holm-Swarm (EHS) tumor (laminin-1) is composed of three

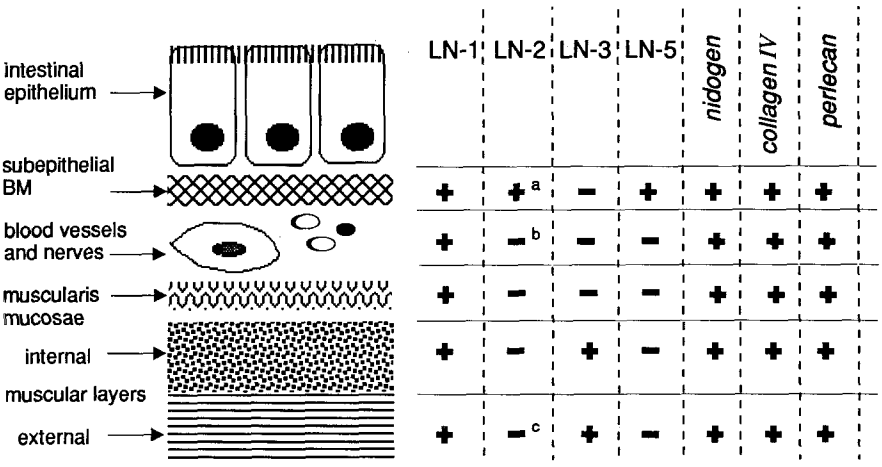

Figure 2. Summary of the distribution of the main basement membrane molecules as revealed by immunofluorescence in the different layers of the adult intestine. Presence (+) or absence (−) of fluorescence. LN-1, LN-2, LN-3 and LN-5 correspond respectively to EHS-laminin, merosin, S-laminin and nicein. Concerning LN-2, it should be pointed out that [a] the staining is restricted to the BM underlying the crypt region; in [b] labeling is found some fibers underneath the crypts and in [c] capillaries of the muscle.

peptidic chains A, B1 and B2[11] recently named $\alpha 1$, $\beta 1$ and $\gamma 1$[20]. Using polyclonal antibodies, laminin-1 was detected immunocytochemically at 12 days of gestation in the rat intestine[127] and at 8 weeks of gestation in the human[10] at the epithelial-mesenchymal junction as well as around a few cellular structures (presumably blood vessels) scattered within the mesenchyme. At a later developmental stage in rodent, just before villus formation, the staining observed in the mesenchyme was intensified and became confined to two distinct areas: the zone immediately beneath the epithelial-mesenchyme interface, and the most peripheral zone of the mesenchyme which will differentiate into muscular layers[127]. Throughout the following developmental period, laminin-1 was still detected at the boundary between epithelial cells and the stromal compartment; during this villus elongation period, the BM staining was however more regularly found at the base of the villi. In the adult, laminin-1 was linearly distributed from crypts to villus tips. By in situ hybridization, laminin $\gamma 1$ messenger RNA in the 12 day fetal mouse intestine was detected exclusively in the mesenchymal cells and preferentially in those immediately adjacent to the epithelium. Thereafter, laminin $\gamma 1$ mRNA expression was found to be strongest in cells forming the muscle layers, the muscularis mucosae and the lamina propria[119].

The study of neosynthesized laminin-1 from rat intestinal segments taken at various stages of development, purified by affinity chromatography on heparin-Sepharose has revealed interesting changes in the level and molecular forms of laminin-1 as a function of intestinal development[122]. Indeed, the maximal biosynthetic activity of laminin occurred in rat intestinal tissues during the gestational period (16–18 days) showing that the fetal intestine synthesizes very large amounts of laminin-1 at the same time as the commencement of intestinal differentiation, i.e. villus emergence and individualization of the smooth muscle layers proceed. Another peak of laminin-1 synthesis, although weaker than the first one, was detectable when crypts formed (zone of the dividing cells) by invagination of the base of the villi into the mucosalconnective tissue. Evaluation of relative proportions of individual laminin polypeptides showed that the intestine, like many organs, produced laminin $\beta 1$ and $\gamma 1$ subunit forms in excess of $\alpha 1$ subunit whatever the developmental stage considered. Interestingly, the ratio of the amounts of $\alpha 1$ and $\beta 1/\gamma 1$ polypeptides varied during morphogenesis, $\alpha 1$ chain level being maximal during villus formation[71,122]. Northern blot analyses partly confirmed these observations; indeed, examination of RNA transcripts for the laminin $\beta 1$ and $\alpha 1$ chains in the developing rat intestine revealed peculiar temporal patterns (fig. 3). Amounts of steady-state $\beta 1$ and $\alpha 1$ mRNA expression were much higher in the fetal intestine compared to the adult mature organ. Surprisingly, the laminin $\alpha 1$ chain probe hybridized to a ~6 kb band and not to the predicted 9.5 kb transcript (fig. 3). We are uncertain about the significance of this small transcript. Yet Vanden Heuvel and Abrahamson[148] recently showed that both 9.5 kb and 6 kb transcripts exist in developing kidney, and that this smaller transcript is enriched in tubular fractions. However, Klein et al.[71] could identify a very weak 10 kb signal for laminin $\alpha 1$ mRNA in total RNA from 13-day-old embryonic intestine but not from later stages. Furthermore, using three different cDNA fragments detecting different regions of laminin $\alpha 1$ chain mRNA, they were able to identify a 10 kb signal from poly(A) + RNA; according to this study, no truncated forms of $\alpha 1$ chain mRNA were expressed in any tissues analyzed at this embryonic stage.

Despite a high level of cell migration and turnover in the adult mature intestine (2 to 6 days according to the species), production of new BM is low. Indeed, laminin

