Primary structure and expression of a novel human laminin α4 chain

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Abstract The complete primary structure of a novel human laminin α4 chain was derived from cDNA clones. The translation product contains a 24-residue signal peptide preceding the mature α4 chain of 1792 residues. The domain structure is similar to that of the recently described α3 chain [Ryan, Tizard, Van Devanter and Carter (1994) J. Biol. Chem. 269, 22779–22787]. Northern analysis of RNA from human fetal and adult tissues revealed developmental regulation of expression. In adult, strong expression was observed in heart as well as lung, ovary, small and large intestines, placenta and liver, whereas weak or no expression was detected in skeletal muscle, kidney, pancreas, testis, prostate or brain. In contrast, fetal lung and kidney revealed high expression. In situ hybridization analysis of human fetal and newborn tissues showed expression of the laminin in α4 chain in certain mesenchymal cells in tissues such as smooth muscle and dermis.

Key words: Basement membrane; Extracellular matrix; cDNA cloning; In situ hybridization; Primary sequence; Laminin

1. Introduction

The laminins are a complex family of extracellular matrix proteins, each comprising three subunit polypeptide chains [1–5]. These chains, which are classified as α, β and γ types, associate through a triple-helical coiled coil forming a cross-shaped molecule with an ~80 nm long rigid rodlike structure and two or three shorter arms. To date, the primary structure of seven genetically distinct laminin chains, α1, α2, α3, β1, β2, β3, γ1 and γ2 has been determined from man [6–16]. Also the sequence of the α1, β1 and γ1 chains from mouse [17–19] and *Drosophila* [20–23], and that of the rat laminin β2 chain have been reported [24]. Evidence for two additional α chains has also been presented [25,26]. The different laminin chains share a certain degree of homology which includes domains with globular structures, rod-like domains containing EGF-like repeats, and domains participating in the coiled coil of the long arm. Additionally, the α chains contain a large C-terminal globular domain with five internal repeat motifs that share homology with corresponding motifs in perlecan [27,28], agrin [29], neurexins [30], *Drosophila crumbs* protein and the sex-hormone binding globulin [31].

The different laminin subunit chains differ extensively with respect to their distribution in vivo which suggests tissue-specific functions. Numerous physiological functions have been proposed for laminins (for review, see [32]), but the roles of different isoforms are still largely unknown. However, recent data on two inherited diseases involving laminin gene defects have shed some light on the function of two isoforms. In *epidermolysis bullosa junctionalis*, a skin blistering disease, mutations were described in the human gene (LAMC2) for the γ2 chain [33,34] demonstrating that this hemidesmosomal protein is essential for attachment of epithelial cells. A mutation was recently also described in the gene for the α2 chain (LAMA2) [35], in *dy* mice with muscular dystrophy, indicating that this chain is important for differentiation or function of muscle fibers. Although the protein defect resides in the short arm structures in these cases, much of the cellular effects of laminins are thought to be mediated through the proteolytic E8 fragment comprising about 60% of the globular G domain of the α chain and a segment of the trimeric coiled coil. For example, this fragment is recognized by the integrin cell surface receptors α6β1 [36], α3β1 [37], α7β1 [38] and α6β4 [39].

In the present study we report the cloning and the cDNA-derived primary structure of a fourth human laminin α chain, termed α4. Expression of this chain was examined in human tissues by Northern analysis and in situ hybridization techniques.

2. Experimental

2.1. Isolation and characterization of cDNA clones

A human fetal lung library in the λgt11 cloning vector (Clontech) was screened using standard techniques [40] with a PCR product obtained from the same library using primers 5′agtaagcttgccactccttca3′ and 5′tccaggatcctgacatgacaga3′ (see Fig. 2). These primers were designed based on two GenBank sequence entries X70904 and Z24848 encoding a laminin-like protein. All primers in this study were synthesized using DNA/RNA synthesizer model 392 (Applied Biosystems). Clone FL64 was chosen for further analysis and subsequently used as a probe to rescreen the library. In this manner a series of overlapping cDNA clones were identified and subcloned into pBluescriptII.SK– or pCRSScript vectors (Stratagene) for sequencing by the chain termination method [41] using a T7 sequencing kit (Pharmacia). All sequence analysis was carried out using the GCG software [42].

2.2. Northern analysis and in situ hybridization

For Northern analysis filters containing RNA from several human tissues (Clontech) were hybridized with the following probes: nucleotides 4600 to 5681, –190 to 1249 and β-actin as a control (see Fig. 2). For in situ hybridization, a PCR product from primers 5′agtaagcttgccactccttca3′ and 5′tccaggatcctgacatgacaga3′ (nucleotides 5440–5681) was digested with *Hin*dIII, and the 106 bp *Hin*dIII fragment was ligated into the pGEM-3Z vector for the production of single stranded RNA probes in both orientations. In situ hybridization alaysis was carried out on 20-week-old human fetal and neonatal tissues according to Cox et al. [43] with minor modifications as described earlier [11].

