

Human Laminin M Chain (Merosin): Complete Primary Structure, Chromosomal Assignment, and Expression of the M and A Chain in Human Fetal Tissues

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Abstract. The primary structure of the human laminin M chain was determined from cDNA clones isolated from human placental libraries. The clones covered a total of 6,942 bp, with 49-bp encoding a 5' end untranslated region and 6,893-bp coding for a translated sequence. The complete human laminin M chain contains a 22-residue signal peptide and 3,088 residues of the mature M chain. The M chain has a domain structure similar to that of the human and mouse A chains. The homology between the two human laminin heavy chains is highest in the short arm region and lowest in the long arm helical domain I + II. Northern blot analysis of human fetal tissues showed that the M

chain was expressed in most tissues such as cardiac muscle, pancreas, lung, spleen, kidney, adrenal gland, skin, testis, meninges, choroid plexus, and some other regions of the brain, but not in liver, thymus, and bone. In situ hybridization localized the expression of the M chain gene to cells of mesenchymal origin. In contrast, expression of the A chain was observed only in kidney, testis, neuroretina and some region of brain as determined by Northern analyses. Epithelial and endothelial cells were negative for both M and A chain gene transcripts. The gene for the human M chain (LAMM) was localized to chromosome 6q22→23.

LAMININS are a family of large extracellular glycoproteins. Laminin, initially isolated from the murine Engelbreth-Holm-Swarm (EHS)¹ tumor, was shown to be a heterotrimeric cross-shaped molecule containing one large A chain of 400 kD, and two smaller B1 and B2 chains of about 200-kD each (Timpl et al., 1979; Cooper et al., 1981). Recent studies have revealed that several genetically distinct subunit chains and consequently several laminin isoforms exist. In addition to the EHS laminin chains, merosin (M chain) a homologue of the A chain (Leivo and Engvall, 1988; Ehrig et al., 1990), s-laminin (S chain) a homologue of the B1 chain (Hunter et al., 1989), and B2t, a truncated homologue of the B2 chain (Kallunki et al., 1992), have been characterized. Recently partial sequence of another B1 chain variant in avian eye was reported (O'Rear, 1992). K-laminin and kalinin are laminin isoforms that are present in epithelial basement membranes. K-laminin contains the B1 and B2

chains and has a third 190-kD chain immunologically distinct from the A chain (Marinkovich et al., 1992b). Kalinin has three subunits of which the largest one is immunologically related to one chain of K-laminin (Rousselle et al., 1991; Marinkovich et al., 1992a). A new systematic nomenclature has been proposed for laminin by Engel et al. (1991) according to which the classical EHS chains are termed Ae, Ble, and B2e chains, the M chain is termed Am, the S chain is termed B1s, and the truncated B2 homologue B2t chain.

The complete amino acid sequence of the human, mouse and *Drosophila* A, B1, and B2 chains (Sasaki et al., 1987, 1988; Sasaki and Yamada, 1987; Pikkarainen et al., 1987, 1988; Montell et al., 1988, 1989; Chi and Hui, 1989; Haaparanta et al., 1991; Nissinen et al., 1991; Kusche-Gullberg et al., 1992), the rat S chain (Hunter et al., 1989) and the human B2t chain (Kallunki et al., 1992) has been determined. All the laminin chains have a characteristic structure with distinct domains containing cysteine-rich repeats, globular regions and helical structures. The long arm of the molecule is formed by a triple coiled coil with a large globule at the end contributed to by the A type chain. The short arms contain globular domains separated by rigid rod-like domains containing several cysteine-rich repeats. The short

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1. Abbreviation used in this paper: EHS, Engelbreth-Holm-Swarm.

arm of the A chain has three globular domains and three rod-like domains.

The laminin M chain (merosin) was first identified as a protein present in basement membranes of trophoblasts, Schwann cells, and striated muscle (Leivo and Engvall, 1988). The carboxyl terminal sequence of the M chain reported by Ehrig et al. (1990) showed high homology with the laminin A chain. Furthermore, the M polypeptide has a similar size as the A chain and associates with the same light chains. The whole molecule was shown to have a cross-like structure similar to that of laminin. A previously reported 300-kD mouse heart laminin chain is likely to represent a species homologue of the M chain (Paulsson and Saladin, 1989; Paulsson et al., 1991).

The different laminin chains have been shown to have quite varying tissue distribution as determined by immunohistochemical studies and Northern and in situ hybridization analyses (Cooper and MacQueen, 1983; Boot-Handford, 1987; Kleinman et al., 1987; Klein et al., 1988, 1990; Engvall et al., 1990; Sanes et al., 1990; Ekblom et al., 1990; Küchler-Ehret et al., 1990; Nissinen et al., 1991; Kallunki et al., 1992). For example, the A and M chains on the one hand, and the B1 and S chains on the other, have been shown to be mutually exclusive. In placenta four different types of heterotrimers have been identified: A-B1-B2, A-S-B2, M-B1-B2, and M-S-B2 (Engvall et al., 1990).

In vitro studies have indicated that laminin mediates a variety of biological functions such as stimulation of cell proliferation, cell adhesion, differentiation, and neurite outgrowth. The cellular activities are thought to be mediated by cell membrane receptors, many of which are members of the integrin family (Ruoslahti, 1991; Mecham, 1991; Hynes, 1992). Laminin molecules can self-aggregate and form a protein meshwork which, together with the type IV collagen network, contributes to the basement membrane supramolecular structure (Yurchenco and Schnitty, 1990; Yurchenco et al., 1992).

In the present study we have isolated cDNA clones providing the complete primary structure of the human laminin M chain. The gene was localized to 6q22→q23. Expression of the laminin M and A chains was shown to vary extensively between tissues as examined by Northern analyses and in situ hybridization.

Materials and Methods

Generation and Characterization of cDNA Clones

cDNA libraries were made from human placental poly(A) RNA. First, RNA was primed with primer ML-1 (nucleotide residues 6917-6942; see Fig. 2) made according to the previously published M chain sequence (Ehrig et al., 1990). The cDNA was prepared with a cDNA synthesis kit (Amersham Int., Buckinghamshire, UK), purified and cloned into a λ gt10 vector (Promega, Madison, WI) using EcoRI/NotI adaptors (Pharmacia, Uppsala, Sweden), and packaged using the Packagene extract system (Promega). Two other primer extension libraries were prepared similarly using primers M-10 (nucleotide residues 4153-4167; see Fig. 2) and ML-5 (nucleotide residues 1028-1050; see Fig. 2). The first library was screened by using the previously characterized merosin cDNA (Ehrig et al., 1990) as probe. The 5' end 1.4-kb EcoRI fragment of clone M1-1 was used to screen the second extension library, and the third cDNA library was screened using a 1.3-kb NotI/AccI fragment of clone M10-22. To obtain clones for the 5' end of the M chain, ML-6 (nucleotides 706-731) primed cDNA was synthesized and EcoRI adaptors (Promega) were ligated to the cDNA. An EcoRI adapter primer and specific primers were used to amplify

the 5' end of the cDNA by PCR. Purified cDNA clones and PCR products were subcloned into Bluescript II (Stratagene, La Jolla, CA) and sequenced from both strands using dideoxy sequencing (Sanger et al., 1977).

Chromosomal Localization

Chromosomal assignment of the human laminin M chain gene (LAMM) was carried out using human-mouse somatic cell hybrids (Shows et al., 1982, 1984) and in situ hybridizations (Cherif et al., 1989; Fan et al., 1990) with the M10-22 cDNA clone as probe.