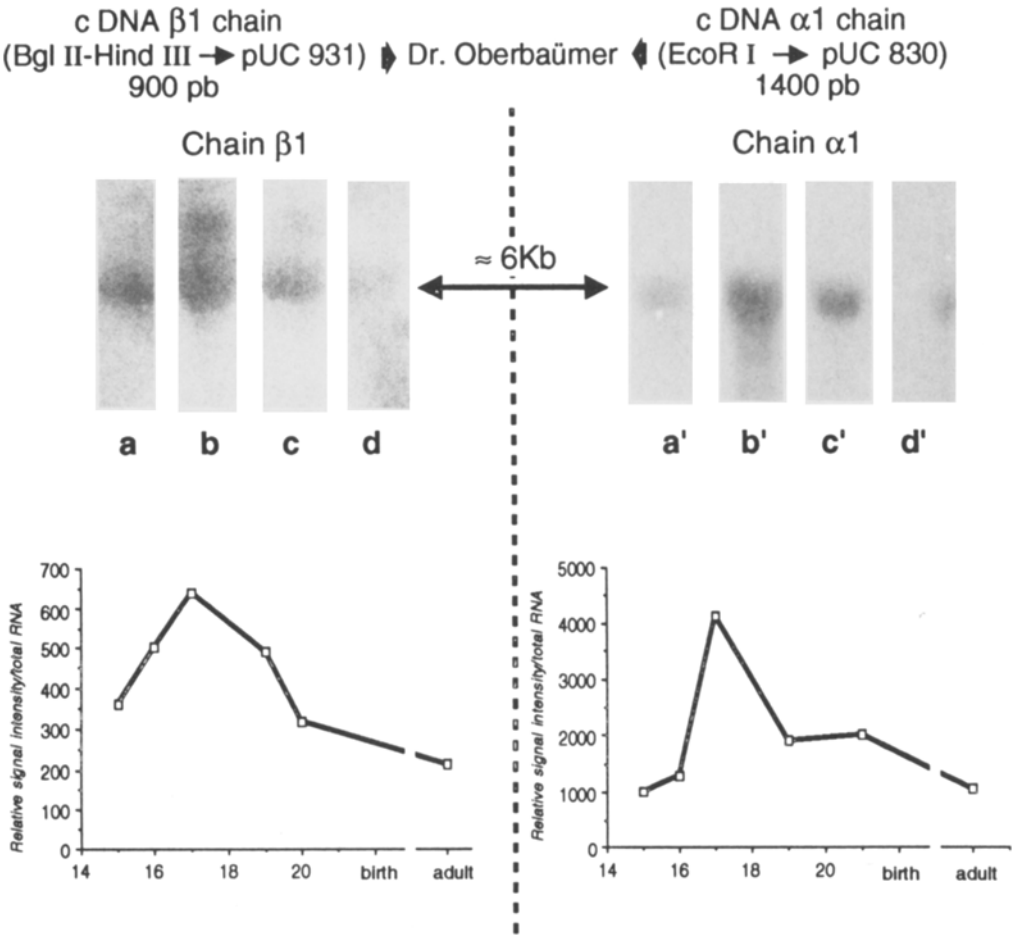

Figure 3. Northern blot analysis of mRNA for laminin $\beta 1$ (a–d) and laminin $\alpha 1$ (a′–d′) chains in RNA isolated from 15-(a, a′), 17-(b, b′), 19-(c, c′) day-old fetal rat intestines and from adult rat intestines (d, d′) (Simo et al. unpubl. results). Integrity of RNA from each preparation was determined using an actin probe. The lower part of the figure depicts semi-quantitative profiles of laminin $\beta 1$ and $\alpha 1$ mRNAs in the developing intestines. RNAs were electrophoresed and transferred onto nitrocellulose filters, and hybridized with the corresponding cDNA probes kindly provided by Dr Oberbäumer[34,96]. The cDNAs were ^{32}P-labeled using the random priming technique; specific activity was between 10^7–10^8 cpm/μg of DNA.

messenger RNA assessed by in situ hybridization[119] and Northern blot (fig. 3) is far lower in the adult than during fetal stages. Moreover, Trier et al.[144] clearly demonstrated by labeling laminin molecules in vivo with anti-laminin IgG that laminin-1 turnover occurs focally in the BM of adult mouse jejunum over weeks; the persistence of some staining along the length of the crypt-villus axis for as long as six weeks provides strong evidence that the BM does not comigrate with its overlying epithelium or underlying myofibroblasts. Immunohistological studies using anti-laminin-1 polyclonal antibodies did not reveal any clear gradient along the crypt-villus axis in adult rodent or human intestine[9,126,127] (fig. 4*A*). Yet, in the adult rodent organ, laminin $\beta 1/\gamma 1$ chains were found homogeneously distributed in the crypt and villus basement membrane, whereas $\alpha 1$ chains were restricted to the crypt zone[122]. Interestingly, in the adult human intestine the $\alpha 1$ chain, found at the subepithelial basement membrane, presented a decreasing gradient of intensity from the tip of the villus to the crypt mouth; no obvious staining was observed in the crypt-cell compartment (fig. 4*B*). These observations point to the potential expression of laminin isoforms along the crypt-villus basement membrane.

From recent studies, it has become apparent that laminin-1 is a member of a family of proteins[20,145]. In fact, molecular biology techniques and availability of specific antibodies have demonstrated 5 laminin variants up to now. These members of the laminin family are expressed in different tissues and at different times during development. An $\alpha 1$ chain variant, the $\alpha 2$ chain (formerly called M chain), has been characterized and shown to be associated with the classically described $\beta 1$ and $\gamma 1$ chains forming the laminin-2 or merosin molecule[41,80]. Laminin-2 was first identified as a protein present in basement membranes of trophoblasts, Schwann cells, and striated muscle[80]. The use of monoclonals against the high molecular weight $\alpha 2$ chain emphasized the heterogeneous composition of laminin

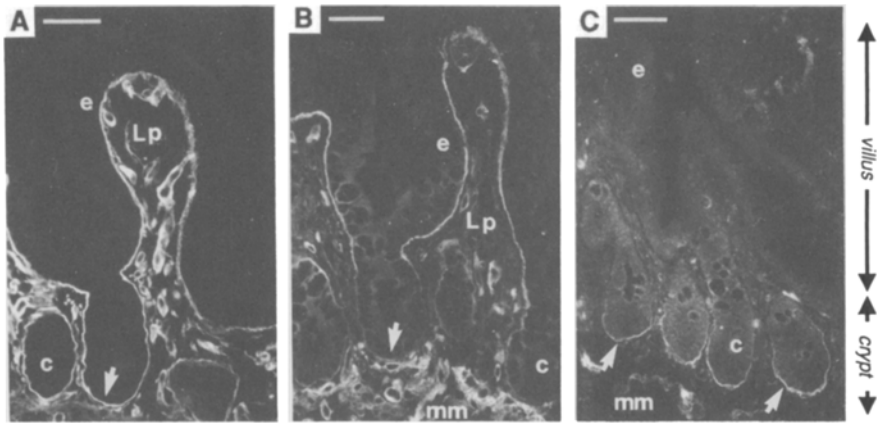

Figure 4. Immunostaining showing the location of *A* laminin-1 detected with a polyclonal antibody, of *B* laminin α1 chain with mAb 4C7, and of *C* laminin α2 chain with mAb 4G9, on cryosections of intestine from a 4 year-old child. e, epithelium; Lp, lamina propria; mm, muscularis mucosae; c, crypts; arrows point to the epithelial/stromal interface in the crypt region. Bar: 50 μm.

molecules in the adult human intestine. α2 chain was exclusively present in the basement membrane at the bottom of the crypts[9,126] (fig. 4*C*) thus depicting a complementary pattern of α1 and α2 chain expression in the human intestine. These data show that at least in the mature human intestine, α1 and α2 chains are mutually exclusive. It is worth noting that α2 chain was only expressed when crypt downgrowths occurred, exhibiting immediately a typical localization in the crypt region[126].

The family of laminin variants also includes a 600 kDa molecular weight glycoprotein termed BM-600, kalinin or nicein[85]. This protein, now called laminin-5, was successfully immunoprecipitated from cultures of keratinocytes from healthy donors but not from patients suffering from a lethal dermatosis, Herlitz junctional epidermolysis[150]. In addition, this molecule has been shown to immunolocalize to anchoring filaments bridging hemidesmosomal structures to lamina densa[108]. Taken together, these data indicate a possible role for laminin-5 in cell-substrate adhesion and BM cohesion. In the small intestine, indirect immunofluorescence studies revealed that, in opposite to laminin-1, laminin-5 was restricted to the subepithelial BM; BM of blood vessels and nerves were not stained (Orian-Rousseau et al., unpubl. data). Yet, a clear increasing gradient of staining was obvious from the mouth of the crypt gland to the villus tip; the staining appeared scarcely if at all detectable in the crypt region. This distribution pattern of laminin-5 was superimposable on one hand to that of HD1 (Fontao et al., unpubl. data), one of the intracellular hemidesmosomal proteins mediating cell adhesion to the extracellular matrix[53] and on the other hand to the β4 integrin subunit[9,126]. The α6β4 integrin heterodimer is known to be localized in hemidesmosomal plaques in cornea and skin[59,136]. However, these hemidesmosomal structures are known to be associated with collagen VII, a major structural component of anchoring fibrils[112] that is absent from the intestine[79]. Thus, it is interesting to note that a certain number of the hemidesmosomal components are mostly located at the basal surface of differentiated cells that migrate up the villi; this suggests that in contrast to the classically described hemidesmosomes, the 'hemidesmosome-like structures' found in the intestine do not function as an anchorage device.

