Tenascin During Gut Development: Appearance in the Mesenchyme, Shift in Molecular Forms, and Dependence on Epithelial–Mesenchymal Interactions

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Abstract. Tenascin, an extracellular matrix protein, is expressed in the mesenchyme around growing epithelia in the embryo. We therefore investigated whether epithelial cells can stimulate expression of tenascin in embryonic mesenchyme. Mesenchyme from the presumptive small intestine was used because it is known that reciprocal epithelial–mesenchymal interactions are important for gut morphogenesis. Rat monoclonal antibodies against mouse tenascin were raised and were found to react specifically with mouse tenascin in ELISA. In supernatants of cultured fibroblasts, the antibodies precipitated two peptides of Mr 260 and 210 kD. One of the antibodies also reacted with these tenascin chains in immunoblots of tissue extracts. We found that tenascin was absent during early stages of gut development, at stages when the mesenchyme is already in contact with the stratified epithelium of the endoderm. Rather, it appeared in the mesenchyme when the homogenous endodermal epithelium differentiated into the heterogenous absorptive epithelium. Tenascin remained present in the stroma of the adult gut, close to the migration pathways of the continuously renewing epithelium. When first detected during intestinal differentiation, the 210-kD component was predominant but at birth the relative amount of the 260-kD component had increased. The expression data suggested that the appearance of tenascin in the mesenchyme was dependent on the presence of epithelium. To test this, isolated gut mesenchymes from 13-d-old mouse embryos were cultured for 24 h either alone or together with epithelial and nonepithelial cells. Whereas mesenchyme cultured alone or in the presence of nonepithelial B16-F1 melanoma cells produced only trace amounts of tenascin, expression was strongly stimulated by the epithelial cell line, Madin–Darby canine kidney (MDCK). We propose that growing and differentiating epithelia produce locally active factors which stimulate synthesis of tenascin in the surrounding mesenchyme.

Tenascin is a mesenchymal matrix glycoprotein with an unusually restricted expression pattern in the developing embryo (10). Tenascin is probably identical to cytotactin (21) and similar proteins from other species have been described in some adult and many embryonic tissues (4, 18, 29). Tenascin is selectively present in the mesenchyme surrounding growing epithelia in organs where the mesenchyme is essential for epithelial development. It has therefore been proposed as a mesenchymal signal transducer in these interactions (10). In the developing kidney tenascin is absent during the first inductive epithelial–mesenchymal interactions (I) and it is therefore probably not a signal transducer during the first inductive interactions in this organ. Other molecules present earlier may be more important in this respect (41). However, tenascin in the kidney appears shortly after the first interaction, suggesting that its expression is a consequence of epithelial–mesenchymal interactions (I). Such a relationship may be common and would explain the restricted distribution of tenascin around growing epithelia in many other tissues. Hence, we propose that actively growing, migrating, and differentiating epithelial sheets can produce factors that stimulate tenascin expression in the nearby mesenchyme. The immunohistochemical data from other embryonic tissues (10) and epithelial tumors (33) fit well with this hypothesis.

To test this possibility, we have examined the expression of tenascin in the developing gut. Data on tenascin expression in this tissue is lacking, although it is known that epithelial–mesenchymal interactions are instrumental for gut development (27, 37, 43). Unlike other epithelia, the gut epithelium undergoes continuous rapid renewal in the adult. New epithelial cells are derived from the proliferating cells of the crypts at the bases of the villi. They then migrate along the villus surface and are finally shed into the gut lumen. The diversity of proliferating, differentiating, and migrating epithelial cells hence makes it possible to test which of these epithelial compartments possibly stimulate expression of tenascin.
To study tenascin expression during gut development, we raised mAbs against mouse tenascin. Two of these mAbs were characterized with respect to their ability to bind tenascin in quantitative ELISA, immunoprecipitations, and immunoblots. Both antibodies recognize a protein that under reducing conditions dissolves into two peptides with Mr's of 210 and 260 kD, and they react with mouse tenascin in immunofluorescence of tissue sections. One of them also recognizes the protein in immunoblots of tissue extracts.

