

# A Truncated Laminin Chain Homologous to the B2 Chain: Structure, Spatial Expression, and Chromosomal Assignment

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**Abstract.** We describe the identification of a novel laminin chain. Overlapping clones were isolated from a human fibrosarcoma HT1080 cell cDNA library spanning a total of 5,200 bp. A second set of clones contained an alternative 3' end sequence giving a total of 4,316 bp. The longer sequence contained an open reading frame for a 1,193-residue-long polypeptide. The alternative sequence was shortened at the carboxyl-terminal end coding for a 1,111-residue-long polypeptide. The amino acid sequence contained 21 amino acids of a putative signal peptide and 1,172 residues or alternatively 1,090 residues of a sequence with five distinct domains homologous to domains I-V in laminin chains. Comparison of the amino acid sequences showed that the novel laminin chain is homologous to the laminin B2 chain. However, the structure of the novel laminin chain isolated here differs significantly from that of the B2 chain in that it has no domain VI

and domains V, IV, and III are shorter, resulting in a truncated laminin chain. The alternative sequence had a shortened domain I/II. In accordance with the current nomenclature, the chain characterized here is termed B2t. Calculation of possible chain interactions of laminin chains with the B2t chain domain I/II indicated that the B2t chain can replace the B2 chain in some laminin molecules. The gene for the laminin B2t chain (LAMB2T) was localized to chromosome 1q25-q31 in close proximity to the laminin B2 chain gene. Northern analysis showed that the B2t chain is expressed in several human fetal tissues but differently from the laminin B1 and B2 chains. By *in situ* hybridization expression of the B2t chain was localized to specific epithelial cells in skin, lung, and kidney as opposed to a general epithelial and endothelial cell expression of the laminin B2 chain in the same tissues.

**L**AMININS are large, basement membrane glycoproteins consisting of three chains connected by an  $\alpha$ -helical coiled-coil domain. The laminin molecule has a cross-like structure with one long arm and three short arms (69). Laminin, first isolated from a murine Engelbreth-Holm-Swarm (EHS)<sup>1</sup> tumor (70), was shown to be a heterotrimer consisting of one heavy A chain of 400 kD and two light chains, B1 and B2, of  $\sim$ 200 kD each (14). The primary structure of the laminin A, B1, and B2 chains has been determined from mouse (1, 60, 61, 62), man (31, 51, 53, 54), and *Drosophila* (12, 26, 48, 49). The laminin chains have a characteristic domain structure with internal repeats (2, 3). The short arms are formed of EGF-like modules and globular domains. The long arm is formed by heptad repeats typical for  $\alpha$ -helical coiled-coil proteins. Diverse biological functions attributed to laminin include stimulation of cell growth and differentiation, and promotion of neurite outgrowth, cell

adhesion, and locomotion. The laminin molecule participates in the assembly of basement membranes through binding to other laminin molecules, type IV collagen, nidogen (entactin), and basement membrane heparan sulfate proteoglycan (2, 69). The cellular activities of laminin are presumably mediated by cell surface receptors, many of which belong to the integrin family (47).

Identification of new laminin chains, s-laminin, a close homologue of the B1 chain (34) and merosin, an A chain homologue (18) has demonstrated that laminin is a considerably more complex protein than previously anticipated. Taking into account the increasing number of laminin subunit chains, Engel et al. (20) have proposed a new terminology for the laminin chains. According to this classification, the classical EHS laminin A chain is termed Ae, merosin Am, the B1 chain B1e, s-laminin B1s, and the B2 chain B2e. This terminology is used in this article. Analysis of laminin isolated from human placenta has demonstrated the existence of isoforms with chain composition Ae-B1e-B2e, Am-B1e-B2e, Ae-B1s-B2e, and Am-B1s-B2e (21). This is supported

1. *Abbreviations used in this paper:* EHS, Engelbreth-Holm-Swarm; HSPG, heparan sulfate proteoglycan.

also by immunostaining of tissue sections which have shown colocalization of these chains in basement membranes (58). In vitro de- and renaturation studies with EHS laminin have shown that the assembly of this laminin is a specific process and only molecules with one Ae chain, one Ble chain, and one B2e chain are formed (35, 36). The carboxyl-terminal parts of laminin chains assemble into a coiled-coil structure. This structure is stabilized by ionic interactions between the chains. Calculations of these interactions show that only heterotrimeric molecules with one A-type chain, one B1-type chain, and one B2-type chain are favorable (20). Studies on the expression of laminin subunit genes and distribution of laminin subunit chains have shown differences in their spatial expression (4, 13, 19, 39, 40, 51). The existence of multiple divergently expressed laminin chains suggests different functions for the laminin isoforms.

In the present work we describe the isolation and characterization of full-length cDNA clones for a new member of the laminin chain family. Despite several unique features, this polypeptide has considerable sequence similarity with the B2e chain, although it is substantially shorter. This chain is therefore termed here B2t (t = truncated). The gene for the laminin B2t chain (*LAMB2T*) was localized to chromosome 1q25→q31 (25), to the same region where the laminin B2e chain gene has been previously localized. The expression of the mRNA for the *LAMB2T* gene was found to be restricted to only a few human fetal tissues, thus differing substantially from the almost ubiquitous expression of the *LAMB1* and *LAMB2* genes. Furthermore, in situ hybridization demonstrated cell specific expression of the B2t chain in certain epithelial cells in skin, lung, and kidney, in contrast to a general expression of the B2e chain in epithelial and endothelial cells in these tissues.

## Materials and Methods

### cDNA Cloning and Sequencing

All cDNA libraries used in this study were made from human fibrosarcoma cell (HT1080) poly(A)-RNA. To obtain clones for the human basement membrane heparan sulfate proteoglycan (HSPG) core protein, a previously described specific library (37) was screened with an end-labeled, degenerate oligonucleotide, h22, based on the sequence GQTLDL from the amino terminus of a short peptide sequence of the human core protein (33). This resulted in the isolation of a clone, HT2-7, which turned out to code for a previously unknown laminin-like chain. The HT2-7 was then used to screen a cDNA library made with both oligo(dT) and random primers. Several overlapping clones were obtained (Fig. 1) and fragments of these clones were used to isolate further clones towards the 5' and 3' ends of the cDNA. To obtain cDNA clones covering the entire 5' end, two primer extension libraries were made using primers B22, complementary to bases 199–222 and B23, complementary to bases 177–197, from the 5' end of the L52 cDNA clone. Yet another HT1080 cDNA library made with an oligo(dT)-primer was used to obtain cDNA clones for the 3' end.

The nucleotide sequence was determined from both strands, either manually using dideoxy sequencing (59) with Sequenase (U.S. Biochemical Corp., Cleveland, OH) or automatically with thermocycle sequencing using AmpliTaq (Perkin-Elmer Cetus Instruments, Norwalk, CT) and an automatic DNA sequencer (A.L.F. Pharmacia, Uppsala, Sweden). The sequences were analyzed using MicroGenie software (Beckman Instruments, Inc., Fullerton, CA).

### Estimation of Interaction Potential of B2t with Other Laminin Chains

To explore whether the B2t chain might be able to assemble with other human laminin chains, interchain ionic interaction factors were calculated for the sequence region 613–1,111 with domains II and I of the other chains:

human Ble chain residues 1,183–1,785 (53), human B2e chain residues 1,035–1,600 (54), and human Ae chain residues 1,561–2,126 (51). By standard algorithms these regions are predicted to have mainly  $\alpha$ -helical structure. In the first step the sequence was ordered in heptad repeats  $[a,b,c,d,e,f,g]_n$  so that positions *a* and *d* are most frequently occupied by hydrophobic residues (Ile, Phe, Val, Leu, Trp, Met, Ala) and *e* and *g* by charged residues (Arg, Lys, Glu, Asp). As already found for the mouse laminin chains, several phase shifts have to be introduced to get an optimal heptad pattern (2). Then the heptad repeats were aligned to adjacent ones assuming a parallel homodimeric configuration as well as those of human Ble, B2e, and Ae assuming a heterodimeric assembly. With respect to chain recognition, the specific distribution of charged residues which can form salt bridges between the chains appears critical. The number of favorable and unfavorable pairs of amino acids in adjacent positions were counted as +1 and -1, respectively. According to the designation of McLachlan and Stewart (46) the residue pairs were considered 2e-1g', 1g-2e', 1g-2a', 2a-1g', 1d-1e', and 1e-1d', where numbers indicate the relative heptad number of chain *n* and *n'*. Their shifts account for the rise of the helix. The interchain ionic interaction factors were calculated by dividing the sum of interactions by the number of heptads/chain.