The other laminin variants, S-laminin (laminin-3), S-merosin (laminin-4) and K-laminin (laminin-6) have not so far been studied in the intestinal organ. However, in a comprehensive study dealing with the expression of laminin variants in the adult human smooth muscle, Glukhova et al.[45] found that S chain, newly called β2 chain, could be detected only in the adult muscular layers. This is in contrast with laminin-1 which is the predominant laminin variant found in the developing human colon and with laminin-2 which is restricted to capillaries in the adult muscle coat. Generally, the nature of the effects of each laminin variant on cell behavior (migration, proliferation, differentiation) remains largely unsolved and is beginning to be analyzed mainly by in vitro model systems.

Type IV collagen, another major structural component of BM, is a triple-helical molecule composed of three α chains. Collagen IV molecules which were assumed to contain two α1 (IV) chains and one α2 (IV) chain are now known to harbor also α3 (IV), α4 (IV), α5 (IV) and α6 (IV) chains[98,147]. To date, five genetically distinct type IV isoforms have been described in mammals[158]. The existence of numerous α chains of collagen IV that can be connected in various arrangements strengthens the complexity of the BM organization. In the adult and developing gut, the expression of subepithelial collagen IV molecules strictly paralleled that of laminin-1[10,127], that is homogenously distributed as development proceeds along the mature crypt-villus axis. In situ hybridization allowed the demonstration of the widespread

presence of α1 (IV) collagen messenger in the intestinal embryonic mesenchyme and in the differentiated mesenchymally-derived compartments (lamina propria, muscular cells . . .). Redistribution of type IV mRNA in the mesenchyme, leading to its confinement just beneath the epithelial/mesenchymal interface, paralleled the segregation of type IV collagen antigens. During villus outgrowth, high levels of mRNA were seen in the protruding villi suggesting that new BM material has to be laid down. Finally, as for laminin in the mature adult organ, no type IV mRNA could be reliably demonstrated by in situ hybridization[126]. Similar data have been produced by Weiser et al.[153], who showed by dot-blot hybridization analyses that there was little, if any, evidence for the presence of the transcripts for collagen α1 (IV) and α2 (IV) chains in the epithelial cells. Furthermore Northern blot hybridization analysis of rat intestinal poly(A)$^+$RNA showed that slight signals were observed and restricted to the lamina propria fraction. Recently, α5 (IV) and α6 (IV) mRNA transcripts of ~7 kb were shown to be expressed in a 24-week human fetal intestine although in low quantities. The fact that a deletion in α6 (IV) collagen gene occurs in inherited smooth muscle tumors, leads to the assumption that these molecules could be critical for normal smooth muscle differentiation[159].

Perlecan. Basement membranes also contain perlecan, a large low-density proteoglycan (PG) composed of heparan-sulfate (HS) side chains which was first isolated and characterized[51] from the EHS tumor. Although heparan sulfate proteoglycans are important ECM components that appear to have multiple functions, precise studies concerning their expression in the intestine are quite rare. Polyclonal antibodies prepared against EHS perlecan and directed against the core protein of the proteoglycan were used to examine the distribution of this BM component in the mature rat intestine and during its morphogenesis[125]. Again, the overall distribution of perlecan is similar to that described previously for the other BM components, laminin-1 and type IV collagen: prominent staining at the subepithelial BM and around muscular cells. However, contrasting with the regular deposition of the latter molecules at all stages of development, changes in the staining pattern of perlecan were observed around birth in the rat intestine; indeed at this period, the labeling of the BM became discontinuous and irregular from the middle to the tip of the villi. Similar transient microheterogeneities in the deposition of BM molecules have been described in other organs undergoing morphogenetic movements (for review see ref. 143). Anti-liver HSPG IgG as well as antibodies raised against HSPG purified from PYS-2 cell cultures were also able to recognize the intestinal subepithelial BM[30,134]. Yet, the PYS-2 HSPG antibodies did not label most smooth muscle tested including that of small intestine[30]. Differences in the size of the HSPG core proteins have been found and recent data provide evidence that minor variants of perlecan appear to be generated by alternative splicing[95]. These data point to structural variations between HSPG molecules in various types of basement membranes, which may account for differences in their overall function.

Entactin/nidogen. Other molecules have been described as associated with the BM. Among them entactin, also called nidogen, is a sulfated multidomain glycoprotein of 150 kDa. Its structure and biological properties have been reviewed recently[25]. Increasing evidence suggests that entactin/nidogen plays an essential role in the assembly of basement membranes forming a link between laminin-1 and type IV networks[5]. Similarly, nidogen mediates the formation of a ternary complex between laminin-1 and the core protein of proteoglycans[5]. Therefore, one can understand that the pattern of expression of entactin/nidogen strictly follows that of other BM molecules[127]. Electron immunohistochemistry revealed that entactin/nidogen was found almost exclusively in the lamina densa[87]. By Northern blot analysis, a significant amount of nidogen mRNA was found in a 12.5 gestational day mouse intestine, the only stage studied[140].

SPARC/BM40 and chondroitin sulfate proteoglycan. Other BM components have been detected in the intestinal subepithelial basements. They concern SPARC/BM40 and chondroitin sulfate proteoglycan (CSPG). SPARC is often considered as a basement membrane protein since it is produced in large amounts by EHS tumor[39]. It has a broad tissue distribution (for review see ref. 98). It is worth noting that in immunohistochemistry performed on various fetal and adult mouse organs, its preferential location was in epithelia exhibiting high rates of turnover such as the gut[111]. Production of core protein-specific monoclonal antibodies that recognize a large high density proteoglycan (bearing 13–22 chondroitin sulfate glycosaminoglycan chains) allowed McCarthy and Couchman[90] to conclude that one CSPG is a constituent of most basement membranes. The subepithelial BM in the gut, as well as basement membranes surrounding smooth muscle cells and blood vessels, were recognized by these antibodies.

Fibronectin and type III procollagen. All the other matrix molecules found in the intestinal lamina propria and smooth muscle layers belong to the family of interstitial molecules. Concerning fibronectin, several studies have localized this glycoprotein to the basement membrane of a variety of tissues (for references see ref. 75). It should be noted that at early stages of development (12 days in the fetal rat), immunostaining of fibronectin was clearly detected as a linear band at the endodermal/mesenchymal interface (unpubl. data). These data are in accordance with those of Laurie et al.[77] showing by electron microscopic immunostaining that type IV col-

lagen, laminin-1, heparan sulfate proteoglycan and fibronectin did not occur in separate layers but were integrated into a common structure. Later on, during formation of villi (17 days of gestation in the rat), fibronectin as well as type III procollagen disappeared from the top of the protruding connective tissue[127]. Finally, in the adult rodent and human intestines, fibronectin exhibited a decreasing gradient of intensity from crypt region towards the top of the villus core[10,105,127]. In the smooth muscle layer of mouse small intestine, fibronectin was localized abundantly in the narrow space between smooth muscle cells[75].