3. Results and discussion

3.1. Primary and domain structure of the laminin α4 chain

Five overlapping cDNA clones isolated and characterized in this study (Fig. 1) together covered a total of 6204 nucleotides, including 190 bp of a 5′ UTR and 566 bp of a 3′ UTR regions,

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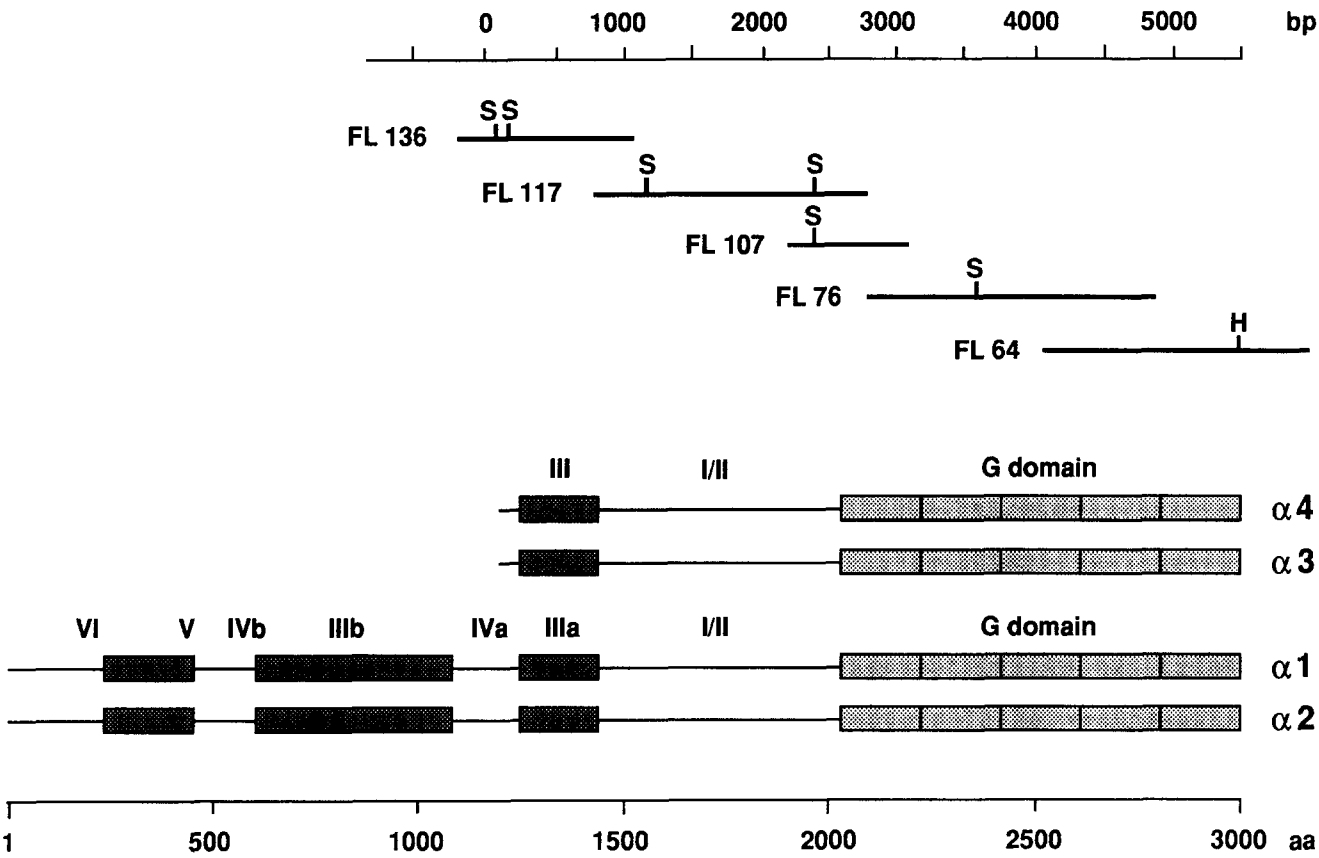

