Amino Acid Sequence of Mouse Tenascin and Differential Expression of Two Tenascin Isoforms during Embryogenesis

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Abstract. We have isolated cDNA clones for mouse tenascin and analyzed expression of tenascin mRNAs during embryonic development of the kidney and gut. The deduced amino acid sequence of the mouse tenascin cDNAs shows a modular structure of repeats similar to chicken and human tenascin. In mouse there are 14.5 cysteine-rich repeats with similarity to the EGF repeat, followed by several repeats with similarity to the type III repeat of fibronectin. A longer variant contains 13 fibronectin type III repeats, whereas a shorter splice variant of mouse tenascin lacks the 5 type III repeats that occur directly after the fifth repeat in the longer variant. Contrary to the chicken and human sequences, mouse tenascin does not contain an RGD sequence in the third type III repeat implicated in cell attachment, or in any other positions. In Northern hybridizations to RNA from primary embryonic fibroblasts, the cDNA clone M 20/1 detects two mRNAs with sizes close to 6 and 8 kb. This, and the other data presented here suggest that the two major mouse tenascin polypeptides arise through an alternative RNA splicing. The two major mRNAs are differentially expressed during development. The 8-kb mRNA is more prominent than the 6-kb mRNA throughout prenatal kidney development, but during postnatal development the ratio of the two mRNAs changes. A different expression pattern is seen in the developing gut where the 6-kb mRNA predominates during embryogenesis with the 8-kb mRNA appearing later. The mRNA data of the developing gut correspond with previous protein data, which showed that the shorter M_r 210,000 polypeptide predominates during earlier developmental stages and the larger M_r 260,000 polypeptide appears later in the embryonic gut (Aufderheide, E., and P. Ekblom. 1988. *J. Cell Biol.* 107:2341–2349).

TENASCIN is a mesenchymal extracellular matrix glycoprotein with a spatially and temporally restricted tissue distribution. In the embryo, it is prominently expressed at sites of epithelial–mesenchymal interactions during organogenesis (for recent reviews; see Erickson and Bourdon, 1989; Ekblom and Aufderheide, 1989). Tenascin is a hexameric glycoprotein with disulphide-linked subunits originally described as myotendinous antigen (Chiquet and Fambrough, 1984*a*,*b*). Tenascin-like molecules have been described from embryonic brain and from gliomas and have been named cytotactin (Grumet et al., 1985), J1 (Kruse et al., 1985; Faissner et al., 1988), and glial-mesenchymal extracellular matrix glycoprotein (GMEM; Bourdon et al., 1983, 1985). It is now clear that cytotactin and tenascin are identical proteins and that GMEM is the human counterpart to tenascin. However, in the original "J1" complex from mouse tissues, tenascin is only one of several proteins (Faissner et al., 1988).

In EM tenascin has a characteristic six-armed structure termed hexabrachion (Erickson and Iglesias, 1984; Erickson and Taylor, 1987). Each arm of the hexabrachion is formed by one subunit, but the length of the individual subunits may vary (Jones et al., 1989; Spring et al., 1989). In the mouse, two major subunits of tenascin with an apparent M_r of 210,000 and 260,000 have been described (Aufderheide and Ekblom, 1988). As in chicken (Spring et al., 1989; Jones et al., 1989), the presence of polypeptides with varying sizes may be due to an alternative splicing of the mRNA, but this has not been directly shown. To study this possibility, and to learn more about the structure of mouse tenascin, we have isolated and analyzed cDNA clones covering the complete coding sequence.

Because of its transient appearance at sites of epithelial–mesenchymal interactions, tenascin may be involved in local tissue interactions during organogenesis (Chiquet-Ehrismann et al., 1986; Aufderheide et al., 1987). It is still unclear how tenascin acts on developing tissues, but it has been shown that binding of tenascin to cell surfaces causes cells to round up (Chiquet-Ehrismann et al., 1986, 1988). Thus, it would be of interest to identify the cell-binding regions of mouse tenascin. It has been suggested that the tripeptide Arg-Gly-Asp/(RGD), present in chicken and human tenascin, is involved in cell binding (Friedlander et al., 1988)

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and that tenascin mediates cell attachment through an RGD-dependent integrin receptor (Bourdon and Ruoslahti, 1989). Other studies, however, have shown that the GRGDS peptide cannot significantly inhibit cell attachment to tenascin (Chiquet-Ehrismann et al., 1988) and that a cell-binding site is located closer to the carboxy terminus away from the region containing the RGD sequence (Spring et al., 1989). To assess the importance of the RGD sequence in tenascin, comparisons with sequences of different species may be revealing. Several of our mouse tenascin cDNA clones span the third fibronectin type III repeat, which in human and chicken contains an RGD sequence (Gulcher et al., 1989; Spring et al., 1989). We report here that the mouse cDNA clones predict an RVD sequence in this location, and that the RGD sequence is lacking also from other parts of the two mouse tenascin mRNA forms we have identified.