Tenascin. The first demonstration of the presence of tenascin (also known as cytotactin and J1) in the intestine was published by Thor et al.[141]. Tenascin is a hexameric glycoprotein with disulphide-linked subunits originally described as myotendinous antigen[22]. This molecule is expressed at the boundary between epithelial cells and the lamina propria in an increasing gradient towards the villus tip in mouse[4,141], rat[128] and human adult intestine[7]. More precisely, tenascin was expressed in association with ECM surrounding subepithelial fibroblasts of the lamina propria[102]. The fact that fibronectin and tenascin display inverse gradients in immunofluorescence intensities led the latter authors to speculate that this shift may trigger the cell shedding process. Further arguments were brought out by experiments using colonic cancer epithelial cells (HT29 cells) that adhered to fibronectin, but not to tenascin[102]. In addition, tenascin reduced the adhesion of the cells to fibronectin[23,102]. During development, the first onset of tenascin was only obvious in the intestine at day 14 in the mouse embryo; at this stage a faint labeling occurred mostly in the peripheral part of the mesenchyme corresponding to the region that will develop into outer muscle layers. From the 17th day of gestation, the increasing gradient from the crypt base to the top of the villus was established, becoming more accentuated in the adult organ[4,103]. A similar expression pattern of tenascin was obvious in the developing human intestine[8]. It is worth noting that the molecular form of tenascin changes as development proceeds[4,8]. When first detected, a 210 kDa chain was strongly expressed, but at birth the relative amount of 260 kDa chain increased becoming more pronounced in the adult intestine. These two tenascin polypeptides arise through an alternative RNA splicing; a 6 kb mRNA predominated during embryogenesis whereas the 8 kb mRNA appeared later[154], contrary to the situation found in kidney.

Collagen. New analytical tools have led to the discovery of the impressive diversity of the collagen family (for review see ref. 147). At present, only sporadic data are available for the intestine, compared to the wide array of these molecules. Among them, type I collagen has been found to be the major collagen type in the adult and developing human small intestine and colon[47,89] followed by type III collagen[114]. High levels of the corresponding mRNAs were mainly observed in the submucosa; no staining occurred in the muscular layers[115]. Although collagen II has traditionally been considered specific for cartilage, the intestinal tissue has been found to contain small amounts of its $\alpha 1$ (II) mRNA; in addition, $\alpha 2$ (XI) collagen seems to be synthesized in this organ, although the signal intensity in the RNAase protection assay was lower than that obtained with collagen II probes[114]. By calculating the relative amounts of various α chains, Graham et al.[47] showed that collagen type V, composed of $\alpha 1$ (V), $\alpha 2$ (V) and to a less extent of $\alpha 3$ (V), constituted a very significant proportion of total collagen in the intestine as compared with other tissues. Cells expressing high amounts of $\alpha 1$ (XIII) collagen mRNA were identified in the mucosal layer of the colon and the small intestine[115]. Various cultured cell lines, including human colonic adenocarcinoma cells are able to express multiple mRNA variants of $\alpha 1$ (XIII) chain[61]. Finally, immunohistochemical studies demonstrate that type XIV collagen is present around smooth muscle cells[21]. Conversely, no type VII collagen or type VIII collagen were expressed in the intestine[79,91,112].

Proteoglycans, large proteins carrying sulfated polysaccharides (glycosaminoglycans: GAGs) are present in virtually all mammalian tissues. The diverse structures of proteoglycans found in different tissue locations reflect their unique biological properties[142,156]. Quantitative as well as qualitative follow up of the GAGs have been performed in the developing gut[16,56,113]. Chemical analysis of purified GAGs revealed that all classical molecules are found in the intestine: hyaluronic acid (HA), heparan sulfate (HS), dermatan sulfate (DS) and chondroitin 4 and 6 sulfate (CS4 and 6). The overall GAG synthesis was maximal during fetal life and declined dramatically after birth, reaching relatively steady values maintained until adult stages[16]. One major alteration in the evolution pattern of the individual GAG species concerns the important drop in HA molecules, which accounts for the overall GAG decrease. Moreover, poorly or even completely unsulfated HS molecules were produced by early fetal intestines. Histochemically, GAGs occurred predominantly in the loose connective tissue of the villi[44,56]. A DS-PG was found located in the same region as well as in the muscle layers[131]. Among leucine-rich interstitial proteoglycans is found lumican, which contains several keratan sulfate chains. This proteoglycan showed a limited distribution in connective tissue, and was expressed in cornea as well as in muscle and intestine[12]. In the intestine, as in other tissues, the cDNA clone to lumican hybridized to a 2.0 kb mRNA, and the molecule was synthesized as a precursor protein. Its functional role remains to be elucidated, although it may regulate collagen fibril assembly through its core protein.

Thrombospondin 3. Finally, thrombospondin 3 (TSP3), belonging to a growing family of cell surface and extracellular matrix molecules, has been found to be expressed in the intestine by in situ hybridization. Its pattern of expression in various organs led to the hypothesis that TSP3 could participate in specifying or maintaining mesenchyme differentiation into muscle, bone and cartilage[104].

The increasing interest in the field of the extracellular microenvironment has led to the finding of a huge diversity in the molecular composition of the ECM. Diversity begins at the DNA level as examplified by molecules composed of chains expressed from distinct genes often distributed on different chromosomes. The second level of diversity arises from the alternative splicing of several genes resulting in different polypeptide chains. In addition, some molecules can be differentially expressed as isoforms (as laminin, type IV collagen) varying in their constituent chains or their glycosylation pattern. As an example, the $\beta 1$ chain of EHS laminin participates in the trimeric association in laminin-2 and laminin-6. Variations in the relative ratios among the major well-defined components, and in the expression of minor molecules further increase the structural organ-specific and spatio-temporal ECM heterogeneity. Finally, the orientation and assembly of the various molecules in a given tissue will direct precise cellular functions (for a review see ref. 98). In the case of the intestinal tissue, it is tempting to speculate that ECM and particularly BM microheterogeneities and asynchronous expression of their constituents during intestinal development and along the crypt-villus axis in the mature organ, may account for the functional diversity of the extracellular microenvironment.

Expression of receptors could contribute to cell movements during morphogenesis and in the adult

The differential expression and functional state of receptors for BM molecules may also contribute to morphogenetic movements and differentiation of epithelial cells during development and cell renewal. A large number of cellular receptors able to bind ECM molecules has been described[55,109,130].

Integrins appear to be the major receptors by which cells attach to the ECM. Integrins are a large family of at least 21 different heterodimers, each consisting of an α- and β-subunit that associate nonconvalently. It is now known that at least five α-subunits – $\alpha 1$, $\alpha 2$, $\alpha 3$, $\alpha 6$, and $\alpha 7$ – can combine with the $\beta 1$-subunit to form heterodimers able to bind to EHS laminin[55]. In addition, $\alpha 6\beta 4$ as well as $\alpha 6\beta 3$ have been reported to function as laminin receptors in several organs and in various cell cultures[78].