Fig. 1. Illustration of cDNA clones for the laminin α4 chain and domain structure of laminin α chains. (Top) Scale in base pairs (bp) and alignment of five overlapping cDNA clones with partial restriction map. Restriction enzyme sites *Sac*I (S) and *Hin*dIII (H) are shown. (Bottom) Domain structure of the human laminin α4 chain and comparison with that of the α3, α1 and α2 chains. Five internal repeats of domain G are indicated by light boxes, and cysteine-rich EGF modules of domains IIIa, IIIb and V are depicted by dark boxes. Scale in amino acids (aa) is shown beneath.

respectively. The open reading frame of 5448 nucleotides (Fig. 2) coded for a laminin α-type chain, characterized by the presence of a G domain with five internal repeat motifs. We refer to this chain as the laminin α4 chain, as it was first proposed by Richards et al. who localized the *LAMA4* gene to 6q21 [25]. There are two in-frame ATG codons for methionine in the 5′ region of the FL136 clone, one at position −149 (see Fig. 2) and the other tentative initiation codon at position 1. We consider the latter ATG codon to represent the translation start site for the following reasons. First, the sequence around the second ATG is in agreement with the consensus sequence of Kozak's [44]. Secondly, the predicted sequence of the first 24 residues are characteristic of a signal peptide which is present in all other laminin chains, and thirdly, the amino acid sequence around position 24 is characteristic for a signal peptidase cleavage site as described by von Heijne [45]. In addition, there is an out of frame ATG at position −14, but the corresponding open reading frame covers only seven amino acid residues. Nevertheless, this ATG codon could regulate the initiation of translation as discussed by Kozak [46]. The calculated mass of the entire translation product is 201,964, and that of the processed α4 chain 199,306.

Comparison with the sequence of other human laminin chains revealed that the domain structure of the α4 chain resembles more that of the α3 chain [13], than of the α1 and α2 chains (Fig. 1) [10,14]. The α4 is slightly larger the human α3 chain which has 1713 residues, but both chains are significantly smaller than the α1 and α2 chains which contain 3075 and 3110 residues, respectively. The C-terminal G-domain of α4 has five repeat motifs as the other laminin α chains. It shares 25.6% and 36.0% sequence identities with corresponding domain of the α1 and α3 chains, respectively. The coiled coil domain I/II, that forms the long arm, has a primary structure with heptad repeats similar to that in the other laminin chains (not shown). While the short arms of α1 and α2 chains contain three globular subdomains separated by three rod-like structures, the α4 chain short arm has only one rod-like segment preceded by a short N-terminal domain (Fig. 1). The rod-like segment is composed of three complete EGF-like repeats flanked by incomplete repeats. This sequence shows closest homology to the domain