### Chromosomal Localization

Human-mouse somatic cell hybrids and procedures used for chromosomally assigning *LAMB2T* gene have been described (37, 64, 65). In situ hybridization was accomplished as reported previously (22, 50, 74).

### Northern Analysis

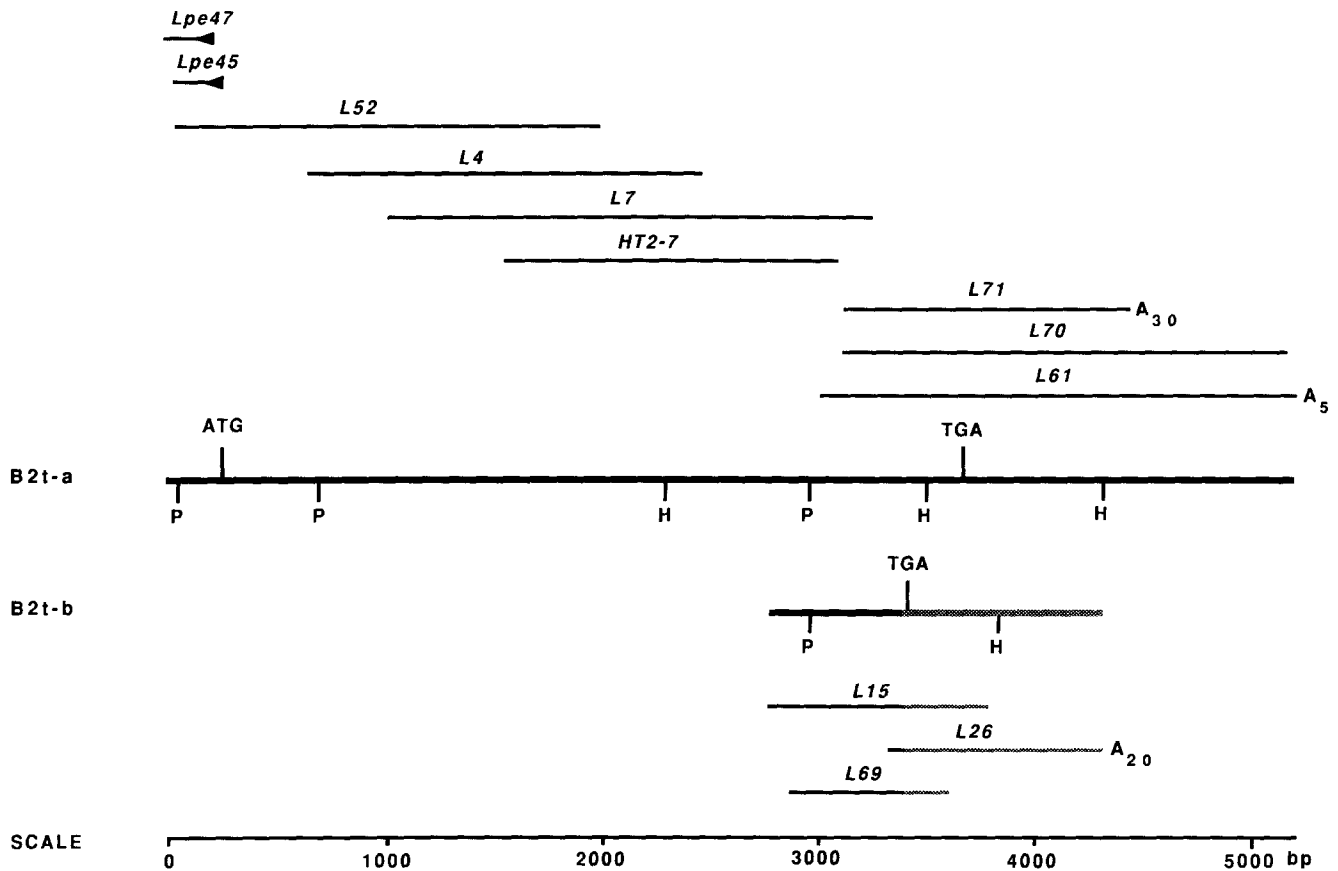
Poly(A) RNA was isolated from human HT1080 fibrosarcoma cells (ATCC, CCL 121) and human choriocarcinoma (JAR) cells (24) as described previously (37). ~5  $\mu$ g of poly(A)-RNA was run in parallel lanes in a 0.7% agarose formaldehyde gel and transferred to a nitrocellulose filter (Sleicher & Schuell, Keene, NH). The filter was cut into strips which were hybridized separately with human cDNA probes for the laminin Ae (51), Ble(53), B2e(54), and the B2t chain identified in this study. The probes were made by nick translation of the insert DNAs to similar specific activity allowing approximate comparisons of transcriptional activity.

Total RNA was isolated from 18–19-wk-old human fetal tissues by routine methods (44) with the appropriate approval of the Ethics committee. Samples containing 10  $\mu$ g of each RNA were run in a 1.2% agarose formaldehyde gel and transferred to a GeneScreenPlus filter (New England Nuclear, Boston, MA). The filter was hybridized sequentially with the different human laminin cDNA probes.

### In Situ Hybridization

To prepare sense and antisense probes for the laminin B2t and laminin B2e chains, which would not crosshybridize, fragments from the least homologous 3' end region of the cDNAs were used. For the laminin B2t chain a PstI-EcoRI fragment of clone L15 (bases 2995–3840, Fig. 1) and for the laminin B2e chain a polymerase chain reaction (PCR) fragment with artificial BamHI-PstI sites (corresponding to bases 105–616 in the 3' non-coding region) (38) were subcloned into pSP64 and pSP65 vectors in sense and antisense orientation. The <sup>32</sup>P-labeled antisense transcripts were shown to recognize only the specific messages for either the laminin B2t or the laminin B2e chain in HT1080 cell RNA (data not shown). For in situ hybridization the antisense and sense probes were labeled with <sup>35</sup>S-UTP (Amersham International, Amersham, UK) using Sp6 RNA polymerase (Promega Biotec, Madison, WI) for 1 h at 37°C. The unlabeled DNA sequences were removed with RNase free DNase (Promega Biotec) and treated with limited alkaline hydrolysis. The unincorporated labeled nucleotides were removed and the probes were precipitated with ethanol, dried, and dissolved in Wilkinsons hybridization buffer (72) with 100 mM DTT to 2 × 10<sup>6</sup> cpm/ml and used for in situ hybridization.

Human fetal tissues from the 17th gestational week were embedded in O.C.T. compound-embedding medium (Miles Laboratories Inc., Elkhart, IN) and 5- $\mu$ m frozen sections were cut on aminoalkylsilane-pretreated objective slides (56). The sections were air-dried and fixed with freshly made 4% paraformaldehyde (PFA) supplemented with 5 mM MgCl<sub>2</sub> in 0.1 M PBS for 15 min at room temperature, dehydrated in alcohol, and air-dried and stored at -70°C. In situ hybridization was performed according to Cox et al. (15) and Wilkinson and Green (72) with some modifications. Briefly, the frozen sections were rehydrated in PBS at room temperature for 5 min, and treated with 0.5  $\mu$ g/ml proteinase K (Sigma Chemical Co., St. Louis, MO) for 7 min at room temperature. The slides were washed with 0.1 M glycine in PBS for 5 min at room temperature, postfixed with freshly made



**Figure 1.** Scheme of 12 cDNA clones encoding the laminin B2t chain. cDNA clones with an arrow tail represent clones made by primer extension. Clone L26 is a mixed clone containing a short region different from the other clones (not shown). Location of the ATG translation initiation signal and the 3'-end TGA translation stop codons present in the two different set of clones are shown. The 3'-end sequence of clones L15, L26, and L69 is illustrated by a gray line. Restriction enzyme sites for *Pst*I (P) and *Hind*III (H) are indicated. Scale in base pairs is shown at the bottom.