Lamin receptors. The most prominent laminin receptors found in the developing intestine and in the adult organ in several species (human, mouse, chicken) are $\alpha 6\beta 1$ and $\alpha 6\beta 4$. These have been found located underlying the epithelium in various segments of the gastrointestinal tract[6,9,18,24,69,73,92,126,133]. The developmental expression of $\alpha 6$ and $\beta 4$ subunits was not coordinated in two species studied (human, mouse), the $\alpha 6$ expression preceding that of $\beta 4$[126]. The fact that the $\beta 4$ subunit was not detected at early stages of morphogenesis when laminin-1 is present, indicates that the $\beta 4$-containing integrins are not involved in the binding of embryonic epithelial cells to BM laminin. Concerning the expression of $\alpha 6$, $\beta 1$ and $\beta 4$ along the crypt-villus axis in the adult organ, it should be pointed out that 1) in the human intestine, all three subunits were expressed at the base of all enterocytes from the base of the crypt to the tip of the villus, while in contrast 2) in the mouse intestine, $\alpha 6$ staining was mainly confined to the crypt cell compartment unlike $\beta 4$ staining which was regularly observed along the crypt-villus axis[9,126]. The differential immunodetection of $\alpha 6$ and $\beta 4$ subunits along the crypt-villus axis in the mouse intestine is intringuing. However, these findings concerning the restricted distribution of the $\alpha 6$ subunit are in accordance with the location of laminin $\alpha 1$ chain, known to have a C-terminal sequence which binds to $\alpha 6\beta 1$ integrins[132]. One can therefore postulate that differentiation occurring along the crypt-villus axis is not only associated with changes in integrin expression but can also be linked to possible changes in receptor conformation, so that the antibody is no longer capable of recognizing it[54]. Furthermore, it has been shown that mouse $\alpha 6$ subunit can exist in two versions, $\alpha 6$A and $\alpha 6$B, which contain structurally distinct cytoplasmic domains; recent findings indicate that many differentiated cell types express $\alpha 6$A[29]. Therefore, one can also postulate that these two $\alpha 6$ isoforms bearing distinct cytoplasmic domains are differentially expressed along the crypt-villus axis in the mouse intestine and/or differ in their affinity for laminin. Integrin subunits $\alpha 1$ and $\alpha 2$ were located more or less regularly along the villus, while $\alpha 3$ subunit was expressed mostly by enterocytes lining the upper villus (refs 6, 24, 73 and authors' unpubl. data). In addition, some evident immunofluorescence staining localized in cell-cell adhesion contacts within the epithelium is even more obvious in the colon compared to the small intestine (ref. 160 and authors' unpubl. data). The new isoform of the laminin integrin, $\alpha 7\beta 1$, was expressed exclusively in the intestinal muscular layers[27].

Receptor binding fibronectin. The $\alpha 5\beta 1$ receptor binding fibronectin has not been detected[24] or is barely detectable[6] at the base of intestinal epithelial cells. Nevertheless, $\alpha v\beta 6$, another fibronectin-binding heterodimer, has been shown to be expressed in small intestinal and colonic epithelium[17]. Finally, the $\alpha 8\beta 1$ integrin, whose ligand is at present unknown, is moderately expressed in the epithelial cell layer[13].

Hyaluronate receptor and non-integrin receptor. There are additional receptors for ECM in the intestine that are worthy of note. The first subset concerns CD44 which is the major cell surface receptor for hyaluronate[3]. This integral membrane glycoprotein was prominently expressed in the BM regions lining exclusively the base of the crypts, corresponding to those regions where proliferation of epithelial cells occurs[2]. More generally, this receptor has been found preferentially expressed on epithelial cells undergoing active cell division[2]. The second subset is a 67 kDa protein, a non-integrin receptor, with high affinity for laminin[130]. Recent data of Rao et al.[106] showed that the 67 kDa mRNA levels were about ten times greater in crypt compared to villus cells in the adult intestine. The fact that the 67 kDa is a common feature of mitotically active cells is corroborated by the fact that its expression was increased in a variety of human adenocarcinomas[26]. Although this 67 kDa laminin receptor is still the subject of controversy, its role in intestinal physiology in the light of present data seems to be rather important.

Dual origin of the subepithelial basement membrane in the developing intestine

New analytical tools and models allowed more recent studies of tissue interactions and BM formation. Deposition and assembly of defined molecules into a basement membrane result from complex mechanisms which are probably unique for each system and depend on the developmental fate, physiological state of the system and environmental conditions[50]. It is now largely accepted that cooperation between various cell types is necessary for BM deposition.

The classic concept of an exclusive epithelial origin of the BM has been revisited since 1977. Indeed, Lipton et al.[82], using cultures of embryonic quail myoblasts, were probably the first to provide evidence for a dual origin of the BM showing the contribution of fibroblasts. Various experimental techniques are currently used to study the expression of basement membrane molecules. They include immunohistochemistry, biochemical approaches, or detection of transcripts by in situ hybridization on isolated tissue compartments or cell lines. Apart from the minor limitations of each model (such as cellular contamination in the case of isolated epithelial or mesenchymal cell preparations, abnormal cell behavior or loss of differentiation of cultured cells, threshold sensitivity), they all have a major drawback. Indeed, the fact that a tissue compartment expresses a given basement membrane molecule does not necessarily imply that this molecule is deposited at the BM region. Autoradiographic studies, which circumvent this problem, unfortunately do not allow discrimination between individual components. The strategy designed by Sariola et al.[116], that is interspecies hybrid glomeruli combined with species-specific antibodies, deserves special attention. The major advantage of this model is that it allows us to distinguish the deposition at the basement membrane level of a single molecule.

To study the tissue origin of BM in the gut we performed similar experiments using recombinants between chick and rodent intestines. As depicted in figure 5, isolation of pure tissue compartments was achieved with embryonic chick intestine and with fetal rat or mouse intestines, and interspecies recombinants were performed. After growth of the grafted implants, species-specific antibodies were applied on cryosections of the developed intestinal hybrid structures. This strategy allowed us to conclude that the intestinal subepithelial BM is composed of molecules produced by both cell populations. This conclusion strengthens the finding that heterotypic cell cooperation is necessary for the formation of a structured BM.

Type IV collagen. We could show that mesenchymal cells are the principal endogeneous source of this molecule[123]. This conclusion is confirmed by the localization of type IV collagen mRNA in the mesenchyme or mesenchyme-derived cellular elements of the lamina propria[124]. Yet, regional differences in basement membrane synthesis and assembly seem to occur, since in the stomach of a 12.5-day mouse embryo the collagen IV transcripts were detected in both epithelium and mesenchyme[140]. The data of Weiser et al.[153] concerning the adult mature organ are in accordance with a mesenchymal origin of collagen IV. These authors clearly showed that the mRNAs for $\alpha 1$ (IV) and $\alpha 2$ (IV) collagen were most abundant in the lamina propria; no collagen IV mRNA were evident in the enterocyte fractions even in oligo (dT)-selected RNA. It has to be noted that, during the reestablishment of the BM in the interspecies reassociations, some type IV collagen can be deposited, although transiently, by the epithelial cells[129]. The surprising but interesting finding of mesenchymal origin of collagen IV is not restricted to the intestinal system. In particular, Marinkovich et al.[84] provide evidence that dermal fibroblasts synthesize and deposit type IV collagen, as well as type VII collagen and laminin, into the basement membrane zone; these authors used as a strategy dual species cultures of bovine keratinocytes and human fibroblasts analyzed by immunofluorescent microscopy with human specific antibodies against BM components.

Perlecan. Unlike collagen IV, HS-PG (or perlecan) deposition at the BM level is achieved by the epithelial compartment; in this case HSPG labeling was exclusively with the antibodies that react specifically with the species from which the epithelial cells were taken[125].