Northern Analysis

Total RNA from 18-19-wk-old human fetal tissues was isolated and samples containing about 10 μ g of each RNA were electrophoresed, transferred to a GeneScreen Plus filter and hybridized with human laminin A or M chain cDNA probes. For quantitative comparisons of expression between different tissues the amounts of RNA were standardized using signals obtained with a human 28S rRNA-specific probe as an internal standard. The autoradiographs were analyzed by an Image Quant laser scanner (Molecular Dynamics, Sunnyvale, CA).

In Situ Hybridization

To obtain sense and antisense probes for in situ hybridization a 260-bp NotI-SalI fragment from A chain cDNA clone C2-12 and a 350 bp XhoI-ClaI fragment from M chain cDNA clone M1-1 were cloned into the Bluescript II vector. Probes were labeled with 35 S-labeled UTP (Amersham Intl.) using T3 and T7 polymerases. Human fetal tissues from the 17th gestational week were used. In situ hybridization was performed essentially according to Cox et al. (1984) and Wilkinson and Green (1990).

Results

Characterization of cDNA Clones and Amino Acid Sequence of the Laminin M Chain

A cDNA clone providing 1,130-amino acid residues from the carboxyl terminal end of the human M chain was reported by Ehrig et al. (1990). This cDNA clone (MER 3') and its sequence were used for priming and screening of the first primer extension library. The longest positive 2.9-kb clone M1-1 (Fig. 1) was further characterized and its 5' end sequence was used to prime and screen the second primer extension library yielding clone M10-22 (3.2 kb). The 5' end of clone M10-22 was similarly used for screening of the third primer extension library resulting in the isolation of clone M5-1 (0.8 kb). Several libraries were made in order to obtain clones spanning the entire 5' end sequence. However, all clones obtained through those efforts were either of similar lengths or shorter than M5-1. Genomic clones that were characterized (data not shown) contained the putative exon 2, but not the coding region for the signal peptide and 5' untranslated region. The 5' end sequences were finally obtained by PCR amplification. The primer ML-6 was used to make cDNA to which EcoRI adaptors were ligated. An EcoRI adaptor primer and two specific primers were then used in PCR to amplify a 300-bp 5' end fragment, M6-16 (Fig. 1), containing sequences for the 5' end untranslated region of the mRNA, the signal peptide and the amino terminal end of the M chain.

The nucleotide sequence of the overlapping cDNA clones and the deduced amino acid sequence are shown in Fig. 2. The previously reported COOH-terminal end amino acid sequence (Ehrig et al., 1990) is also included. The clones generated and characterized in this study covered a total 6,942 bp, consisting of a 49-bp 5' end untranslated region and

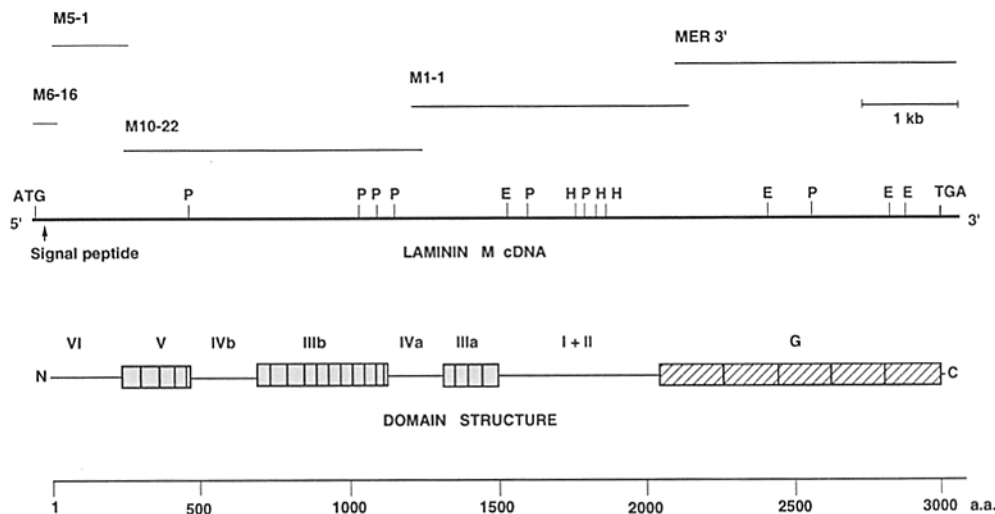


Figure 1. cDNA clones for the human laminin M chain, partial restriction map, and domain structure of the protein. (Top) Alignment of five overlapping cDNA clones and partial restriction map of the cDNA. ATG indicates the translation initiation signal, and TGA the 3'-end translation stop codon. Restriction enzyme sites EcoRI (E), Hind III (H), and Pst I (P) are shown. (Middle) Structure of the protein with domains numbered according to Sasaki et al. (10). Five internal repeats in domain G are indicated by hatched boxes. Domains IIIa, IIIb, and V consisting of cysteine rich EGF modules are shown by shaded boxes. (Bottom) Scale in amino acids (aa).

6,893 bp of an open reading frame. The 5' end sequence has an open reading frame but the sequence ACUACGAUGC around the initiator methionine is in agreement with the Kozak consensus sequence for translation initiation (Kozak, 1991). The putative signal peptide contains 22-amino acids starting with the initiator methionine followed by a hydrophobic leucine-rich sequence. Computer program analysis predicting the signal peptidase cleavage site, based on the method of von Heijne (1986), suggested a cleavage site after Ala22, the mature human M chain thus starting with a glutamine residue as do most laminin chains. Altogether, the laminin M chain contains 3,088-amino acid residues after cleavage of the tentative 22-residue signal peptide.

Domain Structure of the Laminin M Chain and Comparison with the A Chain

The mature human M chain is 30 residues larger than the human A chain which contains 3,058 residues (Nissinen et al., 1991, Haaparanta et al., 1991). Comparison of the two sequences which are aligned in Fig. 3 demonstrates that the domain structure of the M chain is similar to that of the A chain, and that these two laminin heavy chains have considerable homology.

Domains VI (residues 23–386), IVb (residues 528–723), and IVa (residues 1,176–1,379) of the M chain are predicted to form globular structures. Domains V (residues 287–527), IIIb (residues 724–1,175), and IIIa (residues 1,380–1,573) contain cysteine-rich EGF-like repeats and are predicted to have rigid rod-like structures. The numbers of EGF-like repeats is identical in the M and A chains. Domain V has four and one-half repeats, domain IIIb has ten and one-half, and domain IIIa has four repeats. Beck et al. (1990, 1992) count the half repeats as one, and according to that both chains contain 17-cysteine-rich repeats. Domains I+II (residues 1,574–2,153), a part of which has previously been reported (Ehrig et al., 1990), participates together with two B type chains in the formation of a triple coiled-coil structure that forms the long arm of the laminin molecule. In addition, the M chain contains one cysteine residue in this region

which has no counterpart in the A chain or any of the B type chains characterized thus far. The large carboxyl-terminal G domain (residues 2,154–3,110) forms the large globule at the end of the long arm of the laminin molecule.