The expression data on embryonic and adult gut obtained with the antibodies suggested that tenascin expression in the gut mesenchyme may indeed be stimulated by the epithelial cells. This hypothesis was then directly tested by coculturing embryonic gut mesenchymal cells with established cell lines. As judged by immunoprecipitation and immunofluorescence assays using the characterized antibodies, tenascin expression could be strongly stimulated by the epithelial Madin-Darby canine kidney (MDCK) cell line (38) but not by the nonepithelial B16-FI melanoma cell line (20). These results provide experimental evidence that epithelial cells can promote the expression of tenascin in embryonic mesenchyme.

**Materials and Methods**

**Hybridoma Production**

Mouse tenascin, partially purified by affinity chromatography on polyclonal rabbit anti–chick tenascin antibodies coupled to CNBr-Sepharose 4B (Pharmacia Fine Chemicals, Freiburg, FRG) was a kind gift of Dr. Ruth Chiquet-Ehrismann (Friedrich Miescher Institut, Basel, Switzerland). Approximately 20 μg of protein dissolved in PBS and mixed with 1.5 vol of complete Freund's adjuvants (Miles Laboratories, Inc., Bedford, MA) for the first immunization and incomplete Freund's adjuvants for subsequent boosts, were injected intraperitoneally into a male Lou-rat at 40, 27, 12, and 5 d before fusion.

For generation of hybridomas, spleen cells and cells from Peyer's patches were fused with rat YAGl.2.3 myeloma cells (a gift of Dr. R. Kemler, Max-Planck-Institute, Tübingen) as described previously (39). Fusion products were seeded on feeder-layers in 96-well culture dishes (Costar) 1 d after cells had reached confluency and were screened for presence of mAbs by ELISA and immunofluorescence.

**Subcloning and Propagation of Hybridomas**

Cells which produced supernatants with a positive reaction in the ELISA and an immunofluorescence-staining pattern compatible with the one previously seen with polyclonal anti–chicken tenascin antibodies were subcloned by limited dilution, and screened as described above. In addition, immunoglobulin subtypes were identified using a "rat monoclonal typing kit" (ICN Biomedical, Eschwege, FRG). Further propagation of cells and production of supernatants for antibody purification was achieved in DME containing 10% FCS (Biochrom, Berlin, FRG), 10% human endothelial cell supernatant (Costar), and 2.5% Ewing sarcoma growth factor (Costar). Culture supernatants were removed from 96-well plates 1 d after cells had reached confluency and were screened for presence of mAbs by ELISA and immunofluorescence.

**Purification of Monoclonal Antibodies**

Culture medium was diluted 1:4 with 25 mM 2(N-Morpholino)ethanesulfonic acid, pH 4.0, and filtered through a 0.2-μm filter. The filtrate was applied to an HR column (Pharmacia Fine Chemicals) packed with Bakersbond ABx (Baker Chemical Co., Groß-Gerau, FRG) connected to a Pharmacia fast protein liquid chromatography system. The column was then washed with 8 vol of 10 mM 2(N-Morpholino)ethanesulfonic acid, pH 5.6, and eluted with a gradient of 0-100% 1 M Na-Acetate, pH 7.0 (buffer B), over 50 ml. Antibodies eluted at slightly more than 50% buffer B. Their purity was checked by SDS-PAGE using Phastgels 10-15% (Pharmacia Fine Chemicals) and the Pharmacia Phastsystem.

**ELISA**

For the screening procedure and characterization of selected antibodies, 96-well microtiter plates (Falcon Labware, Heidelberg, FRG) were coated with 0.5 μg of immunogen or mouse tenascin. The mouse tenascin was purified on mAbs coupled to CNBr-Sepharose (Pharmacia Fine Chemicals) affinity columns and blocked with Tris-buffered saline (TBS) containing 4% (wt/vol) BSA and 0.05% (vol/vol) Tween 20. Bound antibodies were detected by ELISA (17) using the biotin–streptavidin system (Amersham-Buchler GmbH, Braunschweig, FRG) with horseradish peroxidase as the enzyme and tetramethylbenzidine (Miles Laboratories, Inc./ICN Biomedical) as the substrate. Between all incubations, plates were washed extensively with TBS containing 0.05% Tween 20 by a Scatron Microwash II.