4% PFA-5 mM MgCl<sub>2</sub>, and rinsed in 50% deionized formamide (Merck & Co., Rahway, NJ) and 2× SSC. The sections were acetylated in fresh 0.25% acetic anhydride in 0.1 M triethanolamine for 10 min at room temperature and rinsed again with formamide and 2× SSC. The sections were prehybridized in Wilkinsons hybridization buffer containing 100 mM DTT for 2 h at 50°C. The prehybridization mixture was removed and 30–40 μl of new hybridization buffer with labeled cRNA probes was added and hybridization continued at 52°C for 16–18 h. The posthybridization washes were performed according to Wilkinson and Green (72) followed by autoradiography with an NTB-2 film emulsion (Eastman Kodak Co., Rochester, NY). The sections were exposed under light-safe conditions at 4°C for 7–12 d, developed in a D-19 developer (Eastman Kodak Co.), counterstained with Harris' hematoxylin, dehydrated, and mounted with Permount.

## Results

### Cloning of cDNA Encoding a Distinct Laminin-like Chain

The HT2-7 cDNA clone, isolated by screening a HT1080 cell cDNA library with the degenerate oligonucleotide H22, contained an insert of ~1.5 kb. Initial sequencing of the clone demonstrated that the derived amino acid sequence has substantial similarity with sequences of laminin chains. Although the sequence of the oligonucleotide used for initial screening was based on an amino acid sequence from the human basement membrane HSPG core protein, no significant sequence homology was found between the amino acid se-

quence derived from clone HT2-7 and the sequence of the HSPG core protein from which the oligonucleotide was derived. Some homology is found between the sequence coded by HT2-7 and the laminin-like region of HSPG core protein. On Northern analysis the cDNA hybridized to at least two messages of ~4.5 and 5 kb. Screening of the oligo(dT)/random primed library with the HT2-7 insert resulted in the isolation of overlapping clones L4, L7, and L15. A 5' end fragment of L4 was used to isolate clone L52 from the same library. Clone L15 was used to isolate the 3' end clone L26 from the oligo(dT)/random primed library. To obtain the entire 3' end of the cDNA clone L15 was used to isolate clones L61, L69, L70, and L71 from an oligo(dT) primed library. To obtain clones extending to the 5' end of the mRNA, primer extension libraries were made and screened. This yielded several clones, the largest being Lpe47, reaching only 45 bp upstream of the L52 cDNA clone. Primer extension and isolation and S1 nuclease analysis of genomic clones confirmed that this site corresponds to the initiation of transcription (Kallunki, T., unpublished observation). Illustration of the cDNA clones and their partial restriction map is shown in Fig. 1.

The nucleotide sequence of the overlapping clones and the predicted amino acid sequence are shown in Fig. 2. As illustrated in Figs. 1 and 2 two types of cDNA clones were obtained for the 3' end region. Clones L61, L70, and L71, con-



tained a sequence (Fig. 2 *a*) which gives a total length of 5,200 bp. Clones L15, L26, and L69, contain a sequence (Fig. 2 *b*) which differs at the 3' end giving a total length of 4,316 bp. The first sequence (Fig. 2 *a*) has a 117-bp 5' untranslated region, an open reading frame starting from position 118 with the first ATG codon for methionine and ending with a translation stop codon TGA at position 3,697 and a 1,504-bp 3' untranslated region. The 3' untranslated region contains two conventional polyadenylation signals AAUAAA at positions 4,841 and 5,172. The sequence ends 18 nucleotides after the second signal with a possible poly(A)-tail of only five nucleotides. One of the clones, L71 ends with a poly(A)-tail of 30 nucleotides which begins 15 nucleotides after a slightly unusual polyadenylation signal AUUAAA at position 4,413. The two clones with different polyadenylation sites give mRNAs of 4,463 and 5,200 bases.

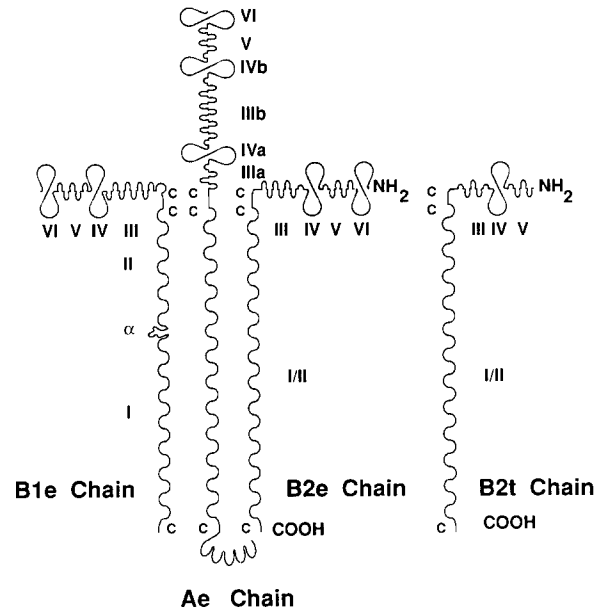
The second and shorter sequence (Fig. 2 *b*) differs in the 3' end starting from position 3,446. It gives an open reading frame ending with a translation termination codon TGA at 3,451 and 866 bp of 3' untranslated region giving the total length of 4,316 bp. Computer homology search revealed that the last 300 nucleotides contain an Alu repeat with a poly(A)-tail at the 3' end.

The 117-nucleotide region preceding the ATG codon has an open reading frame, but we surmise that this ATG codon represents the true translation initiation site for the following reasons. First, extensive screening of primer-extension cDNA libraries did not reveal clones extending further upstream from the Lpe47 cDNA. Second, the sequence surrounding the ATG agrees well with the consensus sequence for the vertebrate translation initiation *cgccA/GcCAUGg* (10) although it has a C following the ATG. Third, this initiation site is also supported by analysis of the amino acid sequence following the methionine, which shows a stretch of hydrophobic amino acids typical for a signal peptide. Computer program analysis predicting the signal peptide cleavage site according to von Hejne (70 *b*) suggested a cleavage site after Ala21.

The open reading frame of the first and longer sequence (Fig. 2 *a*) provides for a 1,193-amino acid polypeptide. The open reading frame of the second sequence (Fig. 2 *b*) provides for 1,111 amino acid residues. After cleavage of the signal peptide the mature polypeptide chain would contain either 1,172 or 1,090 amino acids with a predicted molecular weight of 128,650 or 119,280, respectively, for the longer and shorter sequence. There are six putative Asn-linked glycosylation sites Asn-X-Ser/Thr (Fig. 2). If all of them are used, the molecular/mass could be closer to 140 or 130 kD.

#### Domain Structure of a Truncated Laminin-like Chain

The laminin-like chain identified here has domains similar to those found in laminin chains and the domains are numbered accordingly (Fig. 3). Domain V (residues 28–196) is



Sequence identity of  
Laminin B2t domains  
Human B2e  
vs Human B1e  
Human Ae

	V	IV	III	I/II
Human B2e	55	52	55	34
Human B1e	36	17	40	27
Human Ae	37	28	41	22

formed of three and one half cysteine-rich EGF modules characteristic for laminin. Domain IV (residues 197–381) is a 190-amino acid region containing a single cysteine. This domain is likely to form a globular structure similar to domain IV in laminin. Domain III (residues 382–608) is formed of the second half of an EGF module and four additional modules. Domain I/II of the longer polypeptide (residues 609–1,193) starts with two closely spaced cysteines and contains one cysteine close to the carboxyl terminus. It is an  $\alpha$ -helical domain of 585 amino acids with a heptad repeat structure typical for coiled-coil proteins. In the shorter polypeptide form domain I/II is shortened at the carboxyl terminus by 82 residues.

Alignment of the sequences of the structural domains in the laminin-like chain with similar domains in the laminin Ae, B1e, and B2e chains reveals that there is high sequence

**Figure 2.** Nucleotide sequence of the laminin B2t chain cDNA and the derived amino acid sequence. First line, nucleotide sequence of the cDNA clones. Second line, deduced amino acid sequence. ( $\Delta$ ) The putative signal peptidase cleavage site; (*bent arrows*) borders of structural domains, cysteine residues are circled, and potential attachment sites (Asn-X-Ser/Thr) for oligosaccharides are boxed. (*a*) Nucleotide and derived amino acid sequence of cDNA clones encoding for the 5,200-bp sequence. (*b*) Nucleotide and derived-amino acid sequence of the alternative 3' end sequence from cDNA clones providing the sequence for the total of 4,316 bp. These sequence data are available from EMB/GenBank/DDJB under accession numbers Z15008 (*a*) and Z15009 (*b*).