The amino-terminal domain VI in the M chain has 12, domain IVa two, domain IIIb one, domain I+II has 10 and domain G has 7-amino acid residues more than the A chain. Domain V in the A chain has two residues more than the corresponding domain in the M chain. Comparison of the amino acid sequences of the human laminin M and A chains shows that the overall sequence similarity is 46.6% (Table I) and 58.6% when conservative changes are included (Fig. 3). The sequence identity is highest in the globular domains VI, or 73.9%, although this domain in the M chain contains 12 residues more at the amino-terminus than the A chain. If the additional glutamine rich amino-terminal sequence is excluded, the sequence similarity is 77.4%. All six cysteine sites in this domain are conserved. The amino acid sequence identities of the cysteine-rich domains V, IIIb, and IIIa between the M and A chains are 60.1, 54.9, and 50.2%, respectively. All cysteine residues in these domains are conserved and the length of domains are about the same. The globular domains IVb and IVa of the two chains also have approximately the same number of amino acids, although the sequence similarity is lower (42%). The sequence similarity between A and M chains is lowest in domains I+II or only 32.3%. The sequence identity between domains G is 41.8%. There are 28 putative N-glycosylation sites in the M chain and 34 in the A chain, 10 of these sites are conserved between the two chains. Most putative glycosylation sites are in domains G and I+II. It is noteworthy that the M chain does not contain an RGD sequence which is a recognition site for some integrins (Ruoslahti, 1991; Hynes, 1992).

Chromosomal Assignment of the Human Laminin M Chain Gene (LAMM)

The human laminin M chain gene was mapped to chromosome 6 by hybridization of labeled cDNA clone M10-22 to DNA from a panel of 39 somatic cell hybrids. Hybridization

5300 CTGAAAAAGTGAAGAGCTGTTGGAGAGTCCGGGGGGGAAATGAAGAAATGGAGAAAGATCTCCGGGAAAAATGGCTGACTCAAAAUAUAAUAGTTGATGATGCTTGGAGCTTTTGGAGAGAGCCACAGATAAAATCAGAGAAGCT
1751 L K X V K L L F G E S R G E N E E M E K D L R E K L A D Y K N K V D D A W D L L R E A T D K I R E A

5450 AATGGCTATTTCAGTAAATCAGAAAACATGACTGCATTCAGTGGAGAAAAGAGAGGCTGTGGAGAGCCAAACGAAATGAGAACACTTTAAAGAAAGCAATGACATCTCGATGAAGCCAAACCGCTTCAGATGAAATCAAC
1801 N R L P A V N Q K **N M T** A L E K K K E A V E S G K R Q I E N T L K E G N D I L D E A N R L A D E I N

5600 TCATCATGACATATGTTGAGACATCCAAATTCACCTATGCTGAGGAGCTTAATGATAAATAGATAGCTCCCAAGAAATTAAGCAGGAGAGCTTCCTGAGAGGTTCCCAAGCTGAGAGCCACCCAGTCAAGT
1851 S I I D Y V E D I Q T L L K L P P N S E L N D K I D D L S Q Q E I K D R K L A E K V S Q A E S H A

5750 AATGACTCATCTGCTGCTTTGATGGAAATCCCTGATGAGGCTAAJAAACATCTCTCAATGCCACTGAGGCTTCAAGACTGACAGCAATATTAGGACTATATTGATGAAGCTGAGAAAGTTCCCAAGAAAGCCAAAGATCTTCACAT
1901 **N D S** S A V L D G I L D E A K **N I S** **P N A T** A A F K A Y S N I K D Y I D E A E K V A K E A K D L A H

5900 GAAGTACAAAATGGCAACAGTCTCCGGGGTTTATTAAGGAAGATGCCAAAGGCTGTCTCAGAAAAGCTTCAGGATCTTAAAGCAAGCAAGGATGCAAAATGATGTAAGAAATGAAGACATCTAAATGGCTTAAAGCC
1951 E A T K L A T G P R G L L K E D A K G **C** L Q K S P R I L N E A K K L A N D V K E N E D H L N G L K T

6050 AGGATGAAAATGCTGATGCTAGAAATGGGATCTCTTGGAACTTTGATGACACTTTGGGAAAGTTATCACTTATCCAAATGATCAAGCTGCTTAACTGCAAGCTGTGAGGACAAAGCCAGCAAGCCAGGACACTTAAGAT
2001 R I E N A D A R N G D L L R T L **N D T** L G K L S A I P **N D T** A A K L Q A V K D K A R Q A **N D T** A K D

6200 GTACTGGCAGATTCAGAGCTCCAGAGACCTCGATGCGCTGAAAGAAATACAAATAAATAGCAGACAGCGTCCCAAAACGAAATGCTGTGTAAGATCTCCAAAGAAACAAATCATGGCGATGAGATGCCACTGTCAA
2051 V L A Q I T E L H Q N L D G L K K N Y N K L A D S V A K T N A V V K D P S K N K I I A D A D A T V K

6350 AATTTAGAACAGGAGCTGACCGGCTTAATGATAACTCAACCCATCAAGGAATCTGAGGATAACCTAAGAAAACATCTGAGATAAGGAAATGATAAACCAGCTGGGAAACAGCCCAATCTCTCAAGATATCTGTCTTCA
2101 N L E N A D A R N G D L L R T L **N D T** L G K L S A I P **N D T** E I K E L I N Q A R K Q A N S I K V S V S S

6500 GGAAGTCACTGCAATGCAACATCAAAACAGAAATCAAGAAAGGAGTTCATTAATATATGTTGCTCAAGCTGTAAGACAGCTGTGATCACTCTCTTTTATCTGGAGTCCCAATTTATGACTTTCGGCTATAGAAATGCT
2151 G G D **C** I R T Y K P E I K K G S Y N N I V V N V K T A V A D N L L F Y L G S A K P I D P L A I E M R

6650 AAAGCAAGTCACTGCTCCCTGGAGTGTGGATGCTGGAGTGGAGCTGAGATACCCAGATTTGATTTGATGACTCATATTTGGTACCGTATCGTAGCATCAAGAACTGGGAAATGGAACTATTCTGTGAGAGCCCTGGATGGA
2201 K G X V E A D R N G D L L R T L **N D T** L G K L S A I P **N D T** I D D S Y P D L I D D S Y M Y R I V A S R T G R **N G T** I S I R V S V S S

6800 CCCAAGCCAGCAATGCTCCAGCAGCACACCTTTCAGCTCTCTCCAGGTTACAGATCTAGATGTTGATGCAATGCTGTTTGTGGCTGACTGGGAAATTAAGAAAGGCTGATGCTGTAGCTGTGATAC
2251 P K A S I V P S T H H S T S P P G Y T I L D V D A N A M L F V G G L T G K L K K A D A V R V I T F T