Laminin. Concerning laminin, the third class of BM molecule analyzed, it should be noted that the laminin $\gamma 1$ chain transcripts have been localized to the mes-

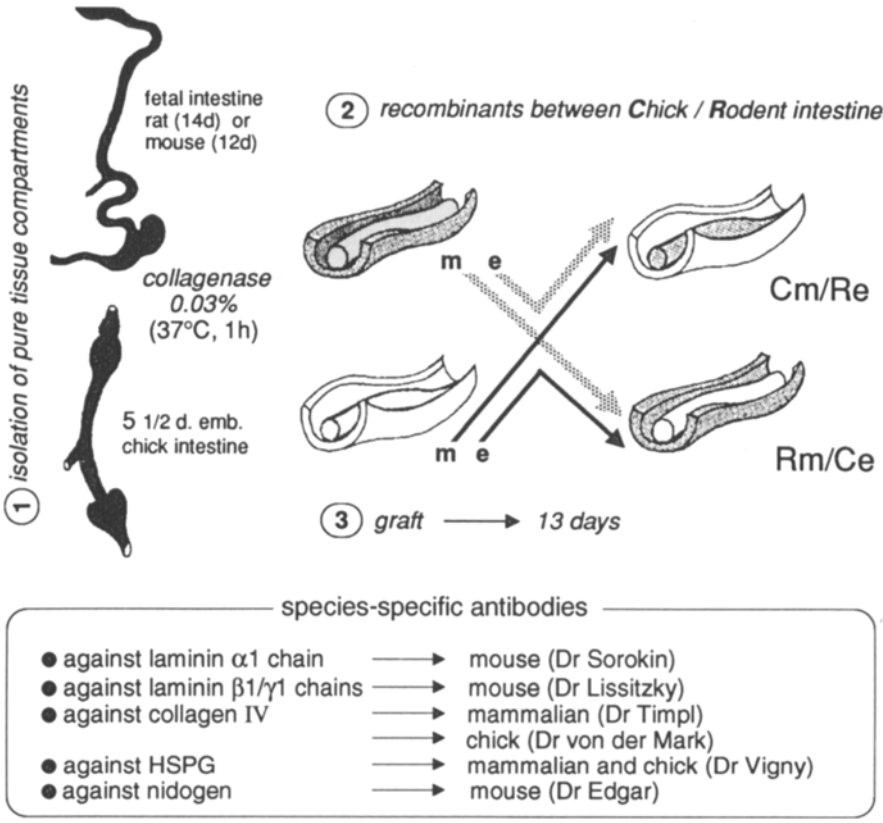

Figure 5. Schematic representation of the experimental procedure used for the study of the cellular origin of BM in the intestine. *1* Isolation of pure tissue compartments from the intestine is performed as follow: 12-day-old or 14-day-old fetal mouse or rat intestines, $5\frac{1}{2}$-day-old embryonic chick intestine are dissected out and incubated in a collagenase solution (1h, 37 °C) to disrupt the BM; *2* after opening the intestinal tube with iris scissors, the endoderm can easily be separated from the mesenchyme, and interspecies recombinants (Cm/Re: chick mesenchyme/rodent (rat or mouse) endoderm; Rm/Ce: rodent mesenchyme/chick endoderm) are performed; *3* they are then grafted into the coelomic cavity of 3-day old chick embryos or under the kidney capsule of adult nude mice to allow the development of the implants up to 13 days. The developed hybrid intestinal segments are taken at various periods of times. The species-specific antibodies recognizing either rodent or chick intestines are applied on cryosections performed through the hybrid intestine.

enchyme of embryonic intestine and in the lamina propria[119]. Yet by the filter hybridization method, messenger mRNAs for laminin $\beta 1$ and $\gamma 1$ chains were found in addition in the epithelial cell fraction in the adult[153]. From the analysis of the interspecies rodent/chick intestines with polyclonal or monoclonal antibodies recognizing rodent but not chick laminin, it can be conclude that laminin-1 has a dual, epithelial as well as mesenchymal, cellular origin[120]. Epithelial cells produce the three constituent chains of laminin during the whole development of the hybrids. In contrast, a precise chronology in the deposition of laminin chains by the mesenchyme could be seen: $\beta 1/\gamma 1$ chains were produced over the whole developmental period, while $\alpha 1$ chain deposition was delayed; this late expression of $\alpha 1$ chain may indicate that inductive influences emanating from the epithelial cells had occurred. Related to these data, expression of laminin $\alpha 1$ transcripts in mesenchymal cells localized immediately beneath the epithelium was found in organs such as gut and lung in the mouse embryo[140]. The asynchrony in the deposition of the constituent chains of laminin can be related to the data reported in the kidney: in this organ, $\beta 1/\gamma 1$ chains were constitutively expressed whereas the onset of $\alpha 1$ chain expression correlated with initiation of kidney tubules morphogenesis[72]. Immunoprecipitation of metabolically labeled molecules produced by the undifferentiated intestinal mesenchyme revealed only $\beta 1/\gamma 1$ chains of laminin[70] that could confirm the hypothesis of an inductive cell interaction process for synthesis of 1 chains; in similar experiments in the isolated endoderm, laminin $\alpha 1$ chain was coprecipitated with $\beta 1/\gamma 1$ chains. As the intestinal tissue is able to produce laminin variants (see above), further studies are necessary to determine their pattern of deposition in the BM. The possibility remains that each individual laminin chain can be secreted independently and be assembled according to a temporo-spatial pattern.

Nidogen/entactin. Found at the subepithelial intestinal BM, this has been shown to be a mesenchymal product (fig. 6) like in other tissues analyzed by in situ hybridization[35,140]. The crucial role of nidogen as an inter-

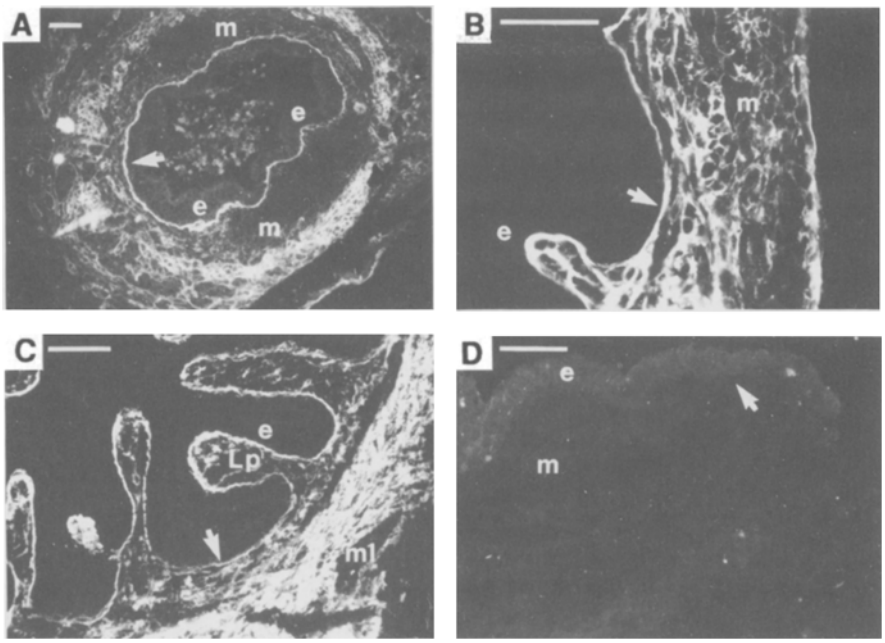

Figure 6. Cellular origin of nidogen (Simon-Assmann et al., unpubl. data). Immunodetection of nidogen molecules with rat monoclonal antibodies[38] recognizing specifically mouse but not chick antigens, on mouse mesenchyme/chick endoderm (*A–C*) and on chick mesenchyme/mouse endoderm (*D*) hydrid intestines at 4 (*A*), 6 (*D*), 10 (*B*) and 13 (*C*) days of development. e, endoderm or epithelium; m, mesenchyme; Lp, lamina propria; ml, muscular layers. Arrows point to the epithelial/stromal interface. Bars: 50 μm.

molecular linker, and of laminin α1 chain shown to be necessary for the formation of BM by MDCK epithelial cells in culture[40], leads to the speculation that both polypeptides play a key role in the formation of a stable basement membrane.

