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SUMMARY

A cDNA clone encoding the B1 chain of human laminin has been isolated from a human endothelial cell cDNA library. With use of this probe and a panel of rodent/human somatic-cell hybrids and in situ hybridization, the gene for the human laminin-B1 chain has been localized to chromosome 7, band q31.

INTRODUCTION

Epithelial cells are separated from underlying connective tissue by an extracellular matrix (Timpl and Martin 1982), which consists of the glycoproteins fibronectin (Yamada and Olden 1978), type IV collagen (Kelfalides 1971), proteoheparin sulfate (Hassell et al. 1980; Kanwar et al. 1981), and laminin (Chung et al. 1979; Timpl et al. 1979; Foidart et al. 1980). Laminin (~1 million daltons), is composed of one A chain (~400,000 daltons) and three B chains (~200,000 daltons) held together by interchain disulfide bonds (Engel et al. 1981; Timpl and Martin 1982). Three species of B chains have been described, and it is likely that laminin may vary with regard to B-chain composition. Rotary-shadowed replicas of native laminin show an asymmetric cross-shaped molecule consisting of one long arm (77 nmol), and three short arms (36 nm) (Engel et al. 1981). Distinct domains within the laminin molecule are involved in the interaction between laminin and other matrix components, as well as

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with a high-affinity cellular laminin receptor. The interaction between cells and laminin mediates the attachment of cells to basement-membrane components (Terranova et al. 1980) and influences cell growth and differentiation (Manthorpe et al. 1983), migration (McCarthy et al. 1983), morphogenesis (Spiegel et al. 1983), neurite outgrowth (Baron van Evercooren et al. 1982; Edgar et al. 1984), and cancer metastases (Terranova et al. 1983). Finally, laminin is the first extracellular-matrix component that appears in developing mouse embryonic kidney (Ekblom et al. 1980), suggesting a role for laminin in the morphogenesis of this organ.

To further explore the biosynthesis of laminin in cellular and developmental systems, we first sought to isolate cDNA clones encoding human laminin. In the present paper we report the isolation of a partial clone for the human laminin B1 chain, its nucleotide sequence, and chromosome location.

MATERIAL AND METHODS

A. Cell Culture, Preparation of Poly(A)-containing RNA, and cDNA Library Construction

Human endothelial cells derived from the umbilical vein (HUVEC) were grown to confluence in 850-cm^2 plastic roller bottles coated with human fibronectin (10 $\mu g/cm^2$) in Medium 199 supplemented with 10% fetal bovine serum and 150 μg crude-preparation bovine brain endothelial cell growth factor (ECGF)/ml (Jaye et al. 1985). Total RNA was extracted from cultured HUVEC cells essentially according to a method described by Chirgwin et al. (1979). Poly(A)-containing RNA (20 μg) was copied into cDNA by means of reverse transcriptase. DNA polymerase I (Klenow fragment) was used for synthesis of the second strand of cDNA (Norgard et al. 1980). Size-fractionated cDNA (≥1 kb) to which *Eco*RI linkers had been added was then inserted into *Eco*RI-cleaved and phosphatase-treated λgt11 arms (Young and Davis 1983*a*, 1983*b*) by means of T4 DNA ligase and packaged according to standard procedures. A library of ~500,000 recombinant phage was produced.

B. Identification of Human Laminin cDNA Clones

One half million plaques of the recombinant HUVEC cDNA library were plated on a lawn of *Escherichia coli* Y1090 and incubated at 42 C. After 4 h of incubation, the plates were overlaid with a nitrocellulose filter that previously had been saturated with 10 mM isopropyl thio β-d-galactopyranoside (IPTG), following which they were incubated at 37 C overnight. The filters were then incubated in a solution of 3% gelatin in Tris-buffered saline (TBS; 20 mM Tris-HCl, pH 7.5, 500 mM NaCl) at room temperature for 1 h. Next, the filters were incubated at room temperature overnight in a 1:100 dilution in TBS of a rabbit antiserum directed against denatured rat laminin. After two 30-min washes with TBS at room temperature, the filters were incubated for 2 h at room temperature in a 1:2,000 dilution of horseradish peroxidase (HRP)–conjugated goat anti-rabbit IgG (Bio-Rad). Approximately 12 plaques gave strong color-development signals, and these were purified to homogeneity by means of

repetition of the above procedure. Phage DNA was prepared according to standard procedures.