2301 **C** M G E T Y F D N K P I G L W N F R E K E G D **C** K G **C** T V S P Q V E D S E C T I Q P D G E G Y A L

2351 V S R P I R W Y P **N I S** T V M F K F R T P S S A L L M Y L A T R D L R D F M S V E L T D G H I K V

2401 S Y D L G S G M A S V V S N Q N H N D G K W K S P T L S R I Q K Q A **N I S** I V D I D T N Q E E N I A

2451 T S S S G N M F G L D L K A D D K I Y F G G L P T L R **N L S** N K A R P E V N L K K Y S G **C** L K D I E

2501 I S R T P Y N I L S S P D Y V G V T K G **C** S L E N V Y T V S F P K P G F V E L S P V P I D V G T E I

2551 **N L S** F S T K **N E S** G I I L L G S G G T P A P P R R K R R Q T G Q A Y Y V I L L N R G R L E V H L S

2601 T G A R T M R K I V I R P E P N L F H D G R E H S V H V E R T R G I F T V Q V D E N R R Y M Q **N L T**

2651 V E Q P I E V K K L F V G G A P P E F Q P S P L R N I P P F E G **C** I W N L V I N S V P M D P A R P V

2701 S F K N A D I G R **C** A H Q K L R E D E D G A A P A E I V I Q P E P V P T P A P T P T P V L T H G P

2751 **C** A A E S E P A L L I G S K Q P G L S R N S H I A I A P D D T K V K N R L T I E L E V R T E A E S G

2801 L L P Y M A A I N H A D P A T V Q L R N G L P Y F S Y D L G S G D T H T M I P T K I N D G Q W H K I

2851 K I M R S K Q E G I L Y V D G A S **N R T** I S P K K A D I L D V V G M L Y V G G L P I **N Y T** T R R I G

2901 P V T Y S I D **C** V R N L H M A E A P A D L E Q P T S S P H V G T **C** F A N A Q R G T Y F D G T G F A

2951 K A V G G F K V G L D L L V E F E P A T T T T T G V L L G I S S Q K M D G K G I E M I D E K L M P H

3001 V D N G A G R P T A V Y D A G V P G H L **C** D G Q W H K V T A N K I K H R I E L T V D G N Q V E A Q S

3051 P N P A S T S A D T N D P V F V G G F P D D L K Q P G L T T S I P F R G **C** I R S L K L T X G T A S H

3101 W R L I L P R P W N

Figure 2. Nucleotide sequence of human laminin M chain cDNA clones and cDNA deduced amino acid sequence of the entire chain. (First line) Nucleotide sequence of cDNA clones characterized in this study. (Second line) Deduced amino acid sequence from the cDNA clones together with the previously determined carboxyl terminal end amino acid sequence (Ehrig et al., 1990). The putative signal peptidase cleavage site is indicated by a triangle. The cysteine residues are circled, and potential attachment sites for asparagine-linked oligosaccharides are boxed. This sequence data is available from EMBL/GenBank/DBJ under accession number Z 26653 (LAMININM).

of the M cDNA clone correlated with the distribution of chromosome 6 (data not shown). In situ hybridization of the cDNA to metaphase chromosomes confirmed the localization of the LAMM gene to chromosome 6, and more precisely to bands 6q22→q23 (Fig. 4).

Expression of M and A Chain Genes in Human Tissues

Relative expression of the M and A chain genes was compared by Northern hybridization using RNA from several 18–19-wk-old human fetal tissues (Fig. 5) and a human 28S rRNA probe as internal standard (not shown). However, comparison of expression between the M and A chain genes was not possible due to different lengths and specific activity of the probes and different exposure times.

As previously reported (Nissinen et al., 1991), the A chain gene has highly restricted expression in human adult tissues.

Signals for the A chain were observed only in brain, meninges, neuroretina, kidney, and testis, while no signals were obtained with RNA from skin, colon, pancreas, adrenal glands, cardiac muscle, lung, thymus, spleen, liver, or calvarial bone, even after long exposures. The relative expression was highest in the neuroretina, and in the brain tissues the A chain gene is expressed in the intermediate zone, cerebellum, olfactory bulb, and weak expression was observed also in choroid plexus.

The M chain gene has a different expression pattern, signals being observed with RNA from most tissues studied except thymus, liver, calvarial bone, colon and ependymal, and intermediate zones of brain. The strongest expression of the M chain gene was seen in pancreas, choroid plexus, cardiac muscle, and meninges.

In Situ Hybridization

The location of M chain mRNA was analyzed by in situ hy-

SP

1 M P G A A G V L L L L L L S G G L G G V Q A Q R P Q Q Q R O S Q A H Q Q R G L F P A V L N L A S N A L I T T N A T G E
 1 M R G G V L L V L L L C V A A Q C
 R Q R G L F P A I L N L A S N A H I S T N A T G E

61 K G P E M Y C K L V E H V P G Q P V R N P Q C R I C N Q N S S N P N Q R H P I T N A I D G K N T W W Q S P S I K N G I E
 44 K G P E M F C K L V E H V P G R P V R N P Q C R I C D G N S A N P R R E R H P I S H A I D G T N N W W Q S P S I Q N G R E

121 Y H V T I T L D L Q Q V F Q I A Y V I V K A A N S P R P G N W I L E R S L D D V E Y K P W Q Y H A V T D T E C L T L Y
 104 Y H W V T I T L D L R Q V F Q V A Y V I I K A A N A P R P G N W I L E R S L D G T T F S P W Q Y Y A V S D S E C L S R Y

181 N I Y P R T G P F S Y A K D D E V I C I T S F Y S K I H P L E N G E I H I S L I N G R P S A D D P S P E L L E F T S A R Y
 164 N I T P R R G P P T Y R A D D E V I C I T S Y S R L V P L E H G E I H T S L I N G R P S A D D L S P K L L E F T S A R Y

241 I R L R F Q R I R T L N A D L M M F A H K D P R E I D P I V T R R Y Y S V K D I S V G G M C I I Y G H A R A C P L D P
 224 I R L R F E R I R T L N A D L M T L S H R E P K E L D P M L P R R Y Y S I K D I S V G G M C I I Y G H A S S C P W D E

301 A T N K S R C E C E H N T C G D S C D Q C C P G F H Q K P W R A G T F L T K T E C E A C N C H G K A E E C Y Y D E N V A
 284 T T K K L Q C Q C E H N T C G E S C N R C C P G Y H Q Q P W R P G T V S S G N T C E A C N C H N K A K D C Y Y D E S V A

361 R R N L S L N I R G K Y I G G V C I N C T Q N T A G I N C E T C T D G F F R P K G V S P N Y P R P C Q P C H C D P I G
 344 K Q K K S L N T A G Q F R G G V C I N C L Q N T M G I N C E T C I D G Y R P H K V S P Y E D E P C R P C N C D P V G

421 S L N E V C V K D E K H A R R G L A P G S C H C K T G F G G V S C D R C A R G Y T G Y P D C K A C N C S G L G S K N
 404 S L S S V C I K D D L H S D L E N G K Q P G C P C K E G Y T G E K C D R C Q L G Y K D Y P T C V S C G C N P V G S A S

479 E D P C F G P C I C K E N V E G G D C S R C K S G F F N L Q E D N W K G C D E C F C S G V S N R C Q S S Y W T Y G K I Q
 464 D E P C T G P C V C K E N V E G K A C D R C K P G F Y N L K E K N P R G C S E C F C F G V S D V C S S L S W P L G Q V N

539 D M S G W Y L T D L P G R I R V A P Q Q D D L D S P Q Q I S I S N A E A R Q A L P H S Y Y W S A P A P Y L G N K L P A
 524 S M S G W L V T D L I S P R K I P S Q Q D A L G G R H Q V S I N N T A V M Q R L A P K Y Y W A A P E A Y L G N K L T A

598 V G G Q L T F T I S Y D L E E E E D T E R V L Q L M I I L E G N D L S I S T A Q D E V Y L H P S E E H T N V L L L K
 583 F G G F L K Y T V S Y D I P V E T V D S N L M S H A D V I I K G N G L T L S T Q A E G L S L Q P Y E E Y L N V V R L V

657 E E S F T I H G T H F P V R R K E F M T V L A N L K R V L L Q I T Y S F G M D A I F R L S S V N L E S A V S Y P T D G
 642 P E N F Q D F H S K R Q I D R D Q L M T V L A N V T H L L I R A T Y N S A K M A L Y R L E S V S L D I A S S N A I D L

716 S I A A A V E V C Q C P P G Y T G S S C E S C W P R H R R V N G T I F G G I C E P C Q C P F G H A E S C D D V T G E C L N
 701 V V A A D V E H C E C P Q G Y T G T S C E S C L S G Y Y R V D G I L F G G I C Q P C E C H G H A A E C N V H G V C I A