Models allowing the study of mechanisms of the role of ECM molecules in heterologous cell-cell interactions

Attempts to investigate the modulation of tissue-specific phenotypes in isolated epithelial cells have failed, mainly due to a rapid loss of cell function in culture[64]. These observations strengthen the idea that survival and differentiation of specific epithelial cells requires a precise molecular microenvironment provided by direct contact with living fibroblasts as in vivo. Indeed, a coculture system has been show to allow enterocytic differentiation starting from embryonic epithelial cells[66]. Endodermal microexplants were seeded onto a confluent feeder layer of fetal intestinal mesenchymal cells or skin fibroblasts. Whatever the cellular substrate, endodermal cells grew and, after about 4 days, covered the fibroblastic cell layer. Elaboration of complete BM comprising type IV collagen, laminin-1, nidogen and perlecan was obvious. The deposition of the BM molecules at the endodermal/fibroblastic interface was progressive and preceded the expression of epithelial differentiation markers, such as lactase. These data have been confirmed by Stallmach et al.[135] and Hahn et al.[49]. However, the latter authors found contrasting behavior of gastric versus intestinal mesenchyme for support of epithelial differentiation in vitro, emphasizing the concept of some regional specificity of the mesenchyme along the intestinal tube (see also Duluc et al.[36]). The induction of epithelial differentiation depends on the contiguity of vital mesenchymal cells, since fibroblast-derived matrices or irradiated fibroblasts were ineffective[66]. In addition, polarization of epithelial cells was not induced by any single type of matrix molecule tested (type I or IV collagen, fibronectin, laminin-1). By contrast, the use of EHS extract to which type I collagen has been added as a substratum led to a burst of cell differentiation accompanied by the expression of digestive enzymes; nevertheless, the survival time of these microexplants was rather limited as compared to coculture conditions[63]. The lack of clear effect such as differentiation when epithelial cells are cultured on isolated matrix molecules seems to be peculiar to the intestinal cells. Indeed, the morphology, behavior and even function of other cell types such as Sertoli cells or mammary epithelial cells in culture are greatly influenced by the extracellular matrix (for reviews see ref. 37, 138). It has clearly been shown that culture of epithelial Sertoli cells on laminin or Matrigel can enhance some differentiation features, such as the cAMP response to FSH[37]. In the case of mammary epithelial cells, concomitant with the organizational changes induced by the ECM, gene expression and synthesis/secretion of milk proteins are also modulated (for reviews see refs 81, 138). It should however be noted that the culture of mouse mammary cells on a floating type I collagen gel allows the cells to deposit their own basement, leading subsequently to

differentiation events[137]. Thus according to these authors, the prime effect of exogenous ECM could be to regulate the production and organization of ECM by the cells themselves.

The most likely explanation for the apparent discrepancy between organs is that de novo biosynthesis by mesenchymal cells is required to form the BM in cooperation with the epithelial cells in the intestine. This idea is perfectly in accordance with in situ observations, showing a close morphological association between mesenchymal or myofibroblastic cells and epithelial cells in the intestine when intensive proliferation and differentiation occur[86,88,97]. Furthermore, coculture experiments eliminating direct contact by placing a Millipore filter in between epithelial and fibroblastic cells did not lead to enterocytic differentiation. The necessity of dynamic and reciprocal heterocellular cooperation in the intestine is also stengthened by the data obtained by Aufderheide and Ekblom[4]. Indeed, in a coculture system, tenascin expression in intestinal mesenchyme was triggered specifically by the epithelial cells.

Other arguments are brought by the effects of glucocorticoids on ECM molecules in epithelial-fibroblastic coculture experiments. Glucocorticoids are responsible for structural and enzymatic changes in the intestine mainly during development; indeed these hormones administered in vivo or added in organ cultures are able to induce precocious maturation of suckling rat intestinal epithelium (for review see refs 52, 64). In the coculture system, glucocorticoids were able to 1) modify the nature and distribution of GAGs synthesized by the fibroblastic cells, 2) induce qualitative changes in laminin molecules synthesized by mesenchyme-derived cells, 3) lead to an accelerated organization of laminin-1 at the epithelial/mesenchymal interface. These modifications accompanied the accelerated maturation of the intestinal embryonic endodermal cells[15,121]. It is important to stress that injections of glucocorticoids to 12-day-old rats led to an increase in type IV (pro)collagen, fibronectin and laminin and their respective mRNA levels; concomitantly a decrease in interstitial collagens, type I and III collagen was noted[152]. Thus, altogether these data suggest that most of the modifications brought by glucocorticoids are subsequent to changes of the ECM from stroma, strengthening the idea that fibroblastic cells are the cellular targets of the hormones. Glucocorticoids have been suggested to influence the stromal layer in other organs as well (for references see ref. 67). Recently, Ekblom et al.[42] showed that glucocorticoids could regulate hematopoiesis by modulating production of tenascin by stromal cells.

The model of heterologous coculture allowed us to provide more direct evidence of the necessity of a well-organized BM for differentiation. Indeed, the addition of polyclonal antibodies to laminin-1 to intestinal endodermal/fibroblastic cocultures led to the inhibition of expression of lactase (an apical differentiation marker). One can postulate that blockage of any other BM molecules would affect differentiation. However, several arguments from the literature point to the major role of laminin, and in particular of the constituent $\alpha 1$ chain for BM assembly, or even for morphogenesis and differentiation. Firstly, in the kidney, the induction of epithelial polarization was inhibited by antibodies towards the COOH-terminal end of laminin $\alpha 1$ chain[72]. Closely similar data were obtained in the lung, although in this case the anti-laminin antibodies that inhibited branching morphogenesis were directed to the cross region of laminin and the globular domains of the $\beta 1/\gamma 1$ chains[118]. Secondly, laminin polymeric networks, formed through relatively low affinity interactions, have been found to make a major contribution to BM architecture[157]. Thirdly, during early embryogenesis some basement membranes possess laminin, but lack type IV collagen[76]. These observations as well as the peculiar location of the $\alpha 1$ chain in the intestine, led us to analyze the role of this laminin constituent using a gene transfer strategy. The expression of antisense RNA in cells is currently widely used for reducing the expression of a targeted polypeptide[28]. This method is particularly useful for the study of vital genes whose elimination by homologous recombination is often lethal. As a model system, we used the Caco2 cells (colonic cancer cells lines) expressing non negligable levels of laminin $\alpha 1$ chain; these cells exhibit phenotypic markers of human fetal small intestinal cells[100]. Immunoblot and immunocytochemistry analysis revealed that the three constituent chains of laminin – $\alpha 1$, $\beta 1$ and $\gamma 1$ – were expressed in Caco2 cells, whereas another colonic cancer cell line, HT29, did not express detectable levels of $\alpha 1$ chain[33]. When cultured on top of fibroblastic cells, Caco2 cells grew as monolayers. At the Caco2 cells/fibroblast interface, a continuous BM was noted as assessed by electron microscopy and immunodetection of collagen IV and laminin. In contrast, the HT29 grew as clusters on fibroblastic cells and no polar deposition of any BM components occurred at regions where HT29 cells and fibroblasts were confronted[14]. The synthesis of laminin was the highest in Caco2 cells as compared to HT29 cells. To study the effect of reduced laminin $\alpha 1$ chain expression on cell behavior, Caco2 cells were transfected with an antisense laminin $\alpha 1$-E3 fragment cDNA construct; clones displaying decreased laminin $\alpha 1$ levels were isolated. As a consequence, these clones accumulated $\beta 1/\gamma 1$ chains intracellularly. In coculture experiments, these deficient cells did not deposit a laminin-containing matrix in contact with fibroblasts; in parallel no collagen IV was found[32]. These preliminary data allowed us to conclude that the $\alpha 1$ chain of laminin contains information required for the formation of a stable laminin-containing basement membrane and for the complete assembly of the matrix network.

