Molecular Cloning and Tissue-specific Expression of a Novel Murine Laminin γ3 Chain*

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A novel laminin γ3 chain was identified from the expressed sequence tag data base at the National Center for Biotechnology Information. A complete cDNA-derived peptide sequence reveals a 1592-amino acid-long primary translation product, including a tentative 33-amino acid-long signal peptide. Comparison with the laminin γ1 chain predicts that the two polypeptides have equal spatial dimensions. In addition, the well conserved domains VI and III(LE4) predict that γ3 containing laminins are able to integrate to the laminin network and also via nidogen connect to other protein networks in the basement membranes. Combination of Northern analysis and in situ hybridization experiments indicate that expression of the γ3 chain is highly tissue- and cell-specific, being significantly strong in capillaries and arterioles of kidney as well as in interstitial Leydig cells of testis.

Laminins are a growing family of large extracellular glycoproteins found in but not confined to basement membranes (1–3). These heterotrimERIC proteins are composed of one α, one β, and one γ type of chain that assemble together through a triple-helical coiled coil motif (4). Today, 10 different laminin chains have been characterized from mammals (5–18) that theoretically could give rise to over 100 different isoforms. This extreme variability reflects the specialization of basement membranes in mature tissues (19, 20) as well as during development (21, 22). Moreover, the heterogeneity in composition provides us a concrete, molecular level handle as to how basement membranes influence cell behavior and differentiation in a given tissue. The characteristic and often delimited symptoms in the various laminin gene defects (23–29) further emphasize the biological significance of the variation within the laminin family.

There are two laminin γ chains established to date (7, 8, 12). The γ2 chain is expressed restrictedly and is mainly confined to epithelial cells (12, 30). Of the eleven characterized isoforms, only laminin-5 (α3β2γ2) contains the γ2 chain (31–33). In contrast, the γ1 chain is widely expressed (12, 22, 34, 35) and takes part in all the other known isoforms (36–41). This dominant position differs from the situation among the α and β chains, both classes having at least one widely expressed, alternative chain (22, 38, 42–45). We, therefore, considered the possibility of the existence of a third γ chain. Such a chain could be particularly interesting with respect to the size of the laminin family. An alternative γ chain would nearly double the number of potential laminin isoforms and, more importantly, proportionally increase the number of identifiable components embedded in the protein meshwork in basement membranes. Clearly, this would significantly improve the resolution of our current basement membrane models that are based on a limited number of distinct proteins (46–49).

In a primary sequence, a coiled coil motif is seen as a seven-amino acid-long repeat (a, b, c, d, e, f, g) in which the residues at positions a and d are predominantly hydrophobic (50, 51). Once the coiled coil is formed, these residues are effectively protected from the polar environment of tissue fluids. While the hydrophobic interactions greatly favor the coiled coil assembly, the specificity of the interaction is often determined by the polar residues running parallel on either side of the hydrophobic core as well as by the polar and charged residues embedded in the core (52, 53).

In this study, we predicted the hydrophobic profile of a γ chain core in the C-terminal part and used this profile to discriminate against other coiled coil sequences that we found from the translated EST‡ data base at National Center for Biotechnology Information (54). Using this strategy, we could identify and further characterize a novel laminin γ3 chain.

MATERIALS AND METHODS

Data Base Search—The EST data base at the National Center for Biotechnology Information (http://www.ncbi.nlm.nih.gov) was searched with tblastn sequence comparison program (55) installed at the National Center for Biotechnology Information server. A C-terminal sequence from the human laminin γ1 chain (8) was used as a query. The probability of finding a match by chance was set to E = 1000, since the sequence homology between laminin chains in the coiled coil regions is usually low, typically about 20–30% identical residues (10, 16–18). Previously uncharacterized translations matching the query were first selected for the presence of a cysteine within the 10 C-terminal residues. To compare the amphipathicity and the hydrophobic profile along the hydrophobic core, the remaining translations were analyzed with the helical wheel program from the Genetics Computer Group software package (56). This permits easy visualization of the aligned residues in a and d positions of successive turns of the helix. A murine EST clone (373279) from the WashU-HHMI Mouse EST Project at the Washington University (57) was found to have a similar hydrophobic profile to the laminin γ1 and to the γ2 chains. Furthermore, the sequence of this clone (GenBank‡ accession number W64443) was found to be similar to that of the laminin γ1 chain by the WashU-HHMI Mouse EST Project. Clone 373279 was purchased from Genome Systems and used

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as a probe for Northern analysis and for screening a mouse testis-specific cDNA library (CLONTECH).