**Tissue Dissection and Organ Culture**

Small intestines were obtained from National Medical Research Institute (NMRJx) 129 hybrid mice. In these hybrids, the first signs of villus formation were seen between days 14 and 15 of gestation (counted from the day of the vaginal plug, day 0). For preparation of intestinal mesenchymes, guts were dissected from 13-d mouse embryos. They were largely freed of the outer muscular layer under a stereo microscope and immersed in PBS with 0.1 mM EDTA for 30 min. The mesenchymal cell layer was then cut open with sharp injection needles, and the endodermal (epithelial) layer was removed. Explant cultures were grown in DME supplemented with 10% FCS. For this purpose, mesenchymes were placed on Nucleopore filters (pore diameter 1.0 μm; Nucleopore, Tübingen, FRG) coated with gelatin in quantitative ELISA, immunoprecipitations, and immunofluorescence.

**Cell Lines**

For coculture experiments, we selected two cell lines not producing tenascin, but with differing morphologies. These were the nonepithelial cell line (B16-FI melanoma) with a fibroblastic morphology but of neuroectodermal origin (20), and the canine kidney epithelial cell line, MDCK, which forms a polarized epithelium (38). Both cell lines were the kind gifts of Drs. W. Birchmeier and J. Behrens (Friedrich-Miescher-Laboratorium, Tübingen, FRG).

**Immunoblotting and Immunoprecipitation**

For immunoblot analysis of tenascin in the developing gut, small intestines were extracted at alkaline pH (11) in a buffer containing 200 mM cyclohexylaminopropene sulfonic acid, pH 11.5, 150 mM NaCl, 1 mM EDTA, 2 mM phenylmethylsulfonyl fluoride (PMSF), 1% trysylol, and 4 mM N-ethylmaleimide. Tissues were dissected from the embryo, briefly washed in ice cold buffer, transferred to approximately five times its own volume of the same buffer, and then disrupted by short ultrasonic treatment (~2 s) using a Bioruptor sonifier with microtip. Extraction was achieved under constant agitation at 4°C for 15 min. The extract was neutralized by addition of NaH2PO4. For Western blotting (46), the extract was boiled with sample buffer and subjected to SDS-PAGE on 5-10% acrylamide gradient gels (31).

Extraction of tenascin for immunoprecipitations from mesenchymes cultured in the transfuser system could efficiently be achieved by sonication of the mesenchymes in 500 μl of NET buffer (1% NP-40, 5 mM EDTA, 50 mM Tris- HCl, pH 8.0, 400 mM NaCl, 1% trysylol, 2 mM PMSF). For metabolic labeling, dissected tissues were incubated for 1 h in methionine-free DME and then for 4 h in the same medium supplemented with [35S]methionine at a final specific activity of 37 MBq (1 mCi) per ml of medium. In the transfuser experiments, [3H]leucine was also added at a final specific activity of 3.7 MBq (0.1 mCi) per ml of medium. Immunoprecipitations of extracts of such labeled tissues were performed as described by Chiquet-Ehrismann et al. (10), except that protein A-Sepharose (Pharmacia Fine Chemicals) was used instead of pansorbin cells, and rabbit

1. Abbreviation used in this paper: MDCK, Madin-Darby canine kidney.

The Journal of Cell Biology, Volume 107, 1988 2342
Results

Specificity of Rat Anti-mouse Tenascin mAbs

Of the nine independent antibody clones that were positive throughout the screening procedure, one with an especially brilliant reaction in immunofluorescence, designated MTn 5 (from the IgM class), and one with a strong reaction in ELISA, designated MTn 12 (IgGt), were selected for further characterization. For this purpose, proteins recognized by the antibodies were immunoprecipitated from supernatants of metabolically labeled fibroblasts. The antibody MTn 12, but not MTn 5, also reacted with tenascin in Western blots (Fig. 5). Specificity of antigen–antibody binding was further examined by the ELISA method. The antibody was shown to bind to the antigen preparation used for immunization (Fig. 2), containing mouse tenascin as well as trace amounts of fibronectin and albumin. No binding was observed with either mouse fibronectin, mouse collagen type I (Fig. 2), or BSA (data not shown). Taken together, the data show that both mAbs react specifically with mouse tenascin.