With the mouse cDNA clones, we studied the expression of tenascin mRNA during late organogenesis of the kidney and intestine. We find that the two mRNAs of close to 6 and 8 kb are differentially expressed both during kidney and gut organogenesis. The presented data strongly suggest that the two major polypeptide subunits of mouse tenascin (Aufderheide and Ekblom, 1988; Ekblom and Aufderheide, 1989) form by an alternative splicing of one mRNA.

Materials and Methods

Polyclonal Antibodies against Mouse Tenascin

Initial attempts to isolate mouse tenascin cDNA clones by screening an expression cDNA library with previously described mAbs MTn 5 and MTn 12 (Aufderheide and Ekblom, 1988) were not successful, and therefore a polyclonal antiserum was made by immunizing rabbits with mouse tenascin (Weller, 1990). Mouse tenascin was purified by affinity chromatography using polyclonal rabbit antichicken tenascin antibodies (Chiquet-Ehrismann et al., 1986).

Cells and Tissues

Hybrid mouse embryos 129× NMRI were used. The day of the vaginal plug was designated as day 0. cDNA libraries were prepared from RNA isolated from kidneys of 14-d-old mouse embryos and from mouse embryonic fibroblasts. To obtain primary cultures of mouse embryonic fibroblasts, the upper parts containing the head, thorax, and parts of the abdomen of 14-d-old mouse embryos were squeezed through a steel grid to dissociate the cells. This suspension was taken into culture in DME (Gibco, Eggenstein, FRG) containing 10% FCS (Biochrom, Berlin, FRG). Adherently growing cells were passaged up to five times and confluent cultures were harvested for RNA extraction. A part of the RNA was used for cDNA synthesis, and another part for Northern blots. To obtain RNA for Northern blots of cells not producing tenascin polypeptides, B16F1 mouse melanoma cells (Fidler and Kripke, 1977; Aufderheide and Ekblom, 1988) were grown in DME containing 10% FCS. Cells were harvested for RNA extraction at confluency.

RNA Isolation

Tissues were frozen on dry ice immediately after microsurgery, and care was taken to perform the microsurgery immediately after decapitation. Tissues or cells were homogenized in 4 M guanidinium thiocyanate (Fluka AG, Buchs, Switzerland) using an Ultra-Turrax T25 homogenizer (IKA-Laborechnik, Staufen i. Br., FRG). The homogenate was squeezed through a 20-gauge needle to shear the high molecular weight DNA. The RNA was then purified by ultracentrifugation (SW41 Ti rotor, 30,000 rpm, 20°C, >20 h) through a 3.3-ml cushion of 5.7 M cesium chloride (Chirgwin et al., 1979). The supernatant was removed by suction and the RNA pellet was dissolved in sterile double-distilled H_2O. Total RNA was harvested by ethanol precipitation. The concentration of the RNA was determined by reading the absorbance at 260 nm. The purity and integrity of the RNA was tested by determining the A_{260}/A_{280} ratio and by agarose gel electrophoresis (Maniatis et al., 1982).