A 1.3-kb cDNA insert was excised from phage 12 DNA by means of digestion with *Eco*RI, further digested with *Pst*I, and subcloned into M13mp11. The chain-termination procedure (Sanger et al. 1977) was employed to determine the nucleotide sequence of the cDNA.

C. In Situ Hybridization

The procedure followed was essentially that of Harper and Saunders (1981). A plasmid subclone of the cDNA insert of phage 12 (pMJ5) was labeled, with use of three tritiated nucleotide triphosphates, to a specific activity of 2.2×10^7 cpm/μg. Hybridization to BrDU-synchronized (50 μg/ml) metaphase chromosomes (Dutrillaux and Viegas-Pequignot 1981) derived from fresh primary lymphocytes was performed at 37 C for 16–18 h in a solution containing 50% formamide, 10% dextran sulfate, 2 × SSCP (1 × SSCP = SSC supplemented with 10 mM sodium phosphate, pH 6.8). The probe concentration range was 0.01–0.15 μg/ml, and sheared salmon-sperm DNA was included at a concentration 500 times that of the probe. Following hybridization, slides were washed five times each at 40 C with 50% formamide in 2 × SSC, and 0.5 × SSC before being dehydrated in an ethanol series. Slides were then dipped in Kodak NTB-2 and allowed to expose at 4 C for 7–50 days. Following development for 15 min in Dektol (1 μg/ml), slides were G-banded by means of staining in Hoechst 33258, exposed to long-wave ultraviolet light for 1 h, and stained with Wright stain according to a modification of a published procedure (Perry and Wolff 1974).

RESULTS

Screening of the recombinant HUVEC cDNA library in λgt11 with rabbit anti-rat laminin antiserum identified 12 strongly reacting plaques. DNA isolated from one of these phage (phage 12) contained a cDNA insert of 1.3 kb, which was further characterized by means of nucleotide sequence analysis. As shown in figure 1, this cDNA fragment corresponds entirely to an open reading frame of 434 amino acids. As shown in figure 2, the amino acid sequence deduced on the basis of this clone shares 91% homology with a mouse laminin-B1-chain cDNA clone (Barlow et al. 1984), authenticating its coding capacity for the B1 chain of laminin. The human clone extends 16 amino acids beyond the NH_2-terminus of the mouse clone and terminates 77 amino acids before the COOH-terminus of the mouse laminin-B1 protein.

Human DNA and DNA from a panel of rodent/human hybrid-cell lines (O'Brien et al. 1982, 1983) was digested with *Eco*RI and analyzed by means of Southern blotting with the human B1-chain cDNA essentially according to a method described by Jaye et al. (1986). In human DNA, a single hybridizing band of 6.3 kb was observed (data not shown), which was then used to score the panel of hybrid-cell lines. As shown in figure 3*a*, 0% discordancy ($P < .001$) was observed between human chromosome 7 or its isozyme marker β-glucuronidase and the 6.3-kb *Eco*RI fragment.

FIG. 1.—Nucleotide sequence of human laminin-B1-chain cDNA and deduced amino acid sequence. The cDNA insert from recombinant 12 was excised from phage DNA by means of digestion with *Eco*RI and further digested with *Pst*I. Restriction fragments corresponding to nucleotides 1–420, 421–947, and 948–1302 were subcloned into appropriate M13 vectors. The nucleotide sequence was determined by means of the chain-termination procedure (Sanger et al. 1977). Flanking *Eco*RI sites are not shown.