Appearance of Tenascin in the Embryonic Gut Mesenchyme Underlying the Differentiating Epithelia

Morphogenesis of the gut starts from the intestinal anlage, which consists of the endoderm, a simple tube of stratified epithelial cells surrounded by mesenchymal cells. In mice used in the present study, this stage was reached on day 13 of embryonic age, the earliest stages investigated. During subsequent development, the mesenchyme forms muscular and connective tissue layers, as well as the stroma within the villi, the lamina propria. The villus epithelium is derived from the endoderm and consists of a single layer of columnar cells (37). To localize tenascin at different stages of intestinal development, frozen sections of intestines were examined by immunofluorescence. In the early gut anlage of a 13-d-old mouse embryo, we found ample expression of fibronectin in the mesenchyme which surrounds the stratified endodermal epithelium (Fig. 3 a), while tenascin could not be detected (Fig. 3 b). The epithelium expressed cytokeratin at this stage, as judged by immunohistological staining for cytokeratin with the TROMA 1 antibody (data not shown). When the first endodermal cells evaginated to form villus primordia, some faint staining with anti-tenascin antibodies was seen.

\[ \text{Graph 1:} \text{Fluorograph of the peptides precipitated by the mAbs MTn 5 (lane 1) and MTn 12 (lane 2), and protein A-Sepharose alone (lane 3). Mouse embryonic fibroblasts at an early passage were labeled for 16 h with 3.7 MDbq/ml of [35S]methionine and the protein precipitated from the conditioned supernatant. Precipitates were separated under reducing conditions by SDS-PADE on a linear gradient gel (5–10% acrylamide).} \]

\[ \text{Graph 2:} \text{ELISA demonstrating specific binding of mAb to tenascin. Bound antibodies were detected by biotinylated goat anti-rat antibodies and a biotin-streptavidin-horseradish peroxidase complex using an enzyme-catalyzed dye reaction. MTn 5 (an IgM) and MTn 12 (an IgGt) bind to tenascin, but not to fibronectin and collagen type I. Wells of a microtiter plate were coated with 0.5 μg of protein per well. (o) Antibody MTn 12/substrate tenascin; (c) MTn 5/tenascin; (Δ) MTn 12/Collagen Type I; (●) MTn 12/fibronectin; (*) MTn 5/fibronectin.} \]
Figure 3. Comparison of fibronectin (a and c) and tenascin (b and d) expression in presumptive small intestine from day 13 (a and b) and 17 (c and d) of embryonic development. In the gut anlage from day 13 of embryonic development, the endoderm (end) forms a hollow tube of stratified epithelial cells which is surrounded by the undifferentiated mesenchyme (mes). Fibronectin is already strongly expressed by this mesenchyme (a), while tenascin can not be detected (b). At day 17 primitive villi have been formed, which already protrude into the lumen. They consist of the mesenchymal core (lamina propria), which is covered by epithelial cells derived from the endoderm. Fibronectin, also at this stage, is evenly distributed throughout the mesenchyme (a). Tenascin is predominantly found in the lamina propria of the villi and in a small band within the outer mesenchyme, probably the presumptive muscularis mucosae (d). Bars, 50 μm.

in the mesenchyme underlying these areas. When the first primitive villi with a lamina propria could be seen, fibronectin was still evenly distributed throughout the mesenchyme (Fig. 3 c), while tenascin was almost exclusively seen within the villi and in a narrow band in the mesenchyme, probably the presumptive muscularis mucosae (Fig. 3 d). In the adult intestine, tenascin was still present (Fig. 4). Whereas fibronectin was apparently present around all cells of the adult intestinal stroma (Fig. 4 a), tenascin was mainly expressed within the villi, i.e., by the lamina propria (Fig. 4 b), and less intensely in the outer muscle layers. Around the crypts of Lieberkühn, where the presumptive epithelial cells are formed by continuous proliferation, tenascin could only be detected in low amounts in the distal regions (Fig. 4 c).