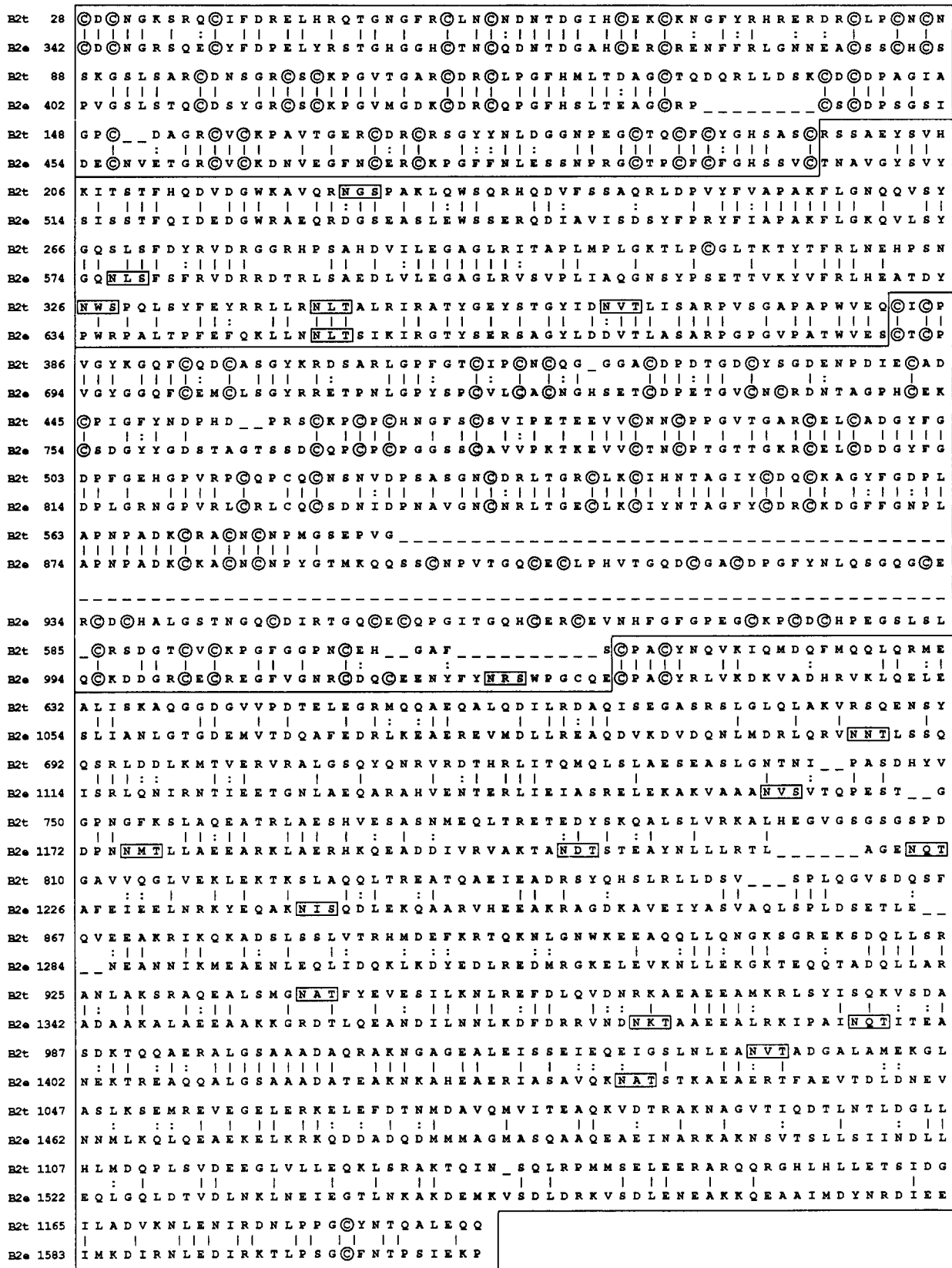


Figure 4. Alignment of the human laminin B2t amino acid sequence with the human laminin B2e (54) amino acid sequence. The structural domains are boxed and indicated by Roman numerals on the right. The amino acid sequence of both chains is numbered from the initiator methionine. All cysteines are circled and the glycosylation sites N-X-T/S are boxed.

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identity with the laminin B2e chain. Domains V, IV, and III are >50% identical between the two chains (Fig. 3). The laminin B1e chain is much less similar, with domain IV showing little similarity. Although the amino acid sequence of the laminin-like chain characterized here demonstrated extensive similarities with the laminin B2e chain, it is considerably shorter. We therefore term this truncated laminin B2e-like chain B2t.

Comparison of the amino acid sequence of the B2t chain with that of the B2e (54) chain shows (Fig. 4) that the B2t chain has several features which clearly distinguish it from the B2e chain. A major feature is that the B2t chain lacks an amino-terminal globular domain VI present in the other B chains which forms the globular structure at the end of the short arms. Another distinct feature is that domains IV, III, and I/II are shorter. The 21-amino acid signal peptide in the B2t chain is followed by domain V. The three EGF-modules can be aligned with modules 2-4 in domain V of the laminin B2e chain, with the exception of an insertion of eight amino acids after the second module in laminin B2t chain. The fourth EGF module is interrupted by a cysteine-free region of ~190 amino acids, domain IV, similarly to the corresponding domain in the laminin Ae and B2e chains. This is followed by domain III, corresponding to domain III in the other laminin chains, containing three complete EGF modules, which can be aligned with modules 7-9 in domain III of the laminin B2e chain (54). The fourth module in the B2t chain contains only six cysteines and it cannot be totally aligned with module 10 in the B2e chain. The laminin B2e chain contains two additional EGF modules in this domain. Similarly to the other laminin chains, the carboxyl-terminal end of the protein, domain I/II, begins with two closely spaced cysteines, which participate in interchain disulfide bonds in the center of the cross in the laminin molecule. Also, as in the other laminin chains, the amino acid sequence of domain I/II can be written into heptad repeats, a structure typical for coiled-coil proteins. However, similarly to the B2e chain the B2t chain has no domain  $\alpha$  which is present in the B1e and B1s chains. The sequence similarity between the B2t and B2e in this domain is lower than that between the other domains. In the longer form B2t chain this domain is 585 residues as compared with 579 residues in the B2e chain. Domain I/II in the shorter form of the B2t chain is 503 residues and has no carboxyl-terminal cysteine residue, which has been shown to participate in the formation of a disulfide bridge between the two B chains in the EHS laminin (52).

There are only six possible glycosylation sites in the B2t chain as opposed to 14 in the B2e chain. In general, the glycosylation sites in laminin chains are concentrated to domain I/II. In the B2e chain there are nine sites in this domain but only two in the B2t chain.

#### ***Tentative Interaction of the Laminin B2t Chain with Other Laminin Chains***

To estimate whether the B2t chain can assemble with itself or other known human laminin chains, we calculated the interchain ionic interaction values for different parallel in-register arrangements (Table I). As found for all other laminin chains studied so far, the negative value of a homodimeric associate indicates this as a rather unfavorable configuration. For human B1e-B1e, B2e-B2e, and Ae-Ae the corresponding

**Table I. Interchain Ionic Interactions for a Parallel, In-Register Chain Arrangement of B2t Domains with Those of Other Human Laminin Chains**

Chain assembly	Interchain interionic interaction factor
B2t-B2t	-0.152
B2t-B2e	+0.013
B2t-B1e	+0.207
B2t-Ae	+0.198
B1e-B1e	-0.105
B2e-B2e	+0.000
Ae-Ae	-0.116

values are -0.105, 0.000, and -0.116, respectively. In contrast, the values for the association of B2t-B1e and B2t-Ae give positive values of over +0.1. Values of similar magnitude have been calculated for the long arm COOH-terminal fragment E8 of mouse EHS tumor laminin (20). In vitro disassembly and reassembly studies performed with the fragment (35, 36) are in complete agreement with the predictions based on the calculations. The low positive value for B2t-B2e interaction suggests this as an unprobable chain arrangement. Accordingly, it seems likely that the B2t chain could replace B2e under certain conditions. The values calculated for the other laminin variant chains, B1s and Am chains, also suggest that they replace their counterparts B1e and Ae, respectively (20).