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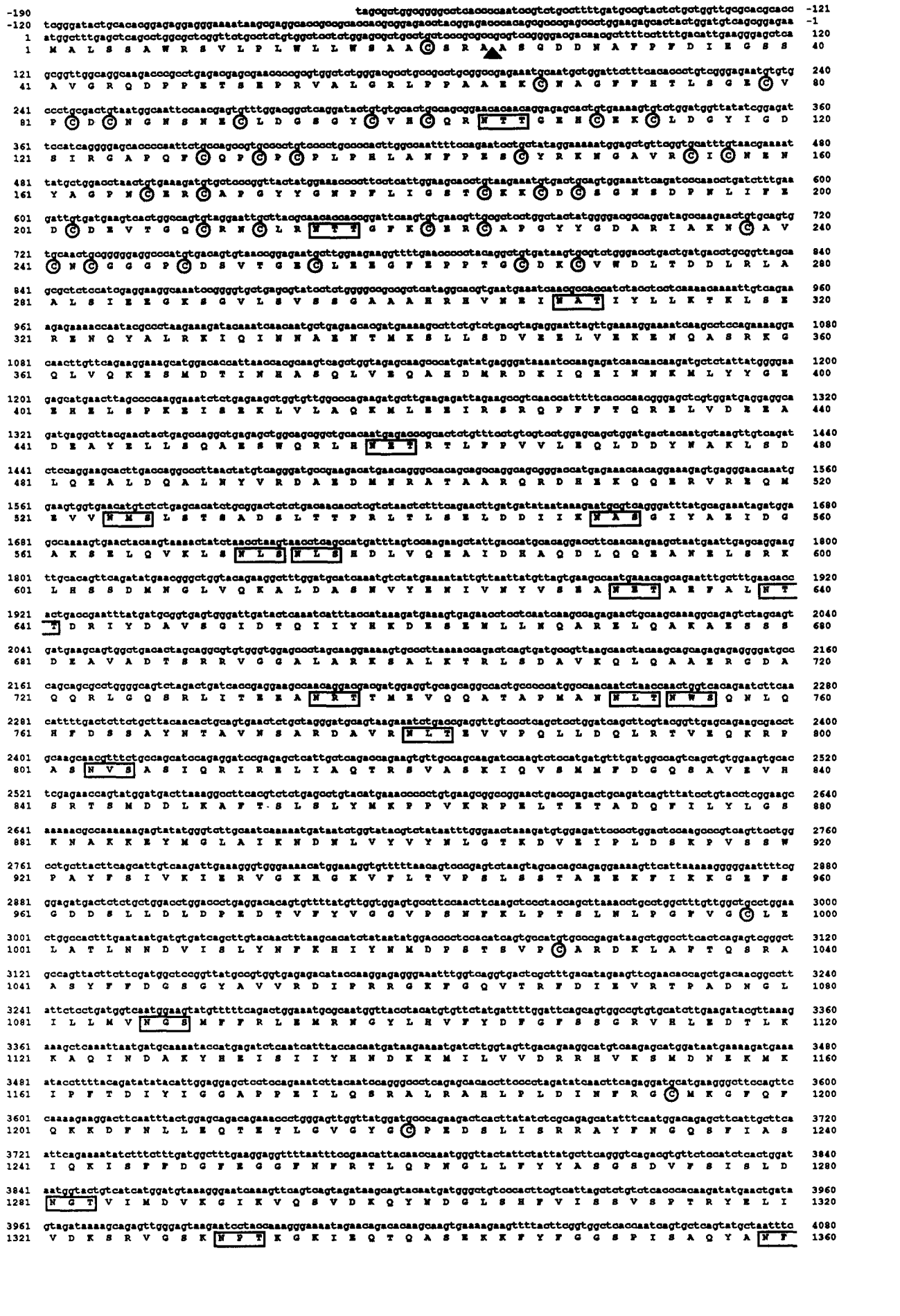
Fig. 2. cDNA nucleotide and derived amino acid sequence for the human laminin α4 chain. First line, nucleotide sequence; second line, deduced amino acid sequence. The translation stop codon TGA is indicated by an asterisk and three potential polyadenylation signals (AATAAA) are underlined. The putative signal peptidase cleavage site is indicated by a triangle (▲). The cysteine residues are circled, and potential attachment sites for asparagine-linked oligosaccharides are boxed.