776 C K D H T G G P Y C D K C L P G F Y G E P T K G T S E D C Q P C A C P L N I P S N N F S P T C H L D R S L G L I C D G C
 760 C A H N T T G V H C E Q C L P G F Y G E P S R G T P G D C Q P C A C P L T I A S N N F S P T C H L N D G D E V V C D W C

836 P V G Y T G P R C E R C A E G Y F G Q P S V P G G S C Q C P C Q C N D N L D F S I P G S C D S L S G S C L I C K P G T T G
 820 A P G Y S G A W C E R C A D G Y Y G N P T V P G E S C V P C D C S G N V D P S E A G H C D S V T G E C L K C L G N T D G

896 R Y C E L C A D G Y F G D A V D A K N C Q P C R C N A G G S F S E V C H S Q T G C E C R A N V Q G Q R C D K C K A G T
 880 A H C E R C A D G Y F G D A V T A K N C R A C E C H V K G S H S A V C H L E T G L C D C K P N V T G Q C D Q C L H G Y

956 F G L Q S A R G C V P C N C N S F G S K S F D C E E S G C Q W C Q P G V T G K K C D R C A H G Y F N F Q E G G C T A C E
 940 Y G L D S G H C C R P C N C S V A G S V S D G C T D E G C C H V P G V A G K R C D R C A H G F Y A Y Q D G S C T P C D

1016 C S H L G N N C D P K T G R C I C P F N T I G E K C S K A P N T W G H S I T T G C K A C N C S T V G S L D F C N V N
 1000 C P H T Q N T C D P E T G E C V C P F H T Q G V K C E E C E D G H W G Y D A E V G C Q A C N C S L V G S T H R C D V V

1076 T G Q C N C H P K F S G A K C T E C S R G H W N Y P R C N L C D C F L P G T D A T C D S E T K K C S C S D Q T G C T
 1060 T G H C Q C K S K F G G R A C V Q C S L G Y R D F P D C V P C D C D L R G T S G D A C N L E Q G L C G C V E E T G A C P

1136 C K V N V E G I H C D R C R P G K F G L D A K N P L G C S S Y C F G T T T C G S E A K G L I R T W V T L K A E Q T I L
 1120 C K E N V F G P Q C N E C R E G T F A L R A D N P L G C S F C F C S G L S H L C S E L E D Y V R T P V T L G S D Q P L L

1196 P L V D E A L Q H T T T K G I V F Q H P E I V A H M D L M R E D L H L E P F Y W K L P E Q P E G K K L M A Y G G K L K Y
 1180 R V V S Q S N L R G T T E G V Y Y Q A P D F L L D A A T V R Q H I R A E P F Y W R L P Q Q F Q G D Q L M A Y G G K L K Y

VI

V

IVb

IIb

IVa

Table I. Similarity of Amino Acid Sequences of the Human Laminin M and A Chains as Aligned in Fig. 3

Domain	Length of aligned sequence	Matches	Matches (conservative substitutions)	Unmatches	Matches/Length %
VI	264	195	25	12	73.9 (83.3)
V	243	146	23	2	60.1 (69.5)
IVb	199	85	28	6	42.7 (56.8)
IIIb	452	248	38	1	54.9 (63.3)
IVa	207	88	33	8	42.5 (58.5)
IIIa	195	98	14	2	50.2 (57.4)
I+II	591	191	79	32	32.3 (45.7)
G	987	413	136	67	41.8 (55.6)
Total	3138	1464	376	130	46.6 (58.6)

tures similar to other laminin chains and it is practically identical to that of the A chain. The amino terminal globular domains VI share the highest homology, although the M chain has additional 12 amino acids at the amino terminus. In fact, domain VI of all known human laminin chains (Pikkarainen et al., 1987, 1988; Nissinen et al., 1991), the mouse A chain (Sasaki et al., 1988), the rat S chain (Hunter et al., 1989), and the *Drosophila* A chain (Kusche-Gullberg et al., 1992) can be aligned so that the cysteine residues, some glycine, serine, proline and arginine residues, and short amino acid sequences RP, TCG and WWQS match in all chains (Fig. 7). A conserved sequence, Y(Y/F)Yxhxdhxh(G/R)G (h: hydrophobic residues, d: D, E, or N) (according to Beck et al., 1992) at the carboxyl terminus of domain VI is also found in the M chain. The function of these conserved sequences is not known, but they might have significance for the role of this domain in laminin self-assembly which is apparently mediated by the amino terminal globular domains (Yurchenco and Schnitty, 1990).



Figure 4. Chromosomal localization of the M chain. The idiogram of chromosome 6 shows the distribution of signals on that chromosome and assignment of the LAMM gene to 6q22→23.

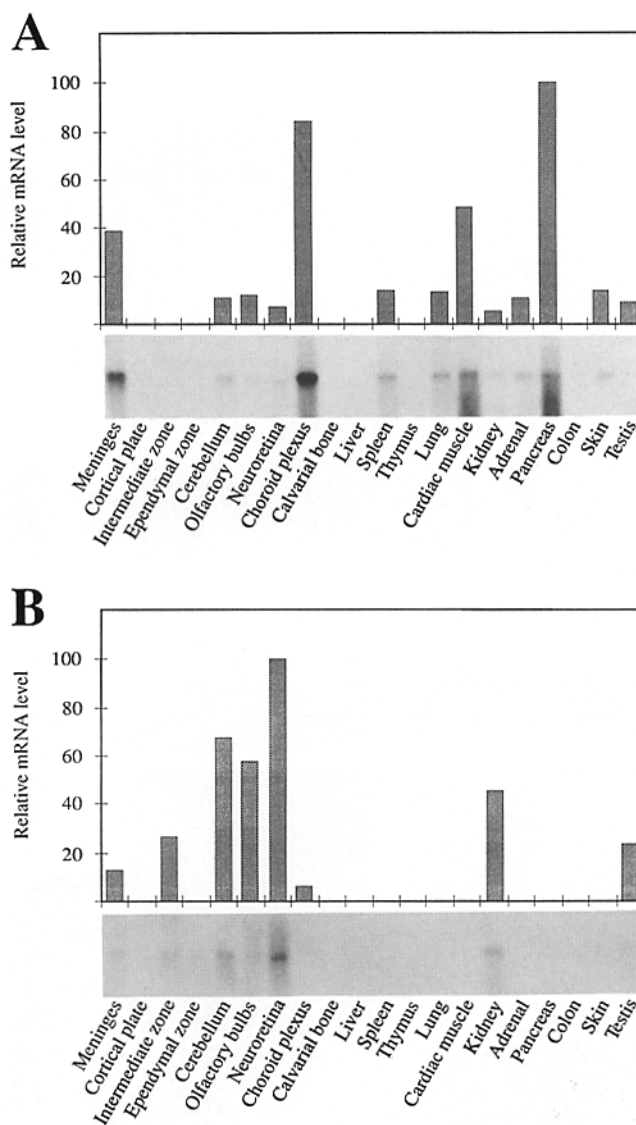


Figure 5. Expression of laminin M (A) and A (B) chains in 17-wk-old human fetal tissues. A Gene Screen Plus filter containing total RNA (~10 µg) was prepared and hybridized with laminin M and A chain probes as well as a 28S RNA probe (internal standard) as described in Materials and Methods. The autoradiographs were scanned and relative intensity of expression is shown for the laminin M and A chains on an arbitrary scale.

