Molecular Cloning of a Novel Laminin Chain, $\alpha 5$, and Widespread Expression in Adult Mouse Tissues

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We have identified a fifth member of the $\alpha$ subfamily of vertebrate laminin chains. Sequence analysis revealed a close relationship of $\alpha 5$ to the only known Drosophila $\alpha$ chain, suggesting that the ancestral $\alpha$ gene was more similar to $\alpha 5$ than to $\alpha 1$–4. Analysis of RNA expression showed that $\alpha 5$ is widely expressed in adult tissues, with highest levels in lung, heart, and kidney. Our results suggest that $\alpha 5$ may be a major laminin chain of adult basal laminae.

Laminins are major glycoproteins of basal laminae throughout the vertebrate body. Originally identified as structural components, it is now clear that laminins are also signaling molecules that regulate the proliferation, motility, and differentiation of the cells they contact (1–3). The first laminin discovered (4, 5), now called laminin-1 (6), is a trimer of related A, B1, and B2 chains (7–9). The subsequent discovery of the novel laminin chains S-laminin (10) and merosin-M (11) revealed that the laminins comprised a larger gene family than initially envisioned. More recently, four additional chains have been cloned (12–19). So far, however, all laminin chains sequenced resemble either A, B1, or B2, and all of the laminins purified are trimers containing an A-like, a B1-like, and a B2-like chain (6, 19–22). Based on these findings, a new nomenclature has been adopted in which laminin chains are divided into $\alpha$ (A-like), $\beta$ (B1-like) and $\gamma$ (B2-like) subfamilies; A, M, B1, S, and B2 are now called $\alpha 1$, $\alpha 2$, $\beta 1$, $\beta 2$, and $\gamma 1$, respectively (6).

Consistent with laminin’s trimeric structure, all basal laminae characterized to date contain at least one $\beta$ and at least one $\gamma$ chain (19, 20, 23–28). For the $\alpha$ chains, on the other hand, the situation is less clear. For example, perineurial basal lamina in peripheral nerve stained poorly with anti-$\alpha 1$ and not at all with anti-$\alpha 2$ (23). Likewise, in kidney, glomerular basement membrane was $\alpha 2$-negative and reacted only moderately well (in human (23)) or not at all (in mouse (29, 30)) with anti-$\alpha 1$. If all laminins are $\alpha/\beta/\gamma$ trimers, these results imply that additional laminin $\alpha$ chains exist. Indeed, several biochemical studies have provided evidence for an $\alpha$-like laminin chain distinct from $\alpha 1$ and $\alpha 2$ (31–33). The recent discoveries of the $\alpha 3$ and $\alpha 4$ chains (15–18) are provocative in this context, but we and others (16, 18) found little $\alpha 3$ or $\alpha 4$ in several tissues with $\alpha 1$- and $\alpha 2$-negative basal laminae.

Accordingly, we undertook a search for additional laminin $\alpha$ chains. Using the polymerase chain reaction, we have identified laminin $\alpha 5$, a novel murine $\alpha$ chain. Sequence analysis reveals that $\alpha 5$ is more similar in domain structure to Drosophila laminin A (34, 35) than it is to mammalian $\alpha 1$–4; the ancestral vertebrate $\alpha$ chain may, therefore, have been more similar to $\alpha 5$ than to $\alpha 1$–4. RNA analysis demonstrates that $\alpha 5$ is widely expressed in adult tissues and thus