**Shift in Tenascin Chain Expression During Gut Development**

Isolated small intestines from various developmental stages were probed by Western blotting for the expression of tenascin. Fig. 5 shows the pattern obtained comparing tissue extracts from day 13 to 18 of embryonic development as well as from newborn and adult stages. An extract from cultured fibroblasts is shown for comparison in Fig. 5, lane f. The relative amount of the heavy (260-kD) and light (210-kD) component changes significantly during the development. While almost no 260-kD band is observed at day 15 of embryonic development, this band becomes more pronounced around birth, and is clearly present in the adult. However, it did not
Figure 4. Distribution of fibronectin and tenascin in the adult duodenum (a and b) and ileum (c). In the duodenum, large areas of intestinal glands (ig) are seen around the lumen. At the base of the villi (c) the crypts of Lieberkühn (cry) can be recognized. In these crypts, new cells are continuously produced by proliferation, which then differentiate and migrate along the villus' surface towards its tip, where they are shed into the lumen. Tenascin is only very weakly expressed around the intestinal glands (b) and the crypts (c), but is prominent in the villus core and especially in the region of the subepithelial fibroblasts (c, arrowhead). Expression of the protein by smooth muscle cells in the muscularis externa is also seen. Fibronectin, in contrast, is present in all mesenchymal regions (a). Bars: (a and b) 30 μm; (c) 60 μm.

become as pronounced as in cultured fibroblasts. In immunoblots as well as in immunoprecipitates from tissue extracts, we frequently observed a resolution of both the upper and the lower component into two bands. This was not observed in tenascin from fibroblasts. While the split of the smaller band has been previously described for chick (8) and human (19) tenascin, the separation of the larger component into two bands seems to be novel.

Induction of Tenascin Expression in Isolated Mesenchymes by Epithelial Cells in Transfilter Culture

The immunofluorescence data showed a relationship between the onset of epithelial differentiation and tenascin expression. This raised the question of whether the mesenchyme starts to expresses tenascin independently of the epithelium, or whether interactions between these two tissues are needed. To study this, we combined isolated 13-d gut mesenchymes with cultures of embryonic cell lines in a transfilter set-up, with tissue and cells separated by the filter. When gut mesenchymes were cultured alone on filters coated with gelatin, or in coculture with B16-F1 melanoma, almost no tenascin could be detected by immunoprecipitation. However, when the mesenchymes were confronted for 24 h with the canine epithelial cell line, MDCK, abundant synthesis of tenascin could be detected, with a clear predominance of the heavy subunit (Fig. 6). Neither the B16-F1 melanoma cells nor the MDCK cells themselves produced tenascin. The results were verified by immunofluorescence analysis of the mesenchyme. The morphological data showed that tenascin was deposited into the matrix of the mesenchyme as fibers around the cells only in those explants which had been cocultured with the MDCK cells (Fig. 7 c) but not
Figure 5. Immunoblot of intestinal tissue extracts and fibroblast lysates with mAb MTh 12. Segments of the intestine directly adjacent to the stomach were extracted in a buffer at pH 11.5. Embryonic mouse fibroblasts were scraped off the plate and directly boiled in sample buffer. Extracts were then separated by SDS-PAGE (linear 5–10% acrylamide gradient), transferred to nitrocellulose, and detected by sequential incubation with the mAb, a biotinylated second antibody, a streptavidin-alkaline phosphate complex, and a color reaction. Extracts are from embryonic days 13 (lane 1), 15 (lane 2), 17 (lane 3), and 18 (lane 4), as well as from newborn (lane 5) and adult (lane 6) tissues. An immunoblot of a fibroblast lysate (lane 7) is shown for comparison.