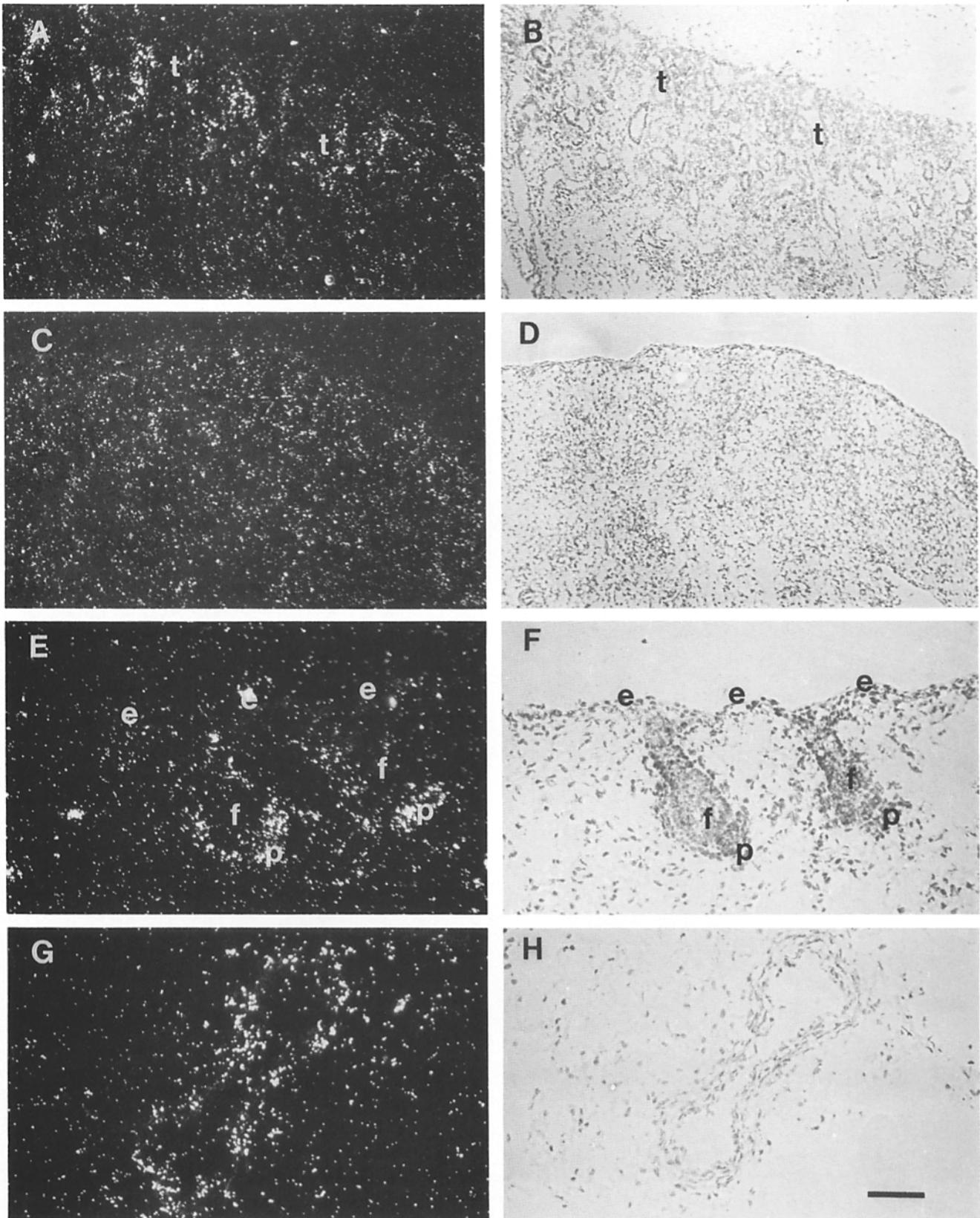


Figure 6. In situ hybridization of the laminin M chain in 17-wk-old fetal tissues. In kidney (*A* and *B*) signals are seen in mesenchymal cells adjacent to condensing pretubular cells and ureter-derived tubules (*t*) in the outer cortex. Secretory tubules of the nephron and blood vessels are negative. In heart muscle (*C* and *D*) signals can be observed in cardiomyocytes throughout the muscle. In section of skin (*E* and *F*) no grains are seen over the epithelial cells of epidermis (*e*), while strong signal can be observed in the condensing papillary mesenchymal cells (*p*) around a developing hair follicles (*f*). In lung (*G* and *H*) signals are present in smooth muscle cells of the peribronchial arterial wall, but alveolar and bronchial cells are negative. Bars: (*A-D*) 200 μm ; (*E-H*) 100 μm .

B1	QEPEFSY	GCAEGSCYPATGDL	LIGRAQKLSVTSTCGL	HKPEPY	CIVS	HLQEDKKCF	CNSQDPYHETLN	PNDSHLIENVTTF	FAPNR	LKI																																	
S	QVPSLDV	PGSCRGSCYPATGDL	LVGRADRLTASSTCGL	HSPQP	CIVS	HLQDEKKCF	LCDSRRPFSARDN	PNNSHRIQNVVTS	FAFQR	RTA																																	
A	RQRGLF	PAILNLASNAHISTNA	TCGE	KGPEM	FCKLV	EHPVGR	PVRNPOCRI	CDGNSANPR	RHPI	SHAIDG	TNN																																
mA	QQRGLF	PAILNLATNAHISANA	TCGE	KGPEM	FCKLV	EHPVGR	PVRHAQCRV	CDGNSNTPR	RHPI	SHAIDG	TNN																																
M	QRPQQR	QSQAHQQRGLF	PAVLNLASNALITTN	TCGE	KGPEM	FCKLV	EHPVGP	VVRNPOCRI	CNQNSNP	NQRHPI	TNAIDG	KNT																															
dA		ELTPPYFN	LATGRKIYATA	TCG	PDTPGPEL	YCKLVG	ANTEHHDHIDYS	VIQGVQ	CDYCD	PTVPER	NHPP	ENAIDG	TEA																														
B2	QAAMDECT	DEGGRP	QRCMPEFVNA	AFNVTVVATN	TCG	TPPEE	YCVQ	TGVTG	VTKSCHL	CDAGQ	PHLQHGAA	FTD	YNNQAD	TT																													
B1	WWQS	ENGVEN	VTIQLDLEAEF	HFTHLIMTF	KTFRPA	AMLIERS	SSDFGKT	WGVYRYFA	YDCEAS	PGISTG	PMKKV	DDI	ICDSRY	SDIEP																													
S	WWQS	ENGVPM	VTIQLDLEAEF	HFTHLIMTF	KTFRPA	AMLIERS	ADFGRT	WRVRYFS	YDCGAD	PGIPLA	PPRRW	DDV	VCESRY	SEIEP																													
A	WWQSP	SIQNGREYH	WVTITL	DLRQVQVAYV	IIKAANA	PRPGN	WILERSLD	GTTFS	PWQYAV	SDSECL	SRNIT	PRRG	PPT	YRADDEV	ICTSY	SRLVP																											
mA	WWQSP	SIQNGREYH	WVTITL	DLRQVQVAYV	IIKAANA	PRPGN	WILERSVD	GVKFK	PWQYAV	SDTECL	TRK	IT	PRRG	PPT	YRADDEV	ICTSY	SKLVP																										
M	WWQSP	SIKNGIEYH	YVTITL	DLOQVQIAYV	IVKAANS	PRPGN	WILERSLD	DVEYK	PWQYAV	HTDTECL	TLN	NIY	PR	TG	PPS	YAKDDEV	ICTSY	SKIHP																									
dA	WWQSP	PLSRGMKFN	EVNLT	INFEQEFH	VAYLFR	MGN	SRPGLW	TLKSTDY	GKTW	TPWQH	FSDT	PAD	CETY	GKDTY	KPITR	DDDV	ICTTEY	SKIVP																									
B2	WWQS	QTLAGVQ	PSSINL	TLHLGK	AFDITV	RLK	HTSR	PESFAI	YKRT	REDG	PWIPYQ	YSGS	CENT	SKANR	GFIRT	GG	DEQ	ALCT	DEF	SDIS																							
B1	STEGEVI	FRA	LDPAFK	IEDPY	SPRIQ	NLLK	ITNLRI	KFVKL	HTLGD	NLL	D	SRMEI	REKY	YAVY	DMV	VRGN																											
S	STEGEVI	YRVL	DP	PIPPY	SSRIQ	NLLK	ITNL	RVN	LR	HTLGD	NLL	D	PRREI	REKY	YAL	YLV	VRGN																										
A	LEHGEI	HTSL	INGR	PSADD	LSPK	L	LEFTS	ARYI	RLR	FR	IR	TLN	ADL	M	TL	SHR	PK	EL	D	P	M	PRR	Y	Y	S	I	K	D	I	S	V	G	G										
mA	LEHGEI	HTSL	INGR	PSADD	PSPOL	L	LEFTS	ARYI	RLR	LR	Q	R	IR	TLN	ADL	M	TL	SHR	DL	R	D	P	I	V	TRR	Y	Y	S	I	K	D	I	S	V	G	G							
M	LENGEI	HISL	INGR	PSADD	PSEL	L	LEFTS	ARYI	RLR	FR	IR	TLN	ADL	M	F	A	H	K	D	P	R	E	I	D	P	I	V	TRR	Y	Y	S	V	K	D	I	S	V	G	G				
dA	LENGEI	PVML	LNER	P	STNY	FN	QEW	TRAT	NV	R	IR	L	R	T	K	N	L	G	H	L	M	S	V	A	R	O	D	P	T	V	TRR	Y	Y	S	I	K	D	I	S	I	G	G	
B2	LTGGNV	AFST	LEGR	PSAY	NFDN	SPV	LQEW	VTAT	D	IR	V	T	L	N	R	L	N	T	F	G	D	E	V	F	N	D	P	K	V	L	K	S	Y	Y	A	I	S	D	F	A	V	G	G