cDNA Library Construction and Cloning of cDNAs Encoding Mouse Tenascin

Poly(A)$^+$ RNA was isolated by twofold oligo(dT)-cellulose chromatography (Collaborative Research/Paesel, Frankfurt, FRG) from primary mouse embryonic fibroblasts and from mouse embryonic kidneys. For cDNA library construction the cDNA synthesis system and the λgt11 cloning system of Amersham-Buchler (Braunschweig, FRG) were used. Two cDNA libraries were made from 5 μg of poly(A)$^+$ RNA using either random or oligo(dT) priming. The libraries were constructed, plated as described (Gubler and Hoffman, 1983; Huynh et al., 1985), and screened with the polyclonal and monoclonal antitenascin antibodies using biotinylated antirat or antirabbit antiserum and streptavidin-biotinylated HRP complex (Amersham-Buchler) to detect immunoreactive fusion proteins (Huynh et al., 1985). The Eco RI insert of the positive phage clone M20/1 isolated from the primary embryonic fibroblast library was purified and subcloned into pGEM-blue (Promega/Genofit, Heidelberg, FRG) and M13mp18/19 (Boehringer, Mannheim, FRG). The M20/1 clone was used for subsequent screening of three additional λgt10 cDNA libraries made from poly(A)$^+$ RNA from mouse primary embryonic fibroblasts. One of the λgt10 libraries was made using specific priming with an oligomer from the 5′ region of clone M20/1 and the cloning kits from Amersham-Buchler (Braunschweig, FRG), the two other λgt10 cDNA libraries were custom made by Stratagene Cloning Systems (La Jolla, CA) using either oligo(dT) or random oligonucleotides as primers. The nucleotide sequences of all isolated clones were determined on both strands (Sanger et al., 1977) using Sequenase™ (USB/Renner, Dannstadt, FRG) and ^{35}S-dATP (Amersham-Buchler, Braunschweig, FRG) after subcloning into pUC vectors (Yannisch-Perron et al., 1985). Several parts of the sequence were determined by using specific oligonucleotides as sequencing primers. The synthetic oligonucleotides were synthesized on a gene assembler (Pharmacia-LKB Biotechnology Inc., Uppsala, Sweden) at the Max-Planck-Institut für Entwicklungsbiologie (Tübingen, FRG). For sequence analysis the MicroGenie™ sequence analysis software (Queen and Korn, 1984) and the UWGCG Sequence Analysis Software Package (Devereux et al., 1984) were used. The mouse sequence was compared with the complete chicken sequence (Spring et al., 1989; Jones et al., 1989) and partial human sequence of tenascin (Gulcher et al., 1989).

Northern Blotting

RNA was subjected to electrophoresis on 1% agarose gels after denaturation with glyoxal (McMaster and Carmichael, 1977). Transfer to hybond N (Amersham-Buchler) was carried out as described by the manufacturer. Preparation of radiolabeled probe using a nick translation kit (BRL, Eggenstein, FRG) or an oligo-labeling kit (Pharmacia, Freiburg, FRG) was done with 30–50 μCi [α-^{32}P]dCTP (Amersham-Buchler) according to the manufacturer's protocols. Hybridization of the filters was carried out as described previously (Mugrauer et al., 1988), except that washing of the filters in 0.1× SSC/0.1% SDS was done at a higher temperature (56–65°C).

Glycosidase Digest of Purified Tenascin

2.5 μg of purified tenascin were digested overnight at 37°C in 100 mM Na-acetate, pH 6.5, 50 mM EDTA, 1% Triton-X-100, 0.2% SDS, 1% β-mercaptoethanol using 20 mU endoglycosidase F (Boehringer). Another aliquot of tenascin was incubated under the same conditions without enzyme. Detection of tenascin by immunoblotting through PAGE and transfer to nitrocellulose using the monoclonal antitenascin antibody MTn12 was carried out as described previously (Aufderheide and Ekblom, 1988).

Results

Isolation of Mouse Tenascin cDNA Clones and Major Features of Mouse Tenascin mRNAs

cDNA libraries in λgt11 prepared from 14 d embryonic mouse kidneys and from primary mouse embryo fibroblasts were screened with the polyclonal antiserum raised against mouse tenascin. In the screening of 1.2 × 10^6 phage

Figure 1. Deduced amino acid sequence of mouse tenascin in a display that shows the modular structure of this protein. The two blocks of contiguous repeats with similarity to EGF (residues 174–620) and fibronectin type III repeat (residues 621–1,791) become apparent. Type III repeats 6–10 (residues 1,072–1,526) are present only in the large subunit of tenascin. The type III repeats are arranged such that the conserved amino acids Trp, Leu, and Thr are aligned. Potential Asn-glycosylation sites, most of which are found in the differentially spliced type III repeats, are underlined. The tripeptide Arg-Val-Asp in the third type III repeat (residues 877–879) is emphasized through double underlining. These sequence data at the nucleotide level are available from EMBL/GenBank/DDBJ under accession number X56304.