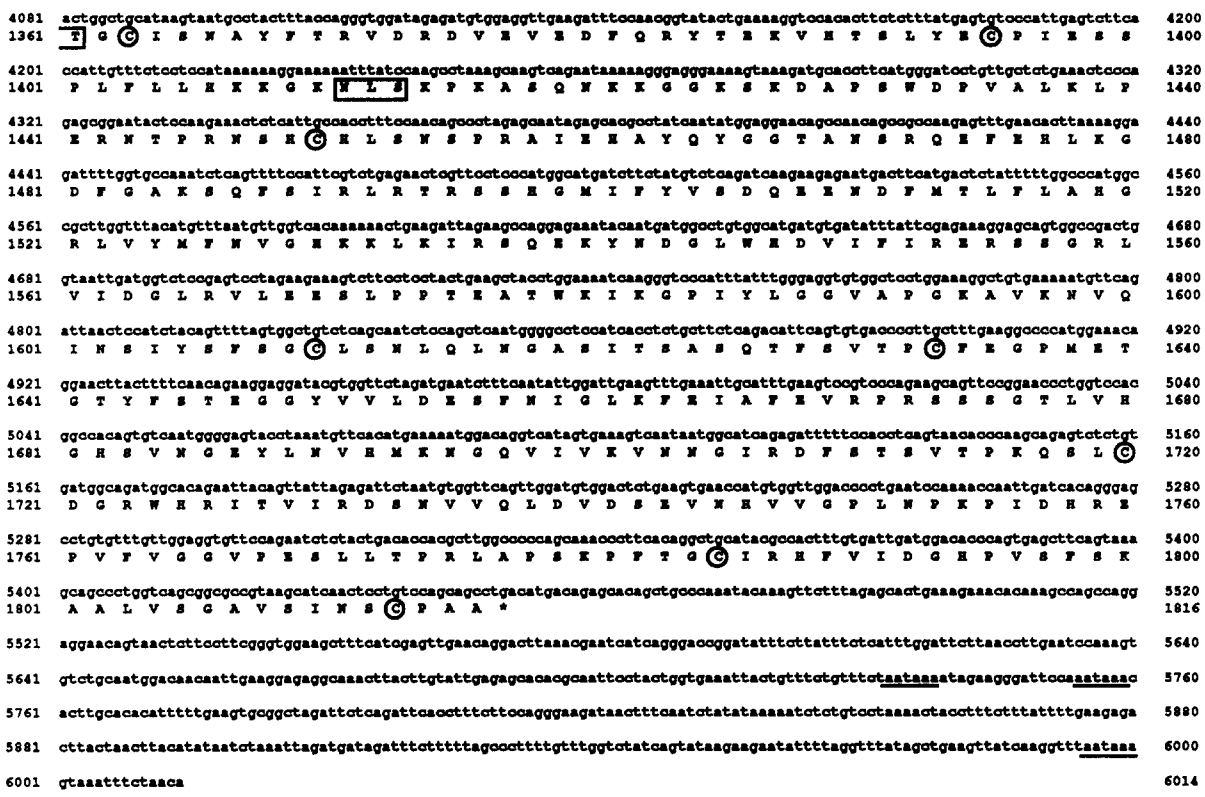


Fig. 2. (*continued*).

IIIb of the α1 chain with 49.3% sequence identity. Comparison with the domain III of the α3 chain reveals 46.3% sequence identity.

3.2. Expression of the laminin α4 chain in human tissues

Expression of the laminin α4 chain was first studied for levels of mRNA using Northern analysis (Fig. 3). In human fetus a strong signal was observed with RNA from lung and a distinct, although somewhat weaker signal was also obtained with kidney RNA. No signals were observed in brain or liver RNA. In human adult RNA the strongest signal was observed in heart, somewhat weaker signals being observed for lung, ovary, placenta and liver. Furthermore, still weaker signals were obtained with RNA from small intestine, colon, skeletal muscle, kidney,

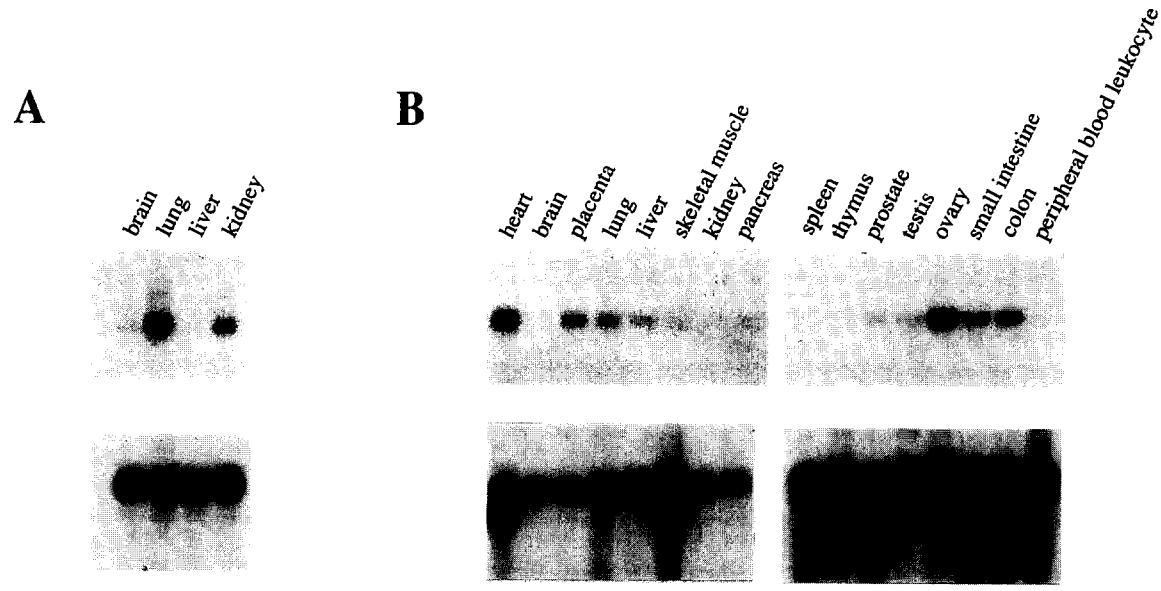


Fig. 3. Northern analysis of the laminin α4 chain with RNA from tissues. A, Fetal. B, Adult. Upper row: laminin α4 chain (6.5 kb), bottom row: β-actin. Note the inverse relationship of the laminin derived band intensities in kidney and liver (A vs. B).