Figure 7. Alignment of domains VI of the known human A- and B-type laminin chains, the rat S chain, the mouse A chain and the *Drosophila* A chain. Amino acids that are identical in half of the chains are shaded, and dark shading indicates conserved change Phe (F) ↔ Tyr (Y).

The number and order of the EGF-like repeats in domains V, IIIb, and IIIa are conserved in the human M chain and, generally, the repeats are very similar to the repeats present in the human and mouse A chains. The repeats in domain V of the human A, M, B1, B2, and B2t chains, the rat S chain, the murine A chain and the *Drosophila* A chain (Pikkarainen et al., 1987, 1988; Sasaki et al., 1988; Hunter et al., 1989; Nissinen et al., 1991; Kallunki et al., 1992; Kusche-Gullberg et al., 1992) can be aligned in order (Fig. 8). The human B2t chain (Kallunki et al., 1992) lacks the first EGF-like repeat, but the rest of the repeats match with repeats of the other chains. In contrast to other laminin chains, the *Drosophila* A chain contains 10 and a half EGF-like repeats in domain V (Kusche-Gullberg et al., 1992; MacKrell et al., 1993). The two first cysteine-rich repeats in the *Drosophila* A chain can be aligned with repeats in the other chains but the rest of domain V differs more, although some similarities are found between repeats 3, 4, 5, and 6 in the *Drosophila* A chain and repeats 3, 4, and 5 in the other chains.

Globular domains IV of the A and B2 type chains have been suggested to have evolved by an insertion between the third and fourth cysteines in one EGF-like repeat, and to be duplicated in A chains to form domains IVb and IVa (Beck et al., 1990, 1992). These domains are present in the M chain and are, thus, well conserved in the A type chains, except for the *Drosophila* A chain which contains only one domain IV. It also has another domain IV* that consists of duplicated sequences that are more similar to the *Drosophila* B1 chain domain IV (Kusche-Gullberg et al., 1992; MacKrell et al., 1993). Domains I + II that form the long arm helical region are similar in the M and A chains and the cysteine pair that is suggested to form interchain disulfide bonds is conserved in the M chain.

Domain G of the M chain consists of five internal repeats that contain 107–178-amino acid residues (Ehrig et al., 1990). These repeats share 30 to 50% similarity when compared with the human or mouse A chain. The *Drosophila* A

chain also has five repeats in the G domain, but there is a large spacer sequence rich in threonine residues between subdomains G3 and G4 (Kusche-Gullberg et al., 1992). Several proteins are known to be homologous to the G domain in the A and M chains. For example, one domain of the HSPG (heparan sulfate proteoglycan) core protein, perlecan, has 33% similarity with the domain G of the human A and M chains (Kallunki and Tryggvason, 1992). Other homologous proteins are sex hormone-binding globulin (Beck et al., 1992), androgen binding protein (Joseph and Baker, 1992) and neurexins (Ushkaryov et al., 1992). Also *Drosophila* proteins *fat*, *slit*, and *crumbs* share similarities with domain G of the M and A chains (Patthy, 1992).

Expression of Laminin M and A Chains in Human Fetal Tissues

In the present study expression of the M chain gene was observed in many tissues known to contain the respective protein from immunohistological studies. However, the strong level of expression at an early embryonic stage contrasts previous immunostaining studies where the M chain was not detected in the mouse embryo (Leivo and Engvall, 1988). The reason for this discrepancy is obscure, but it could be due to some unknown limitation in the antibodies or transcripts may not always be efficiently translated into proteins. The M chain has been reported to appear in mouse muscle tissues first after birth (Leivo and Engvall, 1988) and at adult stages also in some other tissues in several mammalian species (Sanes et al., 1990). The in situ hybridization analyses localized the expression of the M chain gene to myocytes of heart muscle which agrees with several previous studies (Leivo and Engvall, 1988; Paulsson and Saladin, 1989; Klein et al., 1990; Engvall et al., 1990; Paulsson et al., 1991). However, expression was also seen in stromal cells close to condensing mesenchyme in kidney and skin. The M chain has been localized by a monoclonal antibody to a narrow region located between the stromal cells and pretubular condensates in the outer cortex. A good concordance be-

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B1 CFCYGHASECAPVDGFNEEVEGMVHGHCMRHNTKGLNCELMDFYHDL PWRPAEGRNSNACKK
S CFCYGHASOCPAPGAPAHAEQVMHGACICKHNTRGLNCEQCQDFYQDL PWHPAEDGHTHACRK
A CICYGHASSCP WDE TTKLQCCOCEHNTCGESCNRCCPGYHQQPWRPGTVSSGNTCEA
mA CICYGHASSCP WDE EAKQLQCCOCEHNTCGESCDRCCPGYHQQPWRPGTISSGNECEE
M CICYGHARACP LDP ATNKSRCOCEHNTCGDSCDQCCPGFHQKPWRAGTFLTKTECEA
B2 CKCNHGASEC MKN EFDKLVNCKNHNTYGVDCCKLPPFNDRPWRRATAESASECLP
dA CMCNGHADTCD VKDKPSVPRILACRCQHHTCGIQCNCECCPGSEQKKWRONTNARPFNCEP