#### ***Chromosomal Localization of the Laminin B2t Chain Gene (LAMB2T)***

The gene for the laminin B2t chain (LAMB2T) was mapped to chromosome 1 by using a cell hybrid panel of 35 somatic cell hybrids. The hybridization of the partial cDNA clone for LAMB2T correlated with the presence or absence of human chromosome 1 (data not shown). Examination of cell hybrids that retained regions of chromosome 1 localized the gene to 1q12→qter. In situ hybridization of the cDNA to metaphase chromosomes confirmed the localization to chromosome 1 (Fig. 5). The majority of signals localized over bands 1q25→q31.

#### ***Northern Analyses***

Expression of the laminin Ae, B1e, B2e, and B2t chains was compared in cultured cells and human fetal tissues by Northern analysis. The expression pattern of the different laminin chains in the HT1080 fibrosarcoma cells and human chorioncarcinoma, JAR, cells is shown in Fig. 6. In the HT1080 cells, the laminin Ae chain is expressed only at a low level. Hybridization with the B1e, B2e, and B2t chain cDNAs yielded signals of about equal intensity. The B2t cDNA probe revealed two distinct bands of ~4.5 and 5 kb. The JAR cells showed strong signals with the Ae and B1e cDNAs and slightly weaker signal with the B2e cDNA, but no signal with the B2t cDNA. Comparison of B chain expression in a number of human fetal tissues is shown in Fig. 7. The B1e and B2e mRNAs were generally expressed coordinately in most tissues, except in the brain where the ependymal and intermediate zones and cortical plate displayed B1e chain signals much weaker than the B2e chain signal. Expression of the B2t chain mRNA in these three tissues was negligible or absent. In skin the expression of the B1e chain was consider-

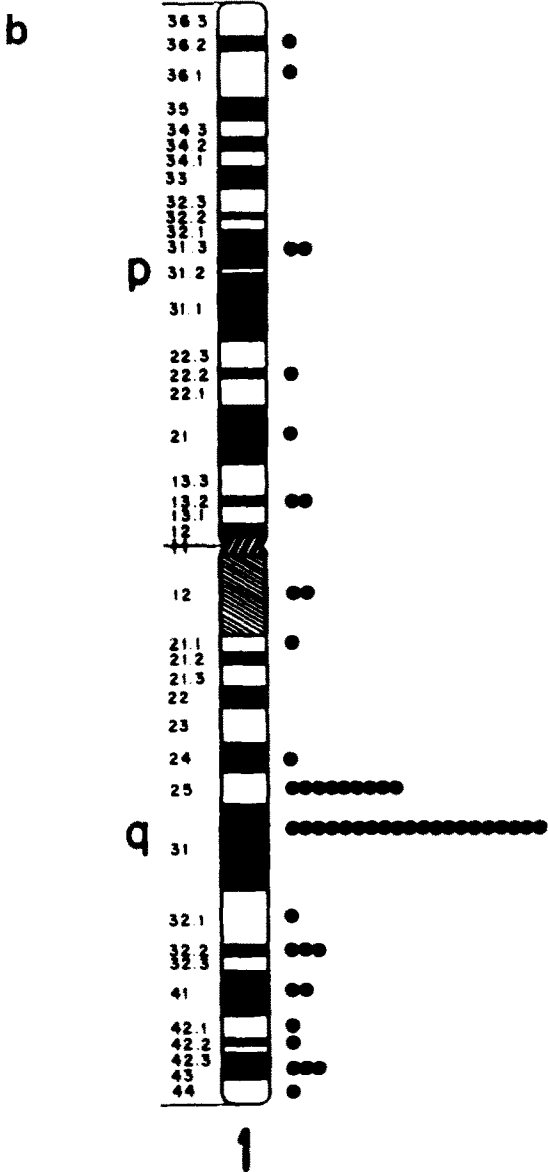
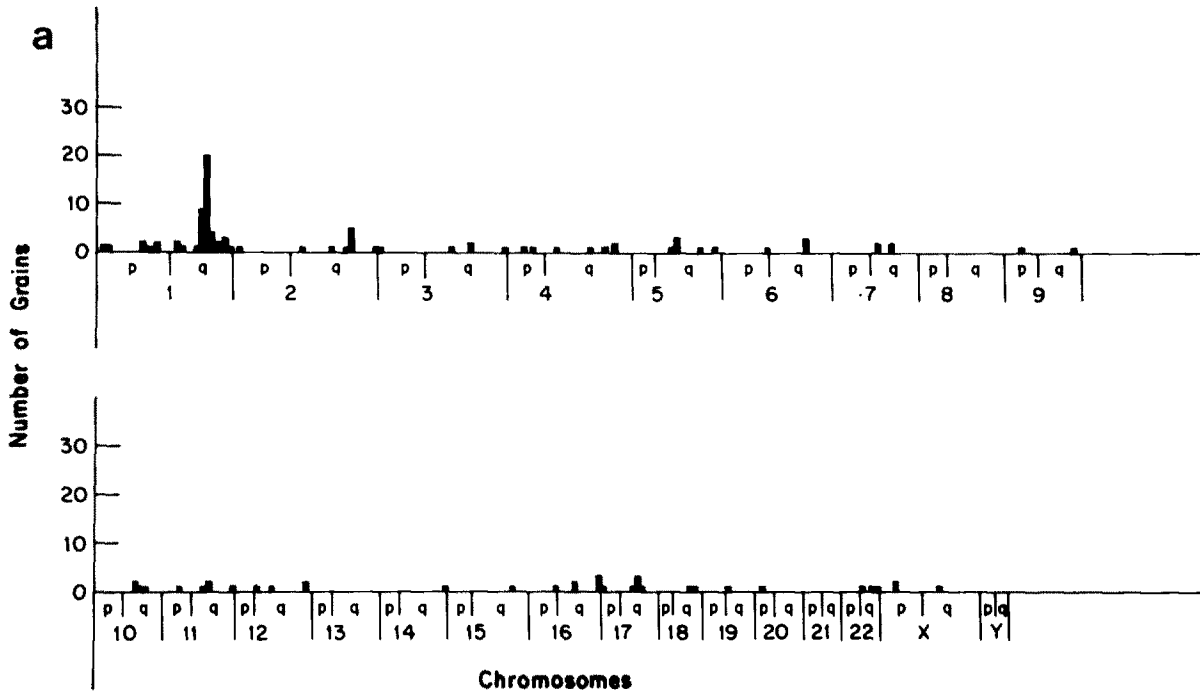
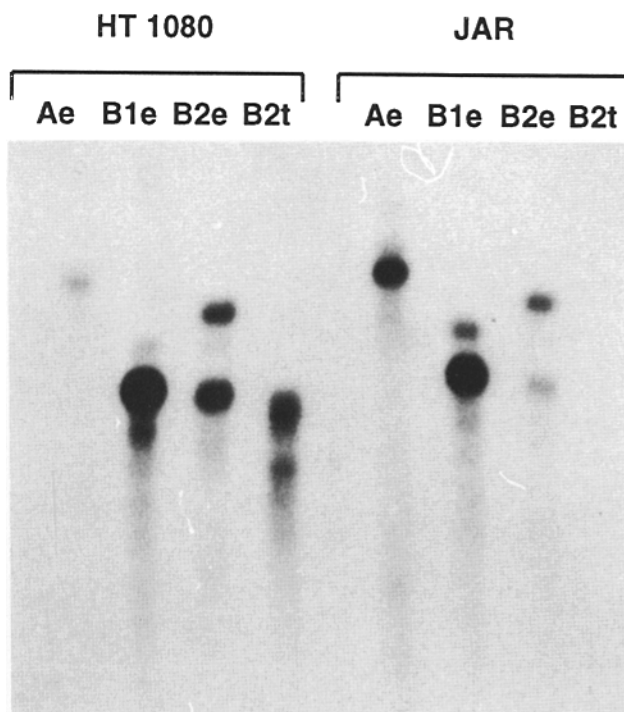


Figure 5. In situ hybridization of the HT2-7 cDNA to metaphase chromosomes. The histogram (a) displays data for all signals counted in 50 metaphases. The idiogram of chromosome 1 (b) shows the distribution of signals on that chromosome and the assignment of LAMB2T gene to 1q25→31.