plaques for immunoreactive tenascin fusion proteins, one positive clone (M20/1) was identified from the random-primed cDNA library made from RNA of mouse embryonic fibroblasts. With the insert of this clone additional clones covering the complete coding sequence of mouse tenascin were isolated. The available clones cover a total of 6,831 bp, including 126 bp of 5′ untranslated and 645 bp of 3′ untranslated sequence. The 3′ end contains an AATAAA sequence 17 bp upstream to the end of the sequence which may serve as a polyadenylation signal. Northern blot data suggest that the full-length mRNA can be close to 8 kb rather than 6.8 kb, but it is likely that we are missing a part of the 5′ leader. Analysis of 12 cDNA clones covering the complete coding sequence of mouse tenascin revealed two splice variants with open reading frames of 2,019 for the larger and 1,564 amino acids for the shorter variant (Fig. 1). The calculated M_r of the primary translational products is 221,842 and 172,181, respectively. Minor additional splice variants may exist but clones that would demonstrate their presence have not been found so far.

The deduced amino acid sequence of mouse tenascin displays a modular structure of contiguous repeats very similar to chicken and human tenascin (Jones et al., 1988, 1989; Pearson et al., 1988; Gulcher et al., 1989; Spring et al., 1989). The two blocks of contiguous repeats with similarity to the EGF repeat and to the type III repeat of fibronectin

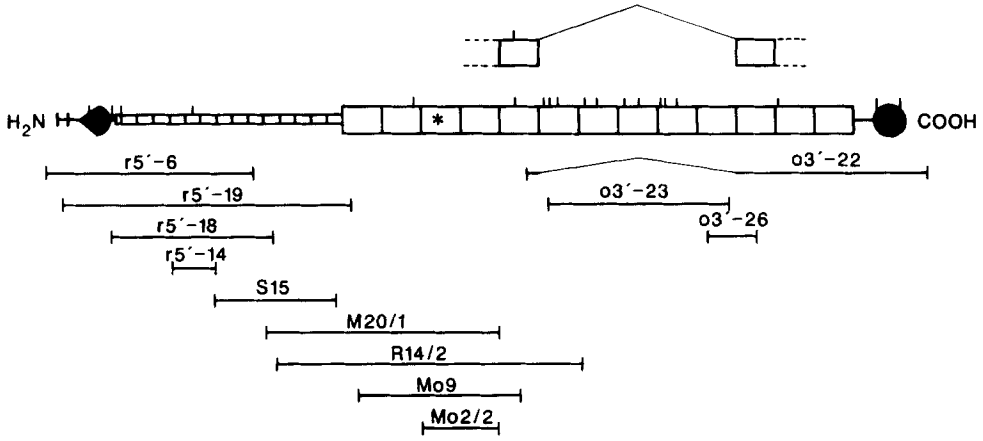

Figure 2. Schematic representation of one polypeptide chain of mouse tenascin showing its modular structure and the relative positions of the cDNA clones. After putative leader and propeptide sequences the amino terminal globular domain of the mature protein is indicated as a segment of a circle. The first block of 14.5 contiguous EGF-like repeats is represented by small rectangles, the second block of 13 repeats with similarity to the type III repeat of fibronectin is indicated with big rectangles. Differential splicing of the type III repeats 6–10 gives rise to the small subunit of mouse tenascin. Clone o3′–22 is derived from the small mRNA and therefore does not contain these repeats. The third type III repeat which contains the tripeptide Arg-Val-Asp in place of the cell attachment sequence Arg-Gly-Asp is marked with an asterisk. At the carboxy terminus a circle represents a domain with similarity to fibrinogen. Potential *N*-glycosylation sites are indicated with thin lines.

become apparent in Fig. 1. The relative positions of the cDNA clones are shown in Fig. 2, where the EGF-like repeats are represented by small rectangles and the subsequent fibronectin type III repeats by big rectangles.

Leader Peptide and EGF-like Repeats

The sequence of mouse tenascin contains a putative leader peptide (residues 1–19) as predicted by the program SIGSEQ (Popowicz and Dash, 1988) using the algorithm of von Heijne (1987). This sequence shows only ~40% sequence identity to chicken tenascin (Spring et al., 1989), whereas the remainder of the amino terminal sequence (residues 20–173) is >70% identical. Both in chicken and mouse tenascin this sequence is followed by a long contiguous block of several cysteine-rich EGF-like repeats, 13.5 in chick and 14.5 in mouse (residues 174–620 in our sequence). Except for the third repeat, the conserved cysteines in the mouse repeats show the same spacing found in the chicken and human sequences (Pearson et al., 1988; Gulcher et al., 1989), and additional amino acids are conserved in the consensus of the human, mouse, and chicken sequences. The repeats appear to be conserved between mouse and human with respect to position: repeats in the same relative positions are ~90% identical on the amino acid level, whereas other combinations show only ~60% identity on average. However, EGF repeats of chicken and mouse tenascin in the same relative positions are not nearly as similar as the human and mouse EGF-like repeats. It must be noted that only partial sequences of the human EGF-like repeats (Gulcher et al., 1989) were available for the comparison.