Discussion

Several lines of evidence support the hypothesis that epithelial cells stimulate nearby mesenchymal cells to produce tenascin. In a previous paper (1) it was shown that tenascin expression appeared in embryonic kidney mesenchyme shortly after the first epithelial–mesenchymal interactions. In the present report, using mAbs, we show that an epithelial cell line can stimulate embryonic gut mesenchyme to produce tenascin. This response of the mesenchyme could be a physiological one since it mimics normal embryonic development. We found that the gut embryonic mesenchyme during normal development starts to produce tenascin when epithelial–mesenchymal interactions occur.

The data described here suggest that cellular cooperation is involved in the control of mesenchymal extracellular matrix synthesis. The importance of heterotypic cellular interactions has been previously clearly shown for the development of the basement membrane of the epithelial cells in many embryonic tissues (2, 3, 15, 16, 28, 30, 42), including the gut (23, 43). Less is known about the possible regulation of the mesenchymal extracellular matrix by the epithelium. This is largely because of a lack of characterized mesenchymal extracellular matrix components with a restricted distribution. Consequently, there have been no probes to study such components. We have here characterized mAbs against mouse tenascin, a mesenchymal protein with a more restricted tissue distribution than other described mesenchymal matrix proteins (5, 7, 10, 11, 34, 35).

With only a few exceptions (11, 24), previous investigations on the expression of tenascin in embryonic tissues have been based largely on immunohistological approaches (1, 10, 34, 35). Analysis of tenascin directly from tissues by immunoblotting or immunoprecipitation was hampered by the lack of an appropriate procedure for extraction of tenascin from tissues (8) and by the fact that the commonly used anti-chicken tenascin antibodies (8, 10) reacted poorly in Western blotting (unpublished observations). Our mAbs overcome this problem and by using a modification of extraction procedures described by Crossin et al. (11), it was possible to detect tenascin directly from tissue extracts in immunoblots. The mAbs reacted with two components of tenascin, with an $M_r$ of 210 and 260 kD, respectively. Previously, tenascin and related proteins such as glioma mesenchymal extracellular matrix antigen (4), hexabrachion (18), and cytotactin (21) could be shown to be composed of a predominant subunit of ~220 kD from chicken (8) and 250–285 kD from human sources (5, 18). Bands of ~210 kD for the human protein (19), which were weak enough to escape discovery in the original publications (18), and bands of ~190–200 kD for chicken tenascin were reported as minor components. Our results on mouse gut development show that the predominance of the larger subunit is not an invariable feature of tenascin. While the 210-kD light chain is strongly predominant at the early developmental stages in the embryo, the 260-kD heavy chain becomes more pronounced around birth and especially in the adult intestine. A similar gradual increase in the relative amount of the heavier chain has been shown for cytotactin in developing chick brain (24). It has
Figure 7. Deposition of tenascin into the extracellular matrix in isolated gut mesenchymes stimulated by MDCK epithelial cells. Isolated mesenchymes were cultured for 24 h on Nuclepore filters (pore size, 1.0 μm), which had been coated on their opposite side with either no cells (a), B16-F1 melanoma cells (b), or MDCK epithelial cells (c). Cryostat sections were stained with mAb M112. Bar, 30 μm.

been shown that there are different forms of cytotactin mRNA (26), suggesting that the detection of isoforms is due to differential synthesis rather than to differences in extractability.

Recently, the distribution of "J-I", a mouse protein that shares some characteristics with tenascin (29), has been described in the adult mouse ileum (45). The distribution of tenascin and J-I in the adult intestine shows similarities, but the molecular mass is somewhat different. In this tissue, J-I consists of peptides with apparent molecular masses of 250 and 170 kD (45). When we purify tenascin by affinity chromatography using our mAbs, we elute a protein of 160-170 kD by high salt conditions, while tenascin can only be eluted under denaturing conditions. If the smaller protein was identical with the second band of "J-I" this would mean that the two bands of "J-I" represent unrelated, though associated, proteins. Recent studies on cytotactin, which shares some similarity with "J-I", also suggest that 160/180-kD peptides recognized by the original antibodies against cytotactin may be unrelated to true tenascin. The more specific antibodies raised against highly purified cytotactin (24) showed a reaction pattern very similar to our mAbs against mouse tenascin. The available evidence thus suggests that larger peptides of tenascin, cytotactin, glioma mesenchymal extracellular matrix antigen, and hexabrachion are identical proteins with two major subunits, whereas the 160/180-kD peptides of "J-I" may represent associated proteins.