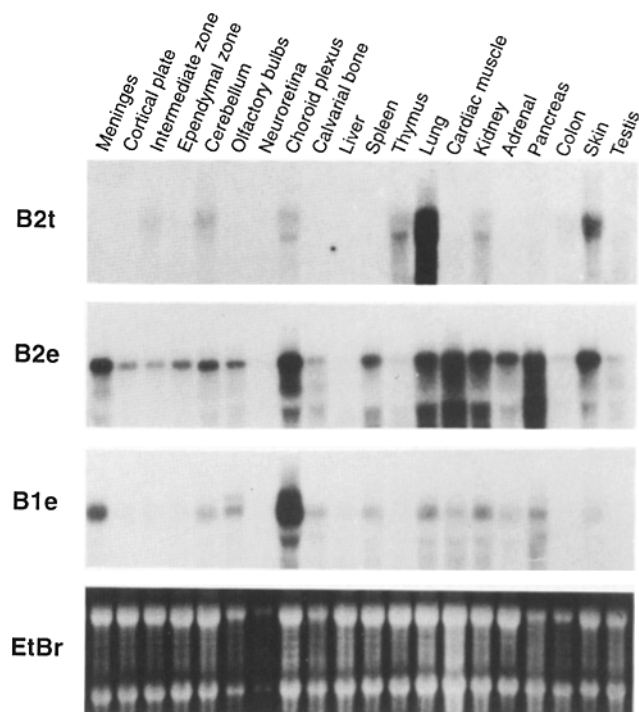


**Figure 6.** Expression of the laminin B chains in cultured cells. Northern analysis of poly(A) RNA from HT1080 fibrosarcoma cells and human choriocarcinoma, JAR, cells. 5  $\mu$ g of poly(A)-enriched RNA was electrophoresed on 0.7% agarose gels and transferred to a nitrocellulose and hybridized with cDNAs for different laminin chains as described in Materials and Methods.

ably lower than that of the B2e and B2t chains. In general, the tissue expression of the B2t chain was considerably more restricted than that of the B1e and B2e chains. The strongest signals were observed in skin and lung, but expression was also seen in kidney, thymus, choroid plexus, cerebellum, and the brain intermediate zone. In contrast, negligible or no signals were seen in the testis, pancreas, adrenal tissue, cardiac muscle, spleen, liver, calvarial bone, neuroretina, olfactory bulbs, brain ependymal zone, cortical plate, or meninges.

#### **Cell-specific Expression of Laminin B2t and B2e Chain mRNAs in Human Fetal Tissues**

The *in situ* hybridization analyses demonstrated highly cell and region specific expression of the B2t chain in 17th-gestational-week human fetal tissues. In the skin, lung, and kidney tissues studies, expression was confined to epithelial cells. In contrast, the B2e chain was expressed both in epithelial and endothelial cells. In skin B2t chain expression was observed in the entire epithelium with particularly strong signals in the appendices and adnexes which form the glands and ducts (Fig. 8, *d* and *e*). The B2e chain was expressed in the epithelium but also in vascular endothelial cells and possibly also in dermal cells (Fig. 8, *A* and *B*). In lung the B2t chain was expressed exclusively in epithelial cells of bronchi and alveoli (Fig. 9, *C* and *D*). The B2e chain was highly expressed in alveoli and also in vascular endothelial cells, but only a faint signal was seen in the bronchial epithelium (Fig. 9, *A* and *B*). In kidney the B2t chain was expressed at low level in the collecting tubules in the medulla (Fig. 10, *C* and *D*). The B2e chain was expressed mainly in secretory



**Figure 7.** Expression of laminin chains in human fetal tissues. Samples of 10  $\mu$ g of total RNA from normal human fetal tissues isolated at ~18–19 gw were run on a 1.2% gel and transferred to a Gene-ScreenPlus filter. The same filter was hybridized with cDNAs for different laminin chains as described in Materials and Methods.

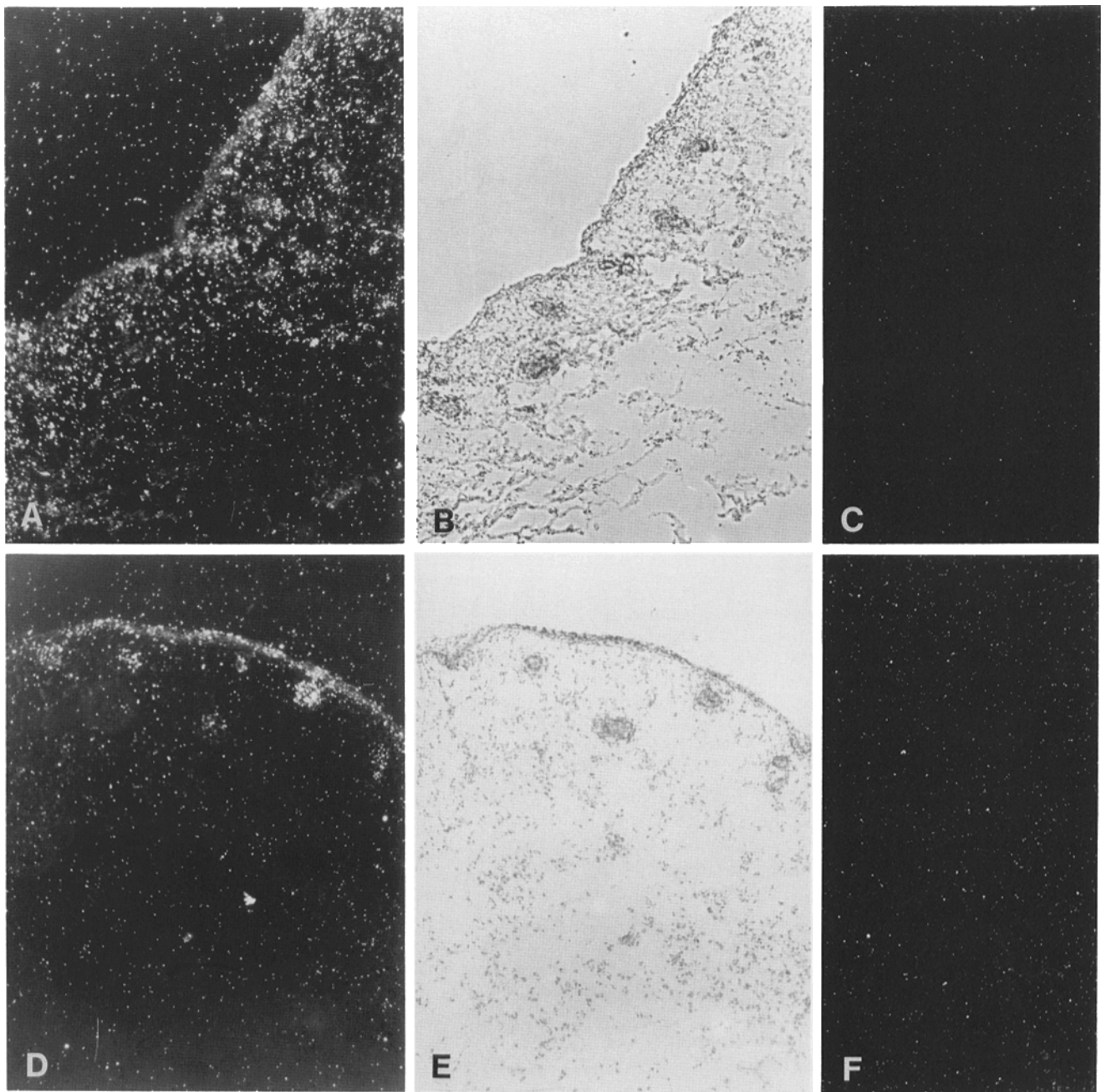
nephrons at the S-shape stage and also in the vascular endothelial cells (Fig. 10, *A* and *B*).

#### **Discussion**

Five genetically distinct laminin chains have been characterized so far. All the previously identified A- or B-type laminin subunit chains have a conserved domain structure and closely similar organization of internal repeats within each type. In the present work we have characterized a previously unidentified laminin chain, extensively resembling the B2e chain. The novel laminin B2t chain differs, however, from the B2e and other B-type chains by having a truncated structure and, consequently, a considerably lower molecular mass than the other B chains (130 vs 190–200 kD). The smaller size of the B2t chain is due to the absence of a domain corresponding to domain VI of the other B chains, but also due to considerably shorter domains V, IV, and III. As such, these differences suggest a distinct biological role for the B2t chain. Additionally the present data indicate that the B2t chain exists in two different forms, the smaller form having a shorter domain I/II giving a molecular mass of 120 kD.