Fibronectin Type III Repeats

As in human and chicken, the EGF-like repeats are followed by several fibronectin type III repeats (residues 621 to 1791). The longer mRNA splice variant of mouse tenascin comprises 13 fibronectin type III repeats, characterized through the rather conserved spacing of tryptophane, leucine, and threonine residues (Fig. 1). The sequence of the chick is somewhat shorter with 11 such elements, whereas the human

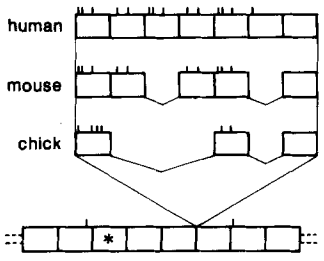

Figure 3. Alignment of the differentially spliced type III repeats of human, mouse, and chick tenascin. These repeats are contiguous in all three species. The three spliced repeats of chick and the five spliced repeats of mouse show higher sequence similarity with certain of the seven repeats of the human tenascin. Thin lines indicate the position of potential *N*-glycosylation sites. The position of these sites has been conserved very well between human and mouse. In chick an additional splice variant has been described which lacks the first and second, but contains the third of the differentially spliced type III repeats.

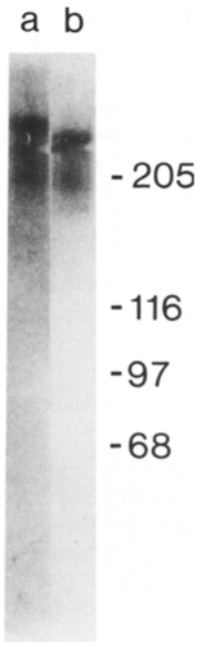

Figure 4. Immunoblot of purified mouse tenascin after treatment with endoglycosidase F. Tenascin purified from the culture supernatant of primary embryonic fibroblasts was treated with (*b*) and without (*a*) endoglycosidase F and transferred to nitrocellulose after gel electrophoresis. Staining with the monoclonal antitenascin antibody MTn12 detects a prominent band at ~260 kD and a weak band of ~210 kD in the undigested sample. In the digested sample the large band has shifted to ~230 kD. The position of the marker proteins is indicated in kilodaltons.

tenascin with 15 such elements is somewhat longer. The fibronectin type III repeats of the different species appear to be conserved with respect to their relative positions: repeats in comparable locations show >70% identity between chicken and mouse, whereas all other combinations give only ~25% identity. The similarity of repeats in comparable locations is even higher (90%) between mouse and human. In each of the three species the smaller mRNA variants form by splicing of type III repeats just after the fifth repeat. In mouse, the potentially differentially spliced fragment comprises five type III repeats, numbers 6–10 (residues 1,072–1,526). In chicken, the differentially spliced fragment is smaller, whereas the human fragment is the largest of the three (Fig. 3). Sequence comparison reveals that certain of the repeats in the differentially spliced sequence of chick and mouse can be aligned with specific repeats of the spliced segment of the human tenascin sequence (Fig. 3).

Glycosylation

Like the human tenascin sequence (Gulcher et al., 1989) the mouse sequence shows a high number of potential Asn-glycosylation sites (Marshall, 1972). It is noteworthy that no less than 10 out of 19 of these sites occur within the differentially spliced segment. The spacing of these glycosylation sites is remarkably well conserved between mouse and human (Fig. 3). Our data from cultured mouse embryonic fibroblasts suggest that these sites are used in the embryo. Digestion with endoglycosidase F, which removes Asn-linked carbohydrate chains, reduced the molecular mass of the larger form of mouse tenascin from M_r 260,000 to ~M_r 230,000 (Fig. 4). This value agrees very well with the size of an unglycosylated polypeptide backbone predicted from the primary sequence (M_r 221,842).