B1 CNCNEHSISCHFDMAVY LATGNVSGGVCDQCQHNMTGRNCEOCKPFYQHPERDIRDPNFCER
S CECNGHSHSCHFDMAVY LASGNVSGGVCDGCQHNTAGRHCCELCPFFYRDPTKMDRDPAACRP
A CNCHNKAKDCYYDESVAKQKSLNTAGQFRGGGVCINCLQNTMGINCETCIDQYRPHKVSYPYEDPCRP
mA CNCHNKAKDCYYDSSVAKERRSLNTAGQYSGGGVVCNCSQNTTGINCETCIDQYRPHKVSYPYDDHPCRP
M CNCHGKAEECYFDENVARRNLSLNIRGKYIGGGVCINCTQNTAGINCETCTDGFPRPKGVSPNYPRPCQP
B2 CDCNGRSQECYFDPELYRST GHGGHCTNCQDNTDGAHCERCENFFRLG NNEACSS
B2t CDCNGKSRQCIEDRELHROT GNGFRCLNCDNTDGIHCEKCKNGFYRHR ERDRCLP
dA CNCHGHSNECKYDDEVNRKGLSLDIHGHYDGGVVCQNCQHNTVGINCNKCKPKYRPGKHWNEDTVCSP

B1 CTCDPAGSQNEG ICDSYT DFSTGLIAGQ CRCKLNVEGEHCDVCKEGFYDLSSDDPFGCKS
S CDCDPMGSQDGG RCDSDH DPVLGLVSGQ CRCKEHVVGTRCQCQRDGFGLSASNPRGCQR
A CNCDPVGSL S VCIKDDLHSDLENGKQPGQ CPCKEGYTGEKCDRCQLGY KDYPT CVS
mA CNCDPVGSL S VCIKDDRHADLANGKWPQO CPCRKGYAGDKCDRCQFGY RGFPN CIP
M CHCDPIGSLN EVCVKDEKHAR RGLAPGS CHCKTGFGGVSCDRRCARGY TGYPD CKA
B2 CHCSPVGSLS TQC DSYGR CSCKPGVMGDKCDRCQPGFHSLTEA GCRP
B2t CNCNKSGSLS ARC DNSGR CSCKPGVTGARCDRLPCFHMILTDA GCTQDORLLDSK
dA QCQDYFFSTGHCEETGNCECRAAFQPPSCDSCAYGYYPNCRECECNLNGTNGYHCE AESGQCPCCKINFAGAYCKQCAEGY YG FP ECKA

B1 CACNPLGTIPGGNPDSETGHICYCKRLVLTGQHCDCQLPEHWGLSNDLD GCRP
S CQCNSRGTVPGGTPCDSSSGTCFCRKLVTGDGCDRCLPGHWGLSHDLL GCRP
A CGCNPVGSASDEPCT GPCVCKENVEGKACDRCKPGFY NLKEKNPRGCSE
mA CDCRTPVGS LNEDPCI EPCLCKNVEGKNCDRCKPGFY NLKERNPEGCSE
M CNCSGLGSK NEDPCF GPCICKENVEGGDCSRCKSGFF NLQEDNWKGCDE
B2 CSCDPSGSI DE CNVETGRVCVKDNVEGFNCERCKPGFF NLESSNPRGCTP
B2t CDCDPAGIA GP CD AGRVCVKPAVTGERCDRCRSGYY NLDGGNPEGCTO
dA CECNKIGSITND CNVTTGECKCLTNFGGDNCERCKHGYP NYP TCSY

B1 CDCDLGGALNNSCFAESGQCSCRPHMIGRQCNEVEPGYY
S CDCDVGALDPOCDEATGQCCPCRPHMIGRR
A CFC FGV SDVC
mA CFC FGV SGVC
M CFC SGV SNRC
B2 CFC FGH SSVC
B2t CFC YGH SASC
dA CDCDNQGTSEIENKQSGQICREGFGGPRCDQCLPGFYNYPDCKP

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Figure 8. Alignment of domains V of the known human A- and B-type chains, the rat S chain, the mouse A chain and the *Drosophila* A chain. Amino acids that are identical in half of the chains are shaded, and dark shading indicates conserved change Phe (F) ↔ Tyr (Y).

tween the M chain mRNA and protein expression is also seen in other embryonic tissues (Leivo, I., and H. Sariola, unpublished results). The strong expression observed in mesenchymal cells located immediately beneath cells at the tip of the developing hair follicle and sebaceous glands indicates the potential role of the M chain in exocrine gland development. Expression of the M chain gene was not found in epithelial or endothelial cells of any of the tissues analyzed. Consequently, it can be concluded that during embryogenesis expression of the M chain is primarily, if not only, the property of cells of mesenchyme origin.

Expression of the A chain gene was shown to be considerably more restricted in human fetal tissues than that of the M chain gene. As previously reported for newborn human tissues (Nissinen et al., 1991) Northern analysis revealed expression of the A chain gene in kidney. We could not locate the expression at this stage of kidney development to specific cells by in situ hybridizations. The A chain has been localized in the kidney to tubular and glomerular basement membranes of adult tissues (Sanes et al., 1990) and in polarized kidney epithelial cells (Holm et al., 1988; Klein et al., 1988; Ekblom, 1990). Klein et al. (1990) reported the detection of A chain mRNA with poly(A) RNA from embryonic heart,

liver, lung, intestine, and laminin containing the A chain has been isolated from skeletal and heart muscle, lung, liver, kidney, and intestine (Paulsson and Saladin, 1989). However, in our studies on tissues from a 17-wk-old no signal for the A chain was observed with total RNA from in lung, heart, or liver, even after long exposures. This discrepancy could be due to in the types of RNAs used or due to differences in temporal expression during development. The intense expression of the A chain gene in neuroretina, olfactory bulbs, and cerebellum, is interesting and indicates its role in brain and nerve development as has been suggested in some previous reports (Edgar et al., 1984, 1988; Sanes et al., 1990; Kleinman et al., 1990). Detailed immunohistological and in situ hybridization analyses on developing brain tissues have been initiated to further analyze the temporal and spatial expression during brain development.

Several studies (Cooper and Mac Queen, 1983; Boot-Handford et al., 1987; Kleinman et al., 1987; Holm et al., 1988; Klein et al., 1988; Engvall et al., 1990; Sanes et al., 1990; Ekblom et al., 1990; Kücherer-Ehret et al., 1990; Nissinen et al., 1991; Kallunki et al., 1992) including the present study have demonstrated variability in both spatial and temporal expression of laminin subunit chains in vivo.

This, in part, implies tissue-specific functions of different laminin isoforms. With regard to the M and A chains, Engvall et al. (1990) and Sanes et al. (1990) showed that they are often mutually exclusive in a distinct type of basement membranes, suggesting that the laminin molecules contain either an M or an A chain as a heavy chain. The present Northern blot and in situ hybridization analyses carried out on RNA from human fetal tissues supported the different tissue distribution of the M and A chains. In particular, the results showed that the M chain gene is expressed in several tissues during embryonic development and possibly only by mesenchymal cells. However, the results also demonstrated that some laminin producing cells and tissues, such as skin and lung epithelia as well as vascular endothelia did not express either gene, or its expression was very weak in these tissues. This suggests that there exist laminin isoforms containing some, as yet, unidentified heavy A type chains. Such isoforms may include kalinin or K-laminin (Marinkovich et al., 1992a,b; Rousselle et al., 1991).

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