#### **Long Arm and Potential Chain Interactions**

To date, there are no protein data available on the B2t chain, and the mode of association of the laminin B2t chain with the other laminin chains could not be determined in the present study. However, the interchain ionic interactions calculations indicated that the B2t chain can replace the homologous B2e chain in laminin molecules. Although the sequence similarity between B2t and B2e is weaker for domain II/I

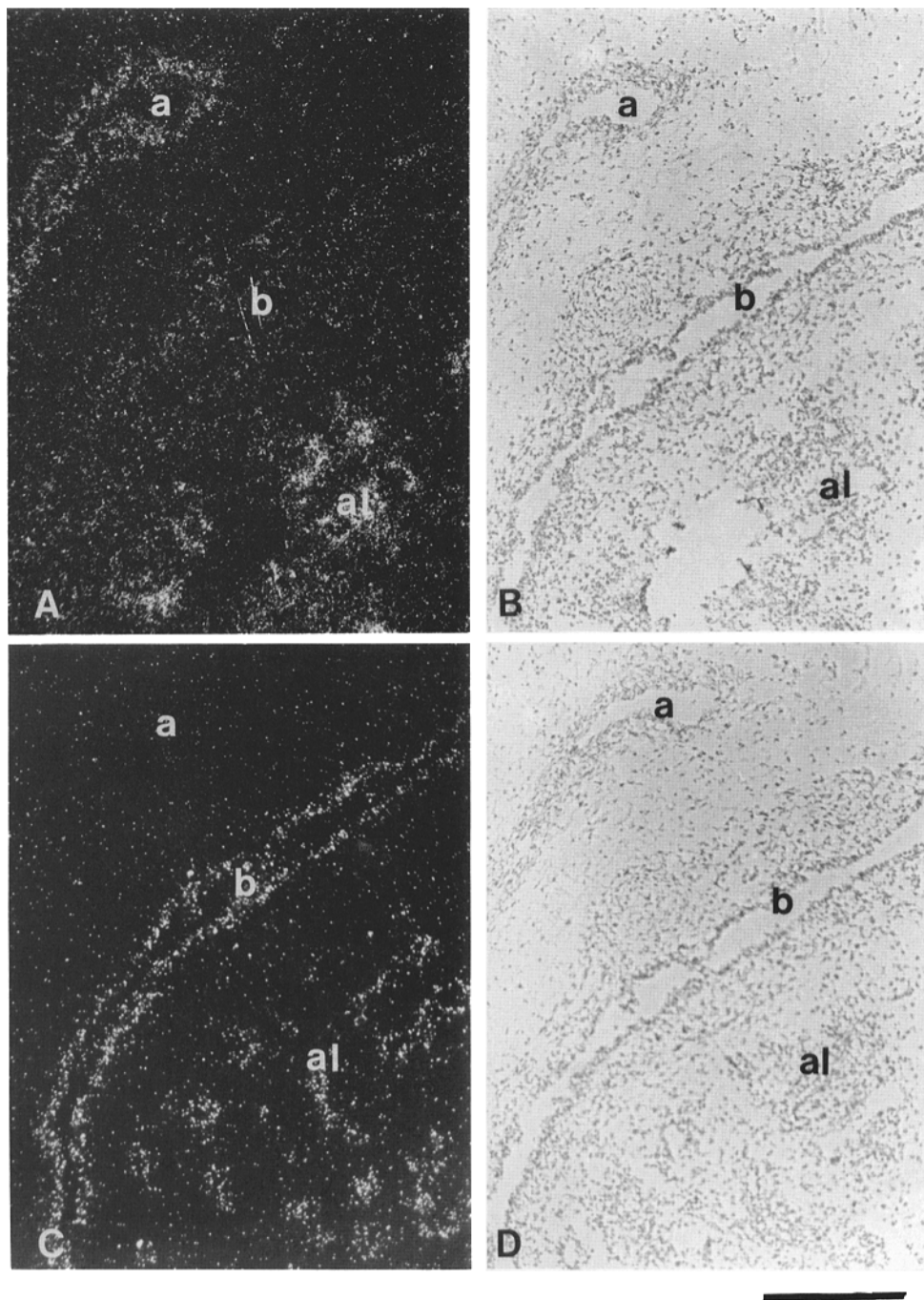


**Figure 8.** Expression of laminin B2e and B2t mRNAs in fetal skin. In situ hybridization with antisense RNA for the B2e chain (*A* and *B*) shows expression in the skin epithelial layer, vascular endothelial cells, and possibly also dermal cells. Signals for the B2t chain can be seen in keratinocytes but they are particularly strong in the appendices and adnexes which form the glands and ducts, while vascular endothelia are negative (*D* and *E*). Sense RNAs for either B2e (*C*) or B2t (*F*) chains used as control show no signals. Bar, 110  $\mu$ m.

than for domains III to V, there are distinct regions showing more or less homology. Residues 613–766 which directly follow the two closely spaced cysteines are most probably located within the center of the cross-like laminin structure and exhibit a sequence identity of  $\sim 37\%$  with the corresponding portion of B2e. Residues 794–908, however, have a rather low similarity, or only  $\sim 15\%$ . Such a value corresponds to a homology which can be expected for random sequences showing a heptad arrangement. It can be concluded from the heptad arrangement that in a complex with B1e this region is arranged around the cysteine-rich domain  $\alpha$  which

is not present in the B2-type chains. The strongest similarity between B2t and B2e,  $\sim 55\%$ , is found at residues 921–1,011 of the B2t chain. This similarity together with the increased density of putative interchain ionic interactions in this region indicates a crucial role for this sequence in laminin chain assembly.

The shorter form of the B2t sequence lacks the carboxyl-terminal region corresponding to fragment 25 K of mouse EHS laminin and also the terminal cysteine which forms a disulfide bridge between B2e and B1e in EHS laminin (52). This sequence was found in three different clones isolated



**Figure 9** Expression of laminin B2e and B2t mRNAs in fetal lung. The B2e antisense probe (*A* and *B*) shows strong signals in endothelial cells of arteries (*a*) and epithelial cells of alveoli (*al*) but negligible signals in the bronchial (*b*) epithelium. The B2t probe (*C* and *D*) shows strong signals both in bronchial and alveolar epithelia, while the arteries are negative. Bar, 110  $\mu$ m.

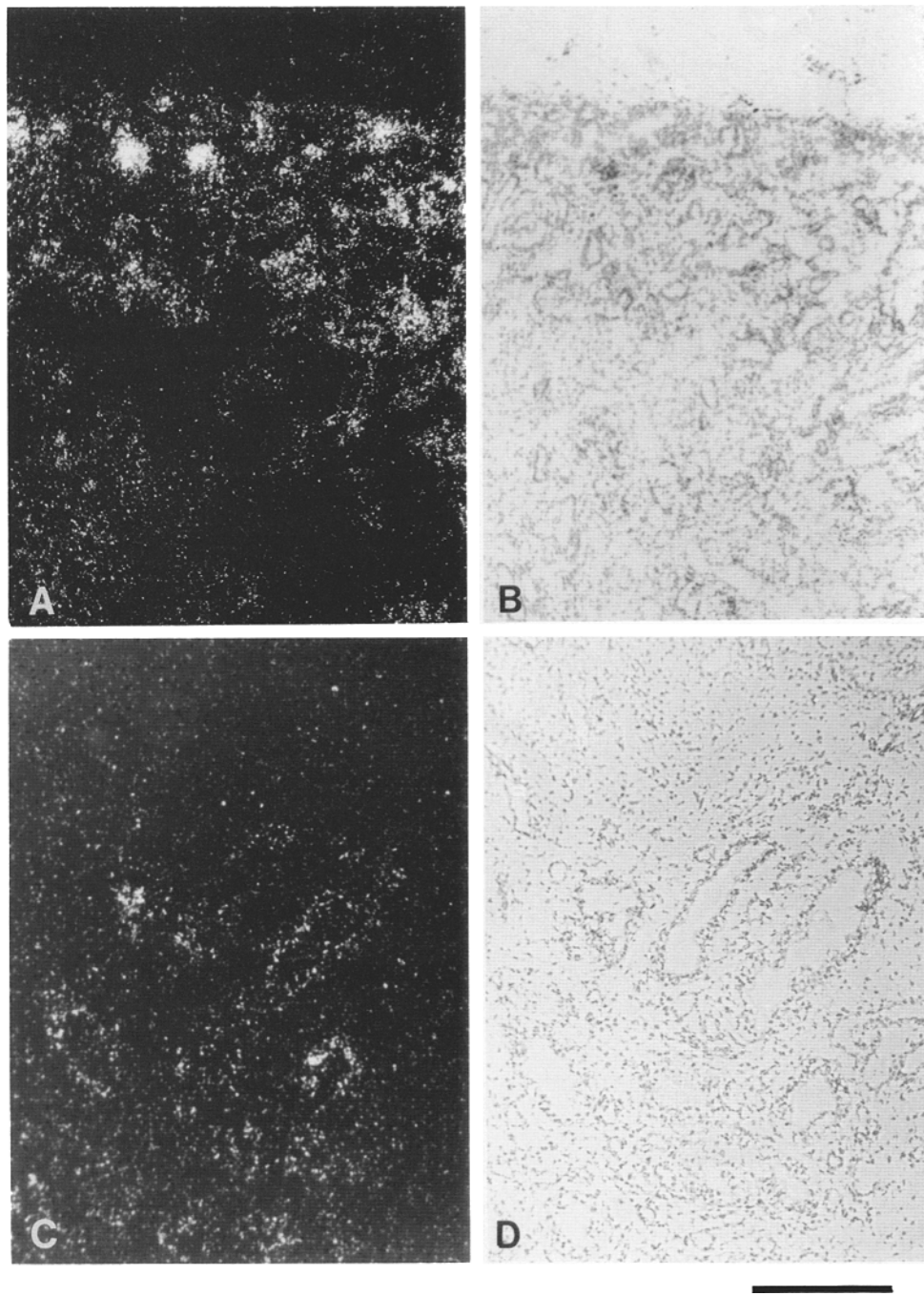
from two different cDNA libraries. This sequence is most likely to be present in some portion of the mRNAs, possibly generated by alternative splicing. At present, the part of the B2t chain gene encoding this region has not been isolated. However, comparison of the sequence of the B2t and B2e chains and comparison to the known exon-intron structure of the laminin B2e (38) chain gene shows that the difference in the 3' end sequence is situated in the exon-intron junction in the homologous sequence of the B2e chain. The shorter B2t form could therefore be generated if the intron corresponding to intron 27 was not spliced out. This hypothesis is currently being investigated.