The presence of different subunits of proteins that affect cell adhesion is not a unique feature of tenascin; it has been noted for several extracellular matrix proteins (25, 36) and some cell adhesion molecules. The chains can be uncoordinate expressed (36). Such variations in composition of the protein may have profound effects on the biological activity of the protein, as shown for the neural cell adhesion molecule (13) and more recently also for laminin, a basement membrane protein (28). It is tempting to speculate that the changes in the different forms of tenascin, shown to occur during gut development, may also lead to changes in the function of the protein.

The appearance of tenascin in the gut mesenchymes was verified by immunohistological means. It is notable that tenascin was not detectable from the early gut anlage, even though the endodermal cells are already characterized as epithelial cells. It then appeared as soon as signs of differentiation towards the intestinal epithelial structures with their diversity of cell types became obvious. It is this diversification that requires epithelial–mesenchymal interactions (22, 32). The results show that tenascin expression during gut development is linked to rather late stages of embryonic differentiation, to the formation of absorptive capacity of epithelial cells. In the adult gut, tenascin expression is also associated with the later stages of epithelial cell differentiation. These findings are in accordance with results from the developing kidney (1).

The expression data both from the developing kidney and gut strongly suggest that tenascin is not mediating the first epithelial–mesenchymal interactions, but rather that it appears in the mesenchyme as a consequence of it. We have previously shown that inhibition of epithelial cell differentiation by an unspecific inhibitor of morphogenesis, heparin, suppresses tenascin expression in the mesenchyme (1). Although this supports the view that epithelial cells influence tenascin production of mesenchyme, the evidence was indirect. We therefore developed a model system, which allowed us to directly probe the effect of epithelial vs. nonepithelial cells. In this system, isolated gut mesenchymes were either cultured alone or in coculture with epithelial (MDCK) cells or nonepithelial cells (B16-F1-melanoma). Tenascin expression in these mesenchymes was specifically triggered by the epithelial, but not the nonepithelial cells. The fact that B16-F1-melanoma cells did not induce tenascin appearance excludes the possibility that the inductive stimulus exerted by the MDCK cells is a nonspecific effect of immortalized cells. Hence, the triggering signal may be specific
for epithelial cells. The fact that MDCK cells, which are derived from dog kidney tubules, can affect mouse intestinal mesenchymes, shows that the signal seems to be conserved between different tissues and species, though the molecular form of tenasin produced after stimulation by MDCK cells is different from the one seen in situ. A similar lack of species and tissue specificity has been demonstrated for the reverse process, the signal transmission from mesenchyme to epithelium, during development of both the gut (27) and the mammary gland (40). However examples of more stringent species and tissue requirements for these interactions have also been reported (12). The coculture model system used here should make it possible to study the effect of both embryonic and malignant epithelial cells and their growth factors on matrix production by mesenchymal cells in more detail.

The role of tenasin in epithelial–mesenchymal interactions remains unknown. It was proposed by Chiquet-Ehrismann et al. (10) that tenasin may support epithelial morphogenesis. It should be kept in mind that tenasin could act primarily on the mesenchyme rather than on the epithelium. Indeed, it was recently shown that tenasin influences the behavior of fibroblast cells. Tenasin can counteract the action of fibronectin and consequently cells attach less avidly to the substrate (9, 24, 34). Embryonic mesenchyme containing tenasin differs morphologically from adult stroma. It is more loose, a feature which may be of importance for a tissue which is rapidly remodelled. One possibility is that tenasin directly loosens up the mesenchymal tissue compartment during the epithelial–mesenchymal interactions. Such an effect may indirectly facilitate cell migration and epithelial branching, two events associated with tenasin expression (10, 35, 44).

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