Deduced Mouse Tenascin Sequence Lacks Arg-Gly-Asp Tripeptide

Both chicken and human tenascin contain the tripeptide sequence Arg-Gly-Asp in the third fibronectin type III repeat (Jones et al., 1989; Spring et al., 1989; Gulcher et al., 1989) and this sequence has been implicated in cell–tenascin interactions (Friedlander et al., 1988; Bourdon and Ruoslahti, 1989). Yet, in the first found mouse clone that covered the third fibronectin type III repeat (M20/1) no such sequence could be found. In view of the postulates that tenascin binds to cells via an RGD sequence the result was highly unexpected. Four independent cDNA clones covering this region were therefore isolated. The clones, isolated from three different cDNA libraries, invariably showed the same nucleotide sequence coding for the tripeptide Arg-Val-Asp rather than Arg-Gly-Asp. Furthermore, no Arg-Gly-Asp sequence was found in any other parts of the mouse tenascin sequence (Fig. 1).

Differential Splicing Gives Rise to Two Major Mouse Tenascin mRNAs

Previously performed immunoprecipitation with monoclonal antitenascin antibodies suggested that mouse embryonic fibroblasts can synthesize two polypeptides of M_r 260,000 and 210,000, whereas mouse B16 melanoma cells did not synthesize any of these polypeptides (Aufderheide and Ekblom, 1988). Our current data suggest that these two major polypeptides form by differential splicing. The insert of clone M20/1 detected two species of mRNA of ~6 and 8 kb in total RNA from primary mouse embryo fibroblasts (Fig. 5, lane *a*), and in accordance with the protein data no signals could be seen in total RNA from B16 mouse melanoma cells (Fig. 5, lane *b*). To further verify that the two RNA species in fibroblasts are splice variants, Northern blots of RNA from fibroblasts were performed with the insert of clone o3′–23, which according to the sequence data should be specific for the larger mouse tenascin variant (Fig. 1). As expected, only the larger 8-kb signal was seen in the fibroblast with cDNA clone o3′–23 (Fig. 5, lane *c*).

Differential Expression of the Two Tenascin mRNAs during Organogenesis In Vivo

Although cultured primary embryonic fibroblasts isolated from the head synthesized both major mRNA variants, in vivo some embryonic mesenchymal cells seem to produce only one of the two major variants. In kidneys from newborn mice only the 8-kb mRNA was prominently expressed, whereas the 6-kb mRNA was expressed only very weakly (Fig. 6). However, during postnatal development, the expression pattern changed drastically. In kidneys of 2-wk postnatal mice the 6-kb message was more predominant whereas the 8-kb mRNA was undetectable (Fig. 6).

A different expression pattern was observed during development of the intestine. Whereas the small mRNA species was more predominant in intestine from 13-d-old embryos, the ratio was reversed in intestine from newborn mice (Fig.

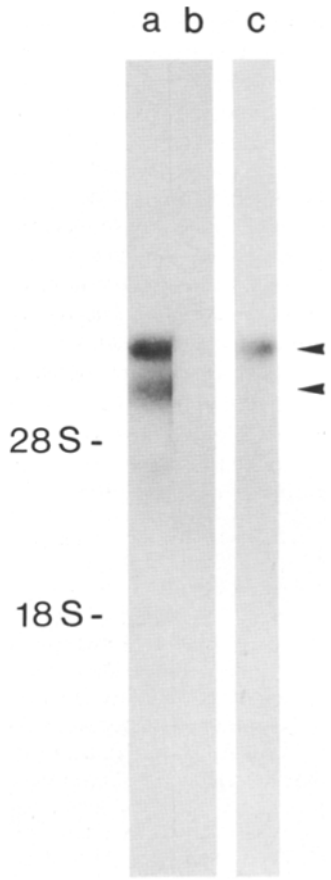

Figure 5. Analysis of the size of tenascin mRNAs by Northern blots. 10 μg total RNA from primary mouse embryonic fibroblasts (*a* and *c*) and from B16 mouse melanoma cells (*b*) were hybridized with clones M20/1 (*a* and *b*) and o3′–23 (*c*). Two signals of close to 6 and 8 kb are detected by clone M20/1 in RNA from fibroblasts known to synthesize tenascin but not in RNA from B16 cells known not to synthesize tenascin protein (Aufderheide and Ekblom, 1988). In contrast, clone o3′–23 detects only the 8-kb mRNA (*c*), indicating that the corresponding sequence is found exclusively in the large splice variant. Autoradiographs were exposed for 48 h (*a* and *b*) and for 16 h (*c*). The positions of 28S and 18S ribosomal RNA are indicated.