Concluding remarks

Morphogenesis and differentiation, which are the processes by which cells 1) shape the detailed architectural features of tissues, and 2) acquire their tissue-specific functions, are characterized by a complex cascade of cellular and biochemical events under precise regulatory controls. Based on the observations and data reported herein we propose that the continuous cell-cell and cell-matrix interactions, during intestinal development and adult cell renewal, are the main driving forces involved in these processes. These data stress the fact that not only epithelial cells but also mesenchymal-like cells act as crucial regulators, together with the ECM which is a highly structured entity continuous with the cell surface and cell interior.

Although several elegant contributions have allowed significant progress in the understanding of gastro-intestinal cell biology[46], few studies have focused on the molecular mechanisms governing the integrated epithelium-mesenchyme unit in the onset and maintenance of gut morphogenesis and differentiation. The precise knowledge of the tissular, cellular and extracellular matrix variations in various tissue systems has led to the vast field of research concerning molecular events involved in these changes. The most important questions in the near future will be to know how 1) the expression of ECM molecules and of their receptors as well as of the degradation enzymes in a given tissue is controlled, 2) tissue- or cell-specific gene expression is regulated by the extracellular microenvironment. As most of these aspects have been and will be approached in cell culture systems, a major challenge will also be to define the actual role in vivo of individual ECM molecules in tissue development and functional maintenance.

Linked to the first question, it is worth noting that the tissue-specific expression, developmental control and coordinate synthesis of the different chains of a given ECM molecule must imply complex interactions of multiple regulatory proteins. In several recent studies, various effectors have been shown to act on ECM production. Among them are glucocorticoids known (see previous section) to influence intestinal maturation through their effect on ECM production by the mesenchymal cells. In a closely similar differentiation inductive system (hematopoietic differentiation), Ekblom et al.[42] have speculated that the hormone-receptor complex acts directly on *cis*-regulatory elements of the targeted gene (tenascin gene in this particular case). Another example of ECM regulation is given by the discovery of retinoic acid (a known morphogen-differentiation inducer)-responsive elements in the laminin $\beta 1$ and type IV collagen promoters, that may modulate gene transcription[19,149]. Moreover, the discovery of a novel specific sequence motif that appears to be unique to the regulatory regions of many genes encoding BM proteins, suggests that it represents an important and potentially unique control element for the coordinate expression and regulation of ECM[19].

ECM (like cell adhesion protein) gene promoters also contain control elements which are targets for homeobox gene products (described for the cytotactin/tenascin gene[58]). The huge number of studies performed during the last decade on these homeotic genes has clearly shown that they are involved in the establishment of the antero-posterior axis in multicellular organisms, and in the patterning of particular tissues. In the intestinal tissue, the following observations are of interest. Firstly, there is a differential expression gradient of several homeotic genes from the anterior small intestine to the distal colon[43,57]. Secondly, the overexpression of the homeobox-containing gene *Hoxa*-4 in transgenic mice led to the abnormal development of the smooth muscle coat and innervation of the terminal gut (megacolon); these alterations were paralleled by an abnormal deposition of BM molecules[139]. Considering those data, it is tempting to speculate that this gene acts on downstream morphogenetic effector genes which could encode ECM molecules.

Cytokines are also good candidates for regulating the extracellular matrix steady state. Among them, TGFβ has been shown to act on the transcription of variety of matrix components and also to up-regulate integrins and inhibit degradation of extracellular matrix[31,101]. More insight into the molecular mechanisms of TGFβ function begins to emerge; for example, activation of collagen transcription occurs through a specific activation element[107]. A variety of other cytokines, acting either on epithelial or on mesenchymal tissues, also display quite marked effects on compositional changes of the entire ECM. The properties of these cytokines have been particularly well analyzed in inflammatory diseases (with an increasing attention towards chronic inflammatory bowel diseases), where they are involved in immunocompetent cell recruitment, fibroblast proliferation and tissue remodeling[93,117,151]. An additional level of cell regulation is brought by the ability of cytokines to bind directly to ECM molecules[1,94]; in this way, ECM molecules function as reservoirs for various growth factors that can be either immediately available to cells when need arises, or undergo conformational changes allowing them to get access to their receptors.

Finally, a link between growth factors and homeobox gene expression could mediate signaling implicated in epithelial-mesenchymal interactions which do not necessarily imply direct contact between tissues. Two recent studies examplify such an interplay. Vainio et al.[146] showed in tooth rudiment that BMP-4 (bone morphogenetic protein 4), a member of the TGFβ superfamily, was able to replace the epithelium in inducing morphological and molecular changes in the dental mes-

enchyme, leading to tooth induction. Furthermore, BMP-4 induced the expression in the presumptive dental mesenchyme of *Msx*-1 and *Msx*-2 homeobox genes which may be involved in the establishment of positional information during tooth organogenesis[146]. In the adult mouse uterus, the expression of *Msx*-1 in the epithelium was shown to be induced by the underlying mesenchyme. This property of the uterine mesenchyme is correlated with the mesenchymal expression of *Wnt*-5, the vertebrate homologue of the Drosophila wingless family of growth factors[99]. Thus, the combination of growth factor-like components and of homeobox-containing genes could play a role in the developmental patterning as well as in maintenance of the adult organ in a morphogenetic responsive state.

Concerning the second important question raised above which dealt with the cellular response to the extracellular microenvironment, the general hypothesis for the mechanism by which ECM regulates gene expression implies the following steps. The first one consists of binding of ECM to specific receptors leading to their clustering. Subsequently, a number of more or less well defined intracellular modifications will occur. The signal transduction, starting from changes in the cytoplasmic domain of the receptors, may involve either changes in the state of assembly of the cytoskeleton or the action of various potential second messengers, implying mostly phosphorylation events (for reviews see refs 1, 60). The final step of the intracellular transduction cascade, whatever its nature, deals with the transcriptional regulation of the responsive genes. A recent review by Lin and Bissell[81] summarizes the current knowledge about this latter aspect in mammary cells, hepatocytes and keratinocytes. They report the presence of ECM/hormone-responsive enhancers in the promoter region of tissue-specific genes, and the activation of specific transcription factors by extracellular signals. The mitogenic response of the C-terminal end of laminin $\alpha 1$ chain long arm includes, as in the response to growth factors, a rapid and transient increase of c-fos and c-jun protooncogenes expression, and DNA binding activity[74].

Almost all the emerging data on the role of individual ECM molecules and on the molecular mechanisms involved in the integrated epithelial-ECM-mesenchymal unit arise from in vitro studies; a major challenge will be now to confirm these findings in situ. An approach to this fundamental question in cell biology and physiology is to analyze the consequences of the overexpression or knock-out of a given molecule on morphogenesis and differentiation processes. Unfortunately, most of the mutations giving deficient expression of ECM molecules or receptors are lethal due to the impairment of major morphogenetic steps in early embryogenesis; thus it is impossible to draw any conclusion about their function in the intestinal tissue. Up to now, two ECM molecule-deficient models have been examined: tenascin knock-out[110] and merosin deficient *dy* mutant mouse[126,155]. They did not reveal any significant modification of the intestinal morphogenesis or crypt/villus formation. Tissue-specific and temporally targeted alterations must now be performed to overcome lethality in transgenic mice or functional redundancy of some of these components.

In conclusion, the benefit of many integrated investigations performed in a wide range of disciplines will most probably lead in the near future to a better understanding of the mechanisms involved in cell-cell communication and of their implication in cell biology, physiology and pathology.

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