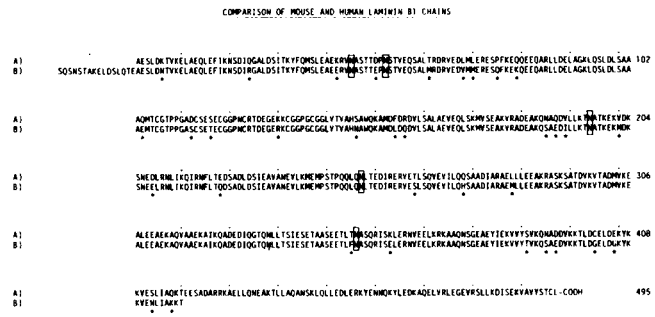


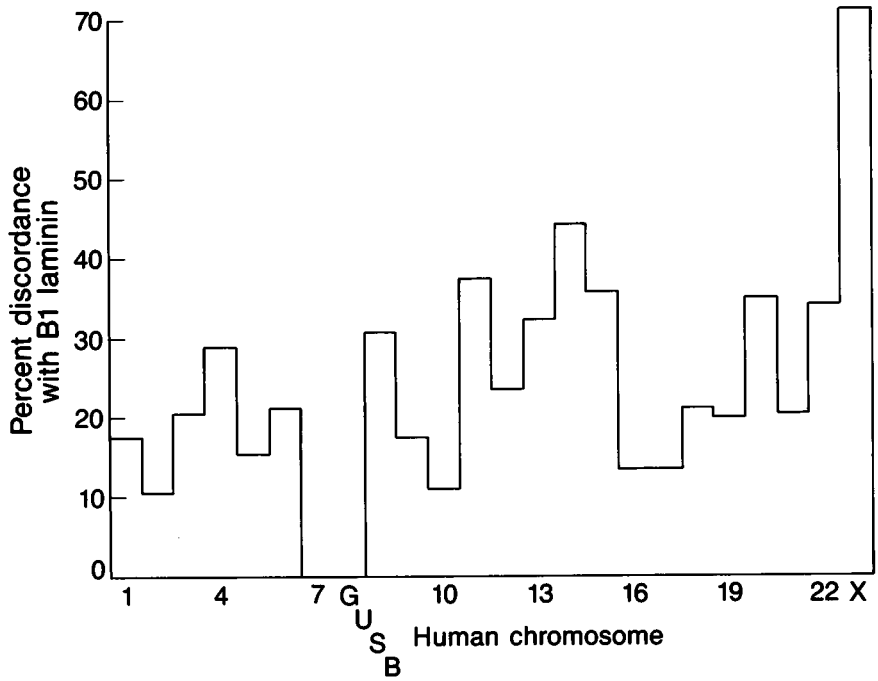
FIG. 2.—Amino acid sequence comparison of human and mouse laminin-B1 chains. Row *A*, mouse laminin (Barlow et al. 1984). Row *B*, human laminin. The numbering system is based upon the mouse data, which extend to the COOH-terminus. Potential *N*-linked glycosylation sites are boxed. Residues that differ between the two species are indicated by asterisks. The one-letter amino acid code is used: A = alanine; C = cysteine; D = aspartic acid; E = glutamic acid; F = phenylalanine; G = glycine; H = histidine; I = isoleucine; K = lysine; L = leucine; M = methionine; N = asparagine; P = proline; Q = glutamine; R = arginine; S = serine; T = threonine; V = valine; W = tryptophan; and Y = tyrosine.

The human laminin-B1 structural gene was further localized by means of in situ hybridization, with use of the cDNA probe, to synchronized human metaphase chromosomes. A total of 239 grains were distributed along the chromosomes of 90 cells examined. Of this total, a significant number of grains, 41, was found over chromosome 7 (13 grains had been expected; $P < .001$) (data not shown). These results confirm the assignment of the human laminin-B1 gene to chromosome 7. Of the grains occurring along chromosome 7, 25 were sublocalized at band q31, and it can be concluded that the laminin-B1 structural gene maps within this region (figs. 3*b*–3*d*).

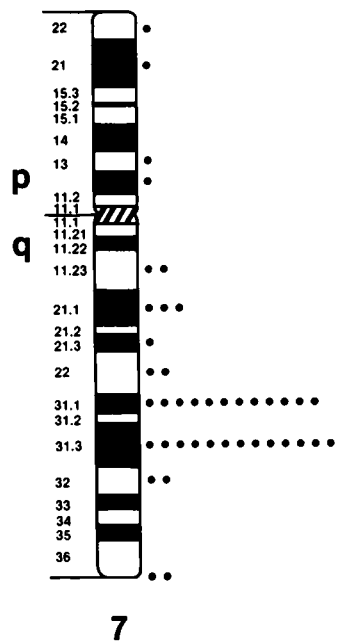
DISCUSSION

We have isolated a human laminin-B1-chain cDNA clone from a human endothelial cell cDNA library. This clone encodes 434 amino acids in the COOH-terminal portion of the human laminin B1 chain and reveals 91% homology with an overlapping partial laminin-B1-chain cDNA clone isolated from mouse parietal endoderm (Barlow et al. 1984).