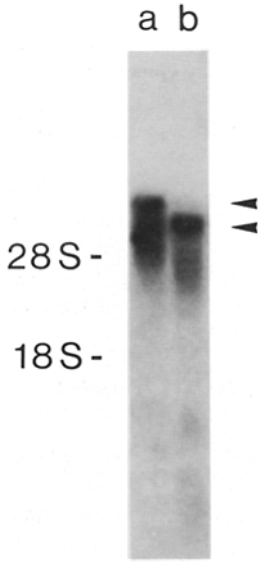

Figure 6. Expression of tenascin mRNAs in developing kidney. 1 μg poly(A^+) RNA from kidneys of newborn (*a*) and 2-wk-old mice (*b*) were hybridized to clone M20/1. In newborn kidneys the 8-kb mRNA species is prominently expressed while the 6-kb species is barely detectable. In the older stage the pattern is reversed with the 6-kb mRNA being expressed much more strongly than the 8-kb form. The autoradiograph was exposed for 24 h.

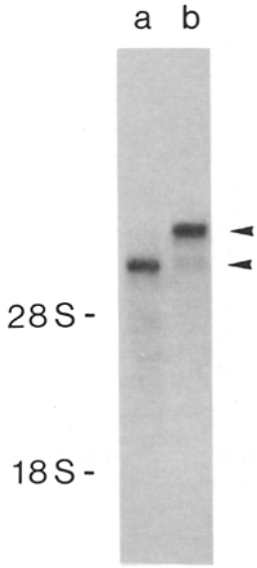

Figure 7. Expression of tenascin mRNAs in developing intestine. 1 μg poly(A^+) RNA from intestine of 13-d-old embryos (*a*) and newborn mice (*b*) were hybridized to clone M20/1. Expression of the tenascin mRNAs shows the opposite pattern: while the ~6-kb mRNA species is prominently expressed in 13-d intestine, its expression declines during the course of development. In the newborn stage the ~8-kb mRNA is expressed much more strongly than the ~6-kb form. The autoradiograph was exposed for 4 d.

7). The mRNA data from intestine match previous protein data well. It has been reported that young embryonic intestine produce predominantly smaller tenascin proteins of M_r 210,000, whereas larger proteins of M_r 260,000 can be detected only at later developmental stages (Aufderheide and Ekblom, 1988).

Discussion

In this study we document some structural characteristics of mouse tenascin, a large glycoprotein expressed prominently in embryonic mesenchyme. We present the primary structure of mouse tenascin as derived from cDNA clones. Biologically significant parts of the molecules are often conserved in evolution, and we therefore here present a comparison between the mouse sequence and the available chicken (Pearson et al., 1988; Jones et al., 1988, 1989; Spring et al., 1989) and partial human sequences (Gulcher et al., 1989). It seems clear from the current data that the two major isoforms of mouse tenascin polypeptides (Aufderheide and Ekblom, 1988) form by differential splicing.

As expected from previous data of tenascin polypeptides, the overall modular structure of mouse tenascin is similar to chicken and human tenascin. The first part of mouse tenascin contains a series of contiguous and highly conserved, cysteine-rich repeats with similarity to the EGF repeat (Carpenter and Cohen, 1979). The spacing of the six cysteine residues follows the same pattern as in the chicken and human sequence; $x_4Cx_3Cx_5Cx_4Cx_1Cx_8C$ (Pearson et al., 1988). It is interesting that the repeats in the same relative position seem to be highly conserved between mouse and human; at the amino acid level they are ~90% identical, whereas other mouse–human combinations show only ~60% identity on average. The biological significance of the EGF-like repeats is unknown at present, but it has been hypothesized that such repeats in the extracellular glycoproteins could act as local mitogens (Panayotou et al., 1989). The availability of cDNA clones that span these tenascin regions should make it easier to test this hypothesis experimentally.

The second part of the mouse contains several contiguous repeats with similarity to the type III repeat of fibronectin described by Kornblihtt et al. (1985). Whereas the mouse EGF elements are ~60% identical, the fibronectin type III repeats show only ~25% identity among each other at the amino acid level. In contrast, a comparison of the repeats between different species shows that each individual repeat displays a high degree of homology with the same repeat from other species. When repeats in similar relative positions are analyzed, the homology between mouse and human is >90%, and between mouse and chicken still >70%. Previous estimations about the size of tenascin polypeptides have suggested that the larger chicken tenascin polypeptide is smaller than the corresponding mouse polypeptide, and that human tenascin can be somewhat larger than mouse tenascin. The available sequence data show that these differences are largely due to the different sizes of the mRNAs. These differences have apparently emerged during evolution by an addition of new fibronectin type III repeats. It is interesting that the differentially spliced fragment begins after the fifth fibronectin type-II repeat in each of the three species.