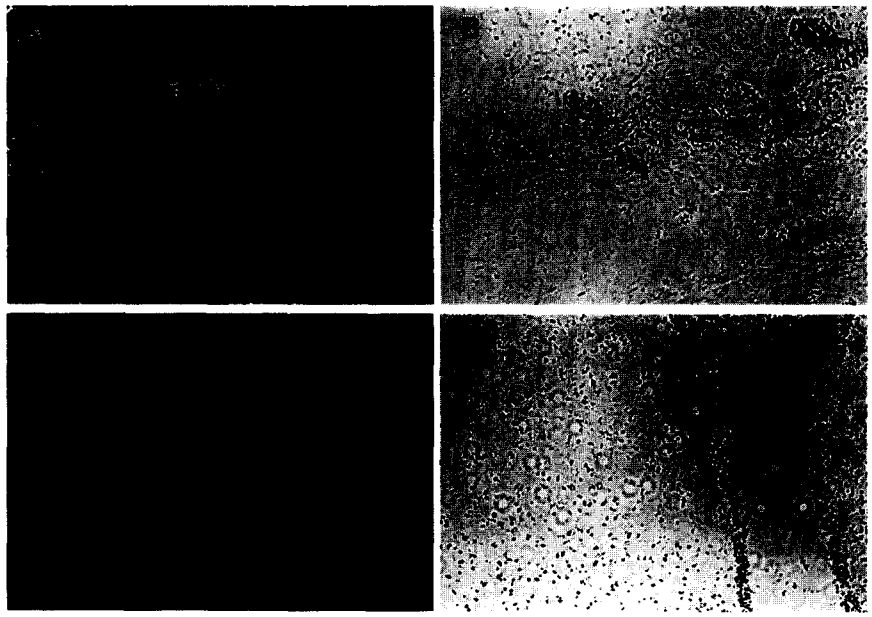


Fig. 4. In situ hybridization of the laminin α4 chain in 17-week-old human fetal tissues. In skin fold (A,B) expression is located in the dermis and subcutaneous fat. In brain (C,D), ependymal cells covering the ventricular surface show clear signals, whereas other regions of the brain tissue remain negative. The sense controls probes did not yield grain density above the negative control without probe (not shown). A and C, dark field; B and D, bright field.

pancreas, testis and prostate. Brain RNA did not yield any signals above background. The changes in liver and kidney expression from fetal to adult stages probably reflect tight developmental regulation of the α1 chain gene.

By in situ hybridization analysis, distinct expression was observed in ependymal embryonic cells lining the ventricular cavity (Fig. 4C,D) while no expression above background was seen in other areas of the brain. In embryonic skin, signals were only observed in the dermis (Fig. 4A,B). In neonatal tissues, strong expression was observed in mesenchymal perialveolar cells as well as in vascular smooth muscle cells (Fig. 5A,B). Alveolar epithelia and endothelial cells were negative. In neonatal skeletal muscle, the muscle fibres themselves did not exhibit expression, but interlobular adipous tissue was faintly labeled (Fig. 5C,D). We did not observe signals above background in neonatal heart of liver tissues.

The limited availability of human tissues hindered exhaustive analysis of the temporal and spatial expression pattern of the

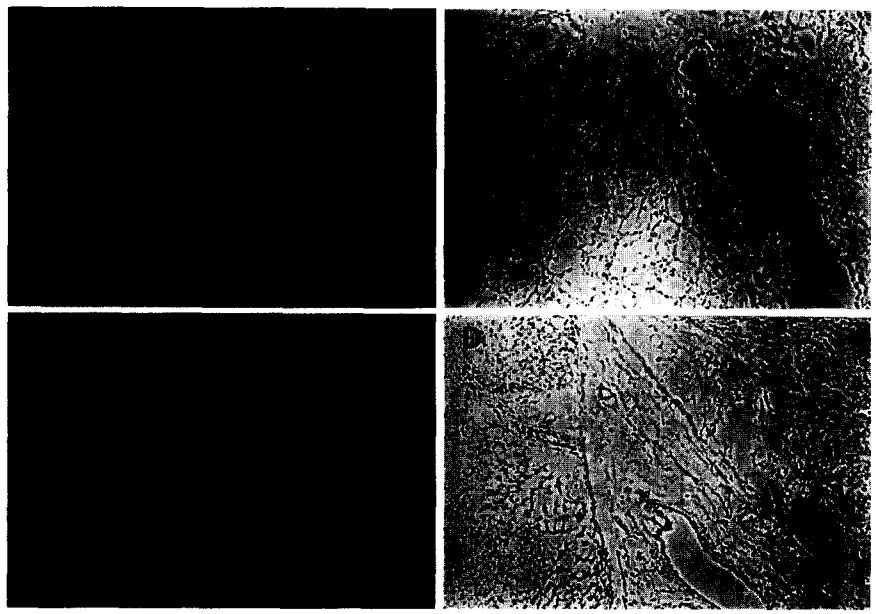


Fig. 5. In situ hybridization expression analysis of the human laminin α4 chain in human neonatal lung and muscle tissues. In lung (A,B) mainly perialveolar and vascular smooth muscle cells are labeled, while epithelial pneumocytes and endothelial cells are negative. In muscle (C,D), the muscle fibers appear to be negative, while interlobular adipous tissue is fainlty labeled (arrow).