Analysis of the amino acid sequences of mouse laminin-B1 and -B2 chains has revealed a striking heptad repeat (Barlow et al. 1984). The principal feature of this heptad is the presence of hydrophobic residues in positions 1 and 4 and of hydrophilic amino acids in positions 2, 3, and 5–7. A similar repeat is observed in proteins that form a double or triple alpha-helical structure, such as myosin (McLachlan and Karn 1982), tropomyosin (McLachlan and Stewart 1975), and fibrinogen (Doolittle et al. 1978). The presence of this heptad repeat in mouse laminin-B1 and -B2 chains has led to the suggestion (Barlow et al. 1984) that, in the native laminin, these chains are folded in a coiled-coil structure. Analysis of the human laminin-B1-chain cDNA confirms the presence of this heptad repeat. A new model for the structure of laminin is suggested by the presence of the heptad repeat (Barlow et al. 1984). In this model, the 200,000-


Percent discordance with B1 laminin
70
60
50
40
30
20
10
0
1
4
7
G
U
S
B
10
13
16
19
22
X
Human chromosome

a


22
21
15.3
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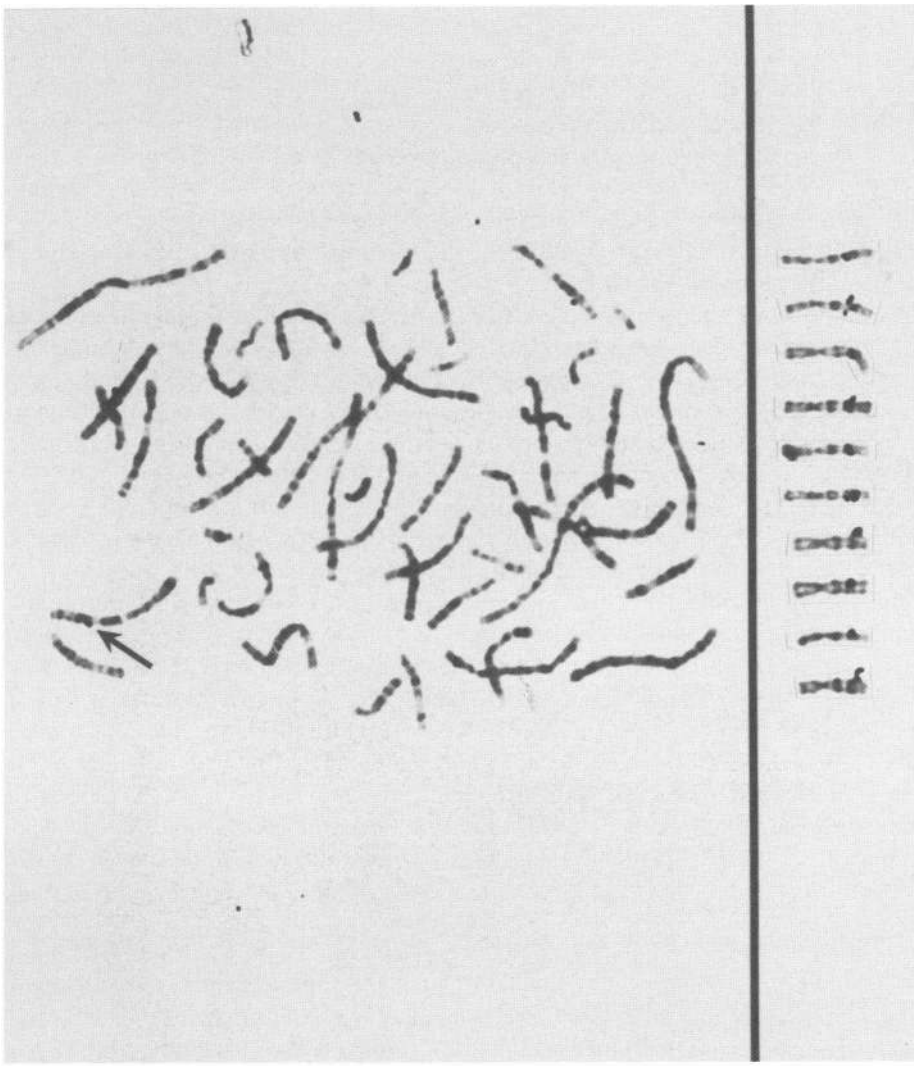
b



c d

FIG. 3.—Gene localization of the human laminin-B1 chain by means of in situ hybridization. *a*, Plot of the frequency of discordancy between human laminin-B1 sequences and each of the 23 human chromosomes in a panel of 41 somatic-cell hybrids. The chromosome scores of hybrids represent the consensus of G-trypsin karyotype and isozyme analysis (O'Brien et al. 1982, 1983). *b*, Idiogram (ISCN 1985, pp. xi, 118) illustrating the grain distribution along chromosome 7. *c*, G-banded metaphase chromosomes with labeled chromosome 7 (arrow). *d*, Series of chromosomes 7 from several different G-banded cells, demonstrating typical labeling pattern.

molecular-weight B chains and the 400,000-molecular-weight A chain would not simply meet at the center of the cross-shaped molecule, but the B chains would bend and coil around the A chain. Chemical, immunological, and physical evidence that supports this model for laminin isolated from the mouse Engelbreth-Holm-Swarm (EHS) tumor has been published (Paulsson et al. 1985). The complete amino acid sequence of the mouse laminin-B1 chain has recently been deduced on the basis of the cDNA sequence (Sasaki et al. 1987). Seven distinct domains are predicted, including cysteine-rich repeats, alpha-helical, and globular structures.

In addition to the B1 and B2 chains, a third subunit of mouse laminin of ~200,000 daltons has been described (Kurkinen et al. 1983). This subunit appears related to the B1 chain, since mRNA obtained by means of hybrid selection with B1 cDNA directs the in vitro synthesis of a doublet as observed on the basis of sodium dodecyl sulfate–polyacrylamide gel electrophoresis (Barlow et al. 1984). These chains are designated B1a and B1b (molecular weight 205,000 and 200,000, respectively). At the present time, it is not clear whether these chains are the products of separate genes or due to processing events at the level of transcription or translation. In an attempt to address this question, we sought to determine the chromosome localization of the human laminin-B1-chain gene(s).

Human DNA and DNA from a panel of rodent/human hybrid-cell lines was digested with *Eco*RI and analyzed by means of Southern blotting with the human B1-chain cDNA. In human DNA, a single hybridizing band of 6.3 kb was observed, which was then used to score the panel of hybrid-cell lines. With this approach, the gene(s) for the B1 chain of human laminin was localized to human chromosome 7 (fig. 3*a*). Confirmation and sublocalization of this assignment was obtained by means of in situ hybridization of metaphase-arrested human chromosomes with a tritium-labeled plasmid containing the B1-chain cDNA. Approximately 17% of all grains were found over chromosome 7. Of the grains on chromosome 7, 60% were observed at band q31 (figs. 3*b*–3*d*). Thus, it can be concluded that the gene for the B1 chain of human laminin is localized to this region—and that the B1a and B1b chains are most likely encoded by the same gene. These data, however, do not eliminate the possibility that separate—but adjacent—genes encode the B1a and B1b chains. The human laminin-B1 gene maps closely to several anonymous (Tsui et al. 1985; Scambler et al. 1986; Buckle et al. 1987) and known sequences, including the met oncogene (White et al. 1985), T cell–receptor B chain (Wainwright et al. 1985) and pro alpha 2(I) collagen gene (Scambler et al. 1985), which demonstrate linkage with the cystic fibrosis locus. Thus the human laminin-B1 gene may demonstrate linkage to the cystic fibrosis locus.

The human laminin-B1-chain cDNA clone that we have isolated and characterized in the present study should prove useful in studying gene regulation in models of development and differentiation. One very interesting question is how the levels of the laminin-A, -B1, and -B2 chains are coordinated. In this regard, it will be interesting to determine the chromosome localization of the A and B2 chains of laminin, once probes become available.

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