For the *in vitro* assembly of laminin chains the presence of the terminal disulfide bond may not be essential, since the same reassembly products can be observed also after reduc-

tion and alkylation of the cysteines (35). The carboxyl-terminal portion of the Be chain which is absent in the short B2t chain may, therefore, not be crucial for the formation of an Ae-Ble-B2t heterotrimer. This is the sequence region where an increased density of favorable interchain ionic interactions between the Ae and Ble chains can be found (not shown). In such a conformation this region might form a two-stranded rope structure similarly to several intracellular proteins, including myosin and intermediate filament proteins. It cannot be excluded, however, that the B2t chain, which lacks the carboxyl-terminal cysteine assembles with some other, as yet unknown, laminin chains.

#### **Unique Domain Structure of the Short Arm**

The absence of domain VI distinguishes the B2t chain from



**Figure 10.** Expression of laminin B2e and B2t mRNAs in embryonic metanephric kidney. In situ hybridization with antisense RNA for the B2e chain (A and B) shows abundant expression in embryonic nephrons. The secretory nephrons are negative for B2t mRNA (C and D) but the collecting tubules in the medulla show clear expression. Bars: (A and B) 110  $\mu\text{m}$ ; (C and D) 110  $\mu\text{m}$ .

all other known laminin chains. This domain which forms the terminal globules of the short arms has been shown to participate in the association of laminin molecules in vitro (8, 63) and recently it has been shown that laminin forms an independent network in basement membranes in vivo (73). Domain VI has also been reported to bind to type IV collagen (11, 42). The absence of domain VI from the B2t chain indicates that it participates in another type of supramolecular structure than molecules containing the classical chains.

Another basement membrane protein, nidogen, has been proposed to form a link between laminin and type IV collagen, binding to both proteins (23). The major binding site for nidogen in laminin has been localized to EGF modules in domain III of laminin B2e chain (28). In the laminin B2t chain, most of the modules corresponding to the ones that

bind nidogen are missing from domain III. However, one of the EGF modules in domain III which shows considerable similarity to a module in domain III in the B2e chain is present. Therefore, it is possible that the B2t chain contains a nidogen binding region. A synthetic decapeptide containing a sequence from the carboxyl-terminal end of the mouse laminin B2e chain has been reported to stimulate neurite outgrowth (43). The region containing this sequence is also missing from the B2t chain. The smaller amount of potential glycosylation sites in laminin B2t chain may also be of significance as glycosylation of laminin has been reported to affect cell attachment, spreading, and neurite outgrowth (5, 16, 17). The binding sites for the integrin type cell surface receptors have been localized to a region in the long arm close to the terminal globule (27, 41, 67, 70a) and also to do-



mains III (29). Recognition of different laminin isoforms by different receptors could be of importance in a number of biological processes. There are already data indicating differences in binding of cells to different laminin molecules (7, 68).

### Chromosomal Assignment of the B2t Chain Gene

The high degree of sequence similarity between the B2t and B2e chains suggests that the genes for these chains have formed through duplication. This idea is supported by the finding that the genes for these subunits are located close to each other on chromosome 1. The gene for the laminin B2t chain was localized in this study to 1q25→q31. Previously, the gene for the laminin B2e chain was localized to the same region (25). However, in the case of the laminin B2e chain gene, the majority of signals localized over 1q25, whereas for the laminin B2t chain, the majority of signals localized over 1q31. The human genes for the basement membrane collagen chains,  $\alpha 1(\text{IV})$  and  $\alpha 2(\text{IV})$  chains have been localized close to each other on chromosome 13 (6, 30) and they have been shown to have a common bidirectional promoter region and transcription from opposite strands (55, 66). This is, however, not the case with the genes for the laminin B2e and B2t chains, which have separate promoters and are probably situated much further apart from each other (T. Kallunki, unpublished data). At the present, the complete exon-intron structure has been elucidated only for the human laminin B1e (71) and B2e chains (38). Although these proteins have a conserved domain structure, the structure of their genes shows extensive divergence, with different exon-intron junctions that do not usually follow the boundaries of structural domains or internal repeats.

### Spatial Expression of B2t and B2e Chain mRNAs

Previous studies have shown a general widespread distribution of the B1e and B2e chains, whereas the B1s chain is present primarily in synapses of motor neurons, renal glomeruli, and arteries (58). The present study showed that the expression of the B2t chain gene differs from that of the B1e and B2e genes regarding spatial expression in human fetal tissues. The Northern analyses confirmed previous observations (4, 40) showing slightly divergent expression of the B1e and B2e genes in human tissues. However, the present results suggest that although there are some differences in the levels of B1e and B2e chain mRNAs, they appear to be expressed in many tissues in a coordinated fashion.

The present in situ hybridization analyses with B2t and B2e chain specific probes demonstrated that the corresponding genes are largely expressed in different cells in tissues where both are expressed as shown by Northern analyses. The major differences are that the B2e chain is expressed in both epithelial cells and vascular endothelial, while expression of B2t is confined to epithelial cells. The particularly strong expression of B2t in epithelial cells of adnexes in skin, lung bronchi, as well as the collecting tubuli in kidney indicates that the B2t chain is associated with specialized basement membranes. The epithelial cells in these regions are characterized by their secretory function. Recently two proteins specific for epithelial basement membranes have been isolated from keratinocyte cultures. Epiligrin has been localized by immunostaining of skin to the basement membranes

of epidermis, sweat glands, and ducts and in lung to the basement membranes of ciliated epithelial cells and submucosal glands in the bronchus (9). Epiligrin colocalizes with integrins  $\alpha 3\beta 1$  and  $\alpha 6\beta 4$  in focal adhesion contacts and stable adhesion contacts (hemidesmosomes). The molecular masses of the subunits of epiligrin have been estimated to be 135, 145, and 170 kD. Kalinin is another protein isolated from skin and keratinocyte cultures with almost same size subunits and tissue localization in skin and lung in epithelial basement membranes with hemidesmosomes (57). The exact relationship of these proteins has not been established. It is possible that the B2t chain described in the present study is related with or even a component of these proteins.

Together, the present and previous data emphasize the existence of a variety of laminin isoforms with distinct localization and possibly different biological functions. These data also indicate that there are still unidentified laminin chains and isoforms. Studies on the molecular assembly and biological functions of these molecules are a major challenge for future studies.

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