laminin α4 chain. Nevertheless, the expression pattern of this laminin chain complements that of the α1, α2 and α3 [14,48] chains. In endothelial cells, the low level of α1 or absence of α3 chain expression as detected by immunohistochemistry [48,49] and the failure to detect α1, α2 or α4 chains by in situ hybridization analysis [14 and this study] might reflect the presence of a unique α chain [26]. The relationship of the α4 chain to the Y shaped laminin produced by adipose cells [50] as well as to laminins 6 and 7 [51] in the skin remains to be shown.

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References

[1] Engel, J. (1992) Biochemistry 31, 10643–10651.
[2] Tryggvason, K. (1993) Curr. Opin. Cell. Biol. 5, 877–882.
[3] Burgeson, R.E., Chiquet, M., Deutzmann, R., Ekblom, P., Engel, J., Kleinman, H., Martin, G.R., Meneguzzi, G., Paulsson, M., Sanes, J., Timpl, R., Tryggvason, K., Yamada, Y. and Yurchenco, P.D. (1994) Matrix Biol. 14, 209–211.
[4] Timpl, R. and Brown, J. (1994) Matrix Biol. 14, 275–281.
[5] Beck, K., Hunter, I. and Engel, J. (1990) FASEB J. 4, 148–160.
[6] Pikkarainen, T., Eddy, R., Fukushima, Y., Byers, M., Shows, T., Pihlajaniemi, T., Saraste, M. and Tryggvason, K. (1987) J. Biol. Chem. 262, 10454–10462.
[7] Pikkarainen, T., Kallunki, T. and Tryggvason, K. (1988) J. Biol. Chem. 263, 6751–6758.
[8] Ehrig, K., Leivo, I., Argraves, W.S., Ruoslahti, E. and Engvall, E. (1989) Proc. Natl. Acad. Sci. USA 85, 3264–3268.
[9] Haaparanta, T., Uitto, J., Ruoslahti, E. and Engvall, E. (1991) Matrix 11, 151–160.
[10] Nissinen, M., Vuolteenaho, R., Boot-Handford, R., Kallunki, P. and Tryggvason, K. (1991) Biochem. J. 276, 369–379.
[11] Kallunki, P., Sainio, K., Eddy, R., Jr., Byers, M., Kallunki, T., Sariola, H., Beck, K., Hirvonen, H., Shows, T.B. and Tryggvason, K. (1992) J. Cell Biol. 119, 679–693.
[12] Gerecke, D.R., Wagman, D.W., Champliaud, M.-F. and Burgeson, R.E. (1994) J. Biol. Chem. 269, 11073–11080.
[13] Ryan, M.C., Tizard, R., VanDevanter, D.R. and Carter, W.G. (1994) J. Biol. Chem. 269, 22779–22787.
[14] Vuolteenaho, R., Nissinen, M., Sainio, K., Byers, M., Eddy, R., Hirvonen, H., Shows, T.B., Sariola, H., Engvall, E. and Tryggvason, K. (1994) J. Cell. Biol. 124, 381–394.
[15] Wewer, U.M., Gerecke, D.R., Durkin, M.E., Kurtz, K.S., Mattei, M.-G., Champliaud, M.-F., Burgeson, R.E. and Albrechtsen, R. (1994) Genomics 24, 243–252.
[16] Iivanainen, A., Vuolteenaho, R., Sainio, K., Janit-Sait, S., Eddy, R., Shows, T.B., Sariola, H. and Tryggvason, K. (1994) Matrix Biol. 14, 489–497.
[17] Sasaki, M., Kato, S., Kohno, K., Martin, G. and Yamada, Y. (1987) Proc. Natl. Acad. Sci. USA 84, 935–939.
[18] Sasaki, M., Kleinman, H.K., Huber, H., Deutzmann, R. and Yamada, Y. (1988) J. Biol. Chem. 263, 16536–16544.
[19] Sasaki, M. and Yamada, Y. (1988) J. Biol. Chem. 262, 17111–17117.
[20] Montell, D.J. and Goodman, C.S. (1988) Cell 53, 463–473.
[21] Chi, H.-C. and Hui, C.-F. (1989) J. Biol. Chem. 264, 1543–1550.
[22] Garrison, K., Mackrell, A.J. and Fessler, J.H. (1991) J. Biol. Chem. 266, 22899–22904.