**Molecular Cloning**—Two methods were used for obtaining additional clones from the same gene as the 373279 clone. First, a mouse testis-specific cDNA library was screened with gene-specific restriction fragments (58). Second, various gene-specific primers and a custom anchor primer 5'-tcgagcggcctcccgggcaggt-3' were used in rapid amplification of cDNA ends for amplifying flanking fragments from embryonic mouse cDNA (E15, CLONTECH). For sequencing, the cDNA fragments were ligated into pBluescript or pCRScript vectors (Stratagene). The cycle sequencing reactions were done with *Taq*-derived polymerase using termination chemistry (Perkin-Elmer) and the extension products resolved with Applied Biosystems 377 and Applied Biosystems 310 automated sequencers. All the oligonucleotides used in this study were synthesized with Applied Biosystems 392 DNA/RNA oligosynthesizer. The sequence data obtained were analyzed with the Genetics Computer Group software (56).

**Northern and in Situ Hybridization Analyses**—An RNA blot with 2 μg/lane of poly(A)-RNA from various adult mouse tissues (CLONTECH) was hybridized according to standard procedures (58). Two probes giving essentially identical results were sequentially applied: a 0.7-kb fragment from the coiled coil region (nucleotides 3585–4263) and a 0.45-kb segment from the 3' UTR. Random priming strategy was applied in labeling the probes (Prime-a-gene, Promega). The filters were hybridized at 42 °C for 16–24 h in a solution containing 50% formamide, 2% SDS, 50 μg/ml of polyvinylpyrrolidone (Pharmacia), 50 μg/ml bovine serum albumin, 50 μg/ml Ficoll 400 (Pharmacia), and 3× SSPE (750 mM NaCl, 50 mM sodium phosphate, pH 7.4, 5 mM EDTA). After washing in 1× SSC + 0.1% SDS for 2× 15 min and in 0.1× SSC + 0.1% SDS for 15 min, Kodak X-OMAT films were exposed to the filter-bound radioactivity for several days at 270 °C using intensifying screens.

In situ hybridization was carried out on tissues from adult mouse essentially as described elsewhere (44, 59, 60). Briefly, tissues were fixed in the fresh 4% paraformaldehyde, dehydrated by a gradient of ethanol, embedded in paraffin, and sectioned. Following postfixation in 4% paraformaldehyde, the sections were incubated in phosphate-buffered saline containing 0.1% active diethyl pyrocarbonate (Sigma), equilibrated in 5× SSC and prehybridized for 2 h at 55 °C. Hybridization was then carried out overnight at 55 °C. For generation of single-stranded RNA probes, a 450-base pair *Pst*I fragment from the 3' UTR of the cDNA was cloned into pGEM3Z (Promega). Antisense and sense riboprobes were labeled with digoxigenin-11-UTP by *in vitro* transcription with T7 and SP6 RNA polymerase (Roche Molecular Biochemicals), respectively. After washing in 5× SSC and prehybridized for 2 h at 55 °C, the sections were incubated with an alkaline phosphatase coupled anti-digoxigenin antibody and stained using nitro blue tetrazolium and 5-bromo-4-chloro-3-indolyl phosphate solutions (Roche Molecular Biochemicals).

**RESULTS AND DISCUSSION**

**Molecular Cloning**—The specificity of laminin chain assembly lies at the coiled coil domain. According to our hypothesis, this is reflected in the chain-specific hydrophobic profiles in the coiled coil core. Similarly, the intraheptad position of the C-terminal cysteine residues in the coiled coil domain is chain-specific. Starting from these premises, the EST data base at the National Center for Biotechnology Information was searched

![Fig. 1. Schematic presentation of the murine laminin γ3 cDNA. Top, scale in base pairs. Bottom: cDNA clones isolated in this study together with the EST clone 373279. Closed arrow = translation initiation codon; open arrow = translation termination codon.](image1)

![Fig. 2. Comparison of the amino acid sequences of the murine laminin γ3 and γ1 chains. Alignment was produced with the fasta program in the GCG package (56). The first letter of Roman numbers indicates the start of domains. *mlang3* = murine laminin γ3 chain; *hlang1* = human laminin γ1 chain (8).](image2)
as described under “Materials and Methods.” The EST clone 373279 from the mouse EST project at the Washington University (see “Materials and Methods”) fulfilled these criteria. Furthermore, the sequence of this clone was found to be significantly similar to that of the murine γ3 chain by the EST project. We then used the clone 373279 as a starting point in the search for overlapping clones. Together with 373279, the identified clones encoded for an open reading frame of 4829 nucleotides that included 53 base pairs of tentative 5′-UTR (Fig. 1).