In the third fibronectin type III repeat of both chicken and human tenascin the tripeptide Arg-Gly-Asp is found (Jones et al., 1988, Gulcher et al., 1989). This sequence is known to be an important part of cell-binding sites in a number of extracellular matrix proteins (Hynes, 1987; Ruoslahti and Pierschbacher, 1987). The involvement of the RGD sequence in cell attachment to tenascin has been postulated, but the issue has remained unclear. Friedlander et al. (1988) reported that the GRGDS-peptide inhibits the attachment of chicken embryonic fibroblasts on tenascin in one attachment assay but fails to do so in another assay. Chiquet-Ehrismann et al. (1988) could not find a dramatic effect of the GRGDS-peptide on the attachment of chicken embryonic fibroblasts to tenascin, although they could demonstrate an inhibitory effect of this peptide on cell attachment to fibronectin. It was also found that a strong cell-binding site is located within 104 amino acids in the tenth and eleventh type III repeat of chicken tenascin (Spring et al., 1989). This is in good agreement with the findings suggesting that a cell-binding site of tenascin is located within the carboxy terminal end of the molecule, far from the fibronectin type III repeat containing the RGD (Chiquet-Ehrismann et al., 1988; Jones et al.,

1988). However, Bourdon and Ruoslahti (1989) have isolated and characterized an RGD-dependent integrin-type tenascin receptor from human glioma cells. Attachment of these cells to tenascin can be inhibited by the GRGDSP peptide, but the isolated tenascin receptor did not bind to GRGDSP-sepharose. In view of these apparent discrepancies, it is interesting that mouse tenascin does not contain an RGD sequence in the third fibronectin type III repeat but instead an RVD sequence. The RVD tripeptide was shown to be nonfunctional in promoting cell attachment in the synthetic peptide RVDSPA (Pierschbacher and Ruoslahti, 1984), which strongly suggests that it is also nonfunctional in mouse tenascin. No RGD sequence was found in any other part of the two mouse tenascin molecules either.

Recently, it was suggested that the tripeptide Leu-Arg-Glu (LRE) could be a cell-binding site for s-laminin, a polypeptide of some basement membranes (Hunter et al., 1989). LRE occurs in human and chicken tenascin, and we here show that this tripeptide is present twice in mouse tenascin, starting from either position 136 or 1498. The first one, but not the second, LRE sequence is found in the chicken sequence. It remains to be seen whether the LRE sequence participates in the binding of tenascin to cells. In light of the studies of the group of Chiquet-Ehrismann this is an unlikely possibility because the cell-binding site was suggested to be located in the tenth or eleventh fibronectin type III repeat (Chiquet-Ehrismann et al., 1988; Spring et al., 1989). It must be concluded that the location of the cell binding site of tenascin is rather unclear at the moment.

Most of the potential glycosylation sites are found in the fibronectin type III repeats. Most notable was the finding that 10 of the sites were found in the repeats of the larger tenascin form. Since glycosidase F treatment reduced the size of the tenascin polypeptides of cultured embryonic fibroblasts by ~30 kD, it seems that these sites are used during embryogenesis. At least in the embryonic kidney the larger 8-kd message, coding for the potentially glycosylated larger tenascin polypeptide, predominates during embryogenesis. The data from the kidney of newborn and postnatal mice raised the possibility that the larger tenascin message is an "embryonic" form of tenascin and this rule may hold for many tissues (Weller, 1990). However, there may be some exceptions and we demonstrate here quite a different expression pattern for the developing gut. In the gut, the smaller mRNA was predominant early and at later developmental stages the larger 8-kb mRNA appeared. In the gut, a continuous renewal of epithelial cells occurs also in the adult stage and one possibility is that expression of the larger message is controlled by factors released by actively growing epithelial cells. Further studies are required to resolve this particular issue, and it may be that there are no simple rules for the expression of the two major splice variants of mouse tenascin. With the available cDNA clones and the sequence data for mouse tenascin, these issues can now be studied in detail.

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