[23] Kusche-Gullberg, M., Garrison, K., Mackrell, A.J., Fessler, L.I. and Fessler, J.H. (1992) EMBO J 11, 4519–4527.
[24] Hunter, D.D., Shah, V., Merlie, J.P. and Sanes, J.R. (1989) Nature 338, 229–234.
[25] Richards, A.J., Al-Imara, L., Carter, N.P., Lloyd, J.C., Leversha, M.A. and Pope, F.M. (1994) Genomics 22, 237–239.
[26] Lindblom, A., Marsh, T., Fauser, C., Engel, J. and Paulsson, M. (1994) Eur. J. Biochem. 219, 383–392.
[27] Noonan, D.M., Horigan, E.A., Ledbetter, S.R., Vogeli, G., Sasaki, M., Yamada, Y. and Hassell, J.R. (1988) J. Biol. Chem. 263, 16379–16387.
[28] Kallunki, P. and Tryggvason, K. (1992) J. Cell Biol. 116, 559–571.
[29] Rupp, F., Payan, D.G., Magill-Solc, C., Cowan, D.M. and Scheller, R.H. (1991) Neuron 6, 811–823.
[30] Ushkaryov, Y.A., Petrenko, A.G., Geppert, M. and Südhof, T.C. (1992) Science 257, 50–56.
[31] Joseph, D.R. and Baker, M.E. (1992) FASEB J. 6, 2477–2481.
[32] Kleinman, H.K., Cannon, F.B., Laurie, G.W., Hassell, J.R., Aumailley, M., Terranova, V.P., Martin, G.R. and DuBois-Dalcq, M. (1985) J. Cell. Biochem. 27, 317–325.
[33] Pulkkinen, L., Christiano, A.M., Airenne, T., Haakana, H., Tryggvason, K. and Uitto, J. (1994) Nature Genet. 6, 293–298.
[34] Aberdam, D., Galliano, M.-F., Vailly, J., Pulkkinen, L., Bonifas, J., Christiano, A.M., Tryggvason, K., Uitto, J., Epstein, E.H. Jr., Ortonne, J.-P. and Meneguzzi, G. (1994) Nature Genet. 6, 299–304.
[35] Xu, H., Wu, X.-R., Wewer, U.M. and Engvall, E. (1994) Nature Genetics 8, 297–302.
[36] Hall, D.E., Reichardt, L.F., Crowley, E., Holley, B., Moezzi, H., Sonnenberg, A. and Damsky, C.H. (1990) J. Cell. Biol. 110, 2175–2184.
[37] Gehlsen, K., Dickerson, K., Argraves, W.S., Engvall, E. and Ruoslahti, E. (1989) J. Biol. Chem. 264, 19034–19038.
[38] Sutherland, A.E., Calarco, P.G. and Damsky, C.H. (1993) Development 119, 1175–1186.
[39] Lee, E.C., Lotz, M.M., Steele, G.D., Jr. and Mercurio, A.M. (1992) J. Cell. Biol. 117, 671–678.
[40] Sambrook, J., Fritsch, E.F. and Maniatis, T. (1989) Molecular Cloning – A Laboratory Manual, 2nd Ed., CSH Laboratory Press, New York.
[41] Sanger, F., Nicklen, S. and Coulson, A.R. (1974) Proc. Natl. Acad. Sci. USA 74, 5463–5467.
[42] Genetics Computer Group (1994) Program Manual for the GCG Pakcage, version 8, Genetics Computer Group, Madison, Wisconsin.
[43] Cox, K.H., DeLeon, D.V., Angerer, L.M. and Angerer, R.C. (1984) Dev. Biol. 101, 485–502.
[44] Kozak, M. (1992) J. Biol. Chem. 266, 19867–19870.
[45] von Heijne, G. (1986) Nucleic Acids Res. 14, 4683–4690.
[46] Kozak, M. (1991) J. Cell. Biol. 115, 887–903.
[47] Aberdam, D., Galliano, M.F., Mattei, M.-G., Pisani-Spadafora, A., Ortonne, J.P. and Meneguzzi, G. (1994) Mamm. Genome 5, 229–233.
[48] Sanes, J.R., Engvall, E., Butkowski, R. and Hunter, D.D. (1990) J. Cell. Biol. 111, 1685–1699.
[49] Klein, G., Ekblom, M., Fecker, L., Timpl, R. and Ekblom, P. (1990) Development 110, 823–837.
[50] Aratani, Y. and Kitagawa, Y. (1988) J. Biol. Chem. 263, 16163–16169.
[51] Marinkovich, M.P., Lundstrum, G.P., Keene, D.R. and Burgeson, R.E. (1992) J. Cell. Biol. 119, 695–703.