Sequence Comparison with Other Laminin γ Chains—Further analysis of the cDNA-derived peptide sequence predicts a 33-amino acid-long signal peptide (61, 62) and a 1559-amino acid-long mature peptide showing a prototype domain structure of a laminin γ chain (Figs. 2 and 3). Comparison with the human γ1 chain reveals 64.0, 62.1, 32.5, 54.3, and 22.0% amino acid identity in domains VI, V, IV, III, and II/I, respectively (8). The corresponding analysis with the human γ2 chain (12) yields 51.5, 34.4, 47.1, and 22.2% for domains V, IV, III, and II/I. Therefore, we name this new chain as laminin γ3 chain in agreement with the established nomenclature (3).

Using the γ1 chain as a model, some predictions about the properties of the γ3 chain can be made. First, it is likely that the dimensions of the two chains are similar. The lengths of the coiled coil domain are 579 (γ1) and 569 (γ3) residues. The globular domains VI and IV are of similar size as well. Furthermore, both chains have 11 copies of laminin repeat motifs (63, 64). Second, the domains to which specific functions have been mapped in the γ1-chain appear to be conserved: domain VI with a calcium-dependent laminin self-assembly activity (49, 65) and nidogen binding site in the fourth laminin repeat in the III domain (66) (see Fig. 3). Taken together, this suggests that the γ3 chain possesses a full networking capability similar to the γ1, but different from the γ2 chain.

Expression of the Laminin γ3 Chain—To investigate the spatial expression in mouse tissues, an RNA blot containing RNA from various adult organs (CLONTECH) was hybridized serially with two laminin γ3 chain specific probes (Fig. 4). The strongest signals were obtained from testis, kidney, and lung, with low level of expression in brain, skeletal, and heart muscle and intestines. Both probes revealed two transcripts of about 6 and 8 kb. The source for the different mRNAs remains un-
known. 3'-rapid amplification of cDNA ends using a primer at position 4748–4771 of the full-length laminin γ3 chain cDNA yielded an about 1.3-kb-long product (not shown). In the case of the γ1 chain, the 3'-UTR contains several polyadenylation signals that probably are alternatively used (67) and thus account for the two mRNAs observed (8).

To further characterize the tissue compartments expressing the γ3 chain, in situ hybridization experiments were performed on adult kidney and testis tissues. In the kidney, strong signals were seen in either afferent or efferent arterioles of the glomeruli (Fig. 5A), and in endothelial cells of other arteries (Fig. 5, B and C), while cells of the actual glomeruli only showed modest staining above background (Fig. 5, A and B). Tubular or interstitial cells did not exhibit signals, but capillaries located between tubular structures were clearly positive for expression (Fig. 5D). In the testsis, the strongest signals were seen in the Leydig cells located between the seminiferous tubuli (Fig. 6, A–C). The sertoli and spermatogenic cells were negative. Staining with the sense probe did not yield any distinct staining (Fig. 6D).

The expression pattern of the laminin γ3 chain overlaps with that of the other γ chains. In kidney, several cell types express the γ1 chain (12, 68). The γ2 chain expression is confined to the epithelial cells (12, 68). In testis, the laminin γ1 chain is found in the seminiferous tubules (69). However, little is known about the expression of laminin γ chains by Leydig cells.

In general, the results of the in situ hybridization analyses indicate that endothelial cells of arteries and capillaries, as well as interstitial cells in certain tissues, express the γ3 chain. However, expression is apparently not significant in all endothelial cells, as tissues rich in blood vessels and capillaries, such as cardiac and skeletal muscles, do not exhibit strong expression, e.g. as is the case for the laminin α4 chain (22, 44, 45). Further studies are needed to show in detail the expression pattern in epithelia. The present results indicate that, with the exception of Leydig cells in the testes, mesenchymal cells are not major producers of laminin containing the γ3 chain.

By the virtue of their coexpression with the γ3 chain, the α2, α3, α4, and α5 chains could take part in the assembly of a γ3 chain containing laminin (22, 30, 38, 42, 44, 45). Since both the β1 and the β2 chains are also expressed at these sites (34, 35, 38, 42, 43), at least eight hitherto uncharacterized laminin isoforms could collectively reside in these tissues. These tentative isoforms are likely to integrate into the basement membrane protein meshwork via the conserved networking promoting domains VI (49, 65) and III(LE4) (66, 70).

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FIG. 6. Expression of the laminin γ3 chain in adult mouse testis as determined by in situ hybridization. A, staining with the antisense probe is seen between the seminiferous tubules (arrow). Sertoli or spermatogenic cells are negative. S, seminiferous tubule. B, schematic illustration of the section in A, showing several seminiferous tubuli (S) containing Sertoli and spermatogenic cells located in the tubules above the basement membrane. Leydig cells are located in the interstitial space filling the interstitium between adjacent tubules (dark pink areas). C, magnification of the box in A shows that the expression is present in Leydig cells. D, staining with the negative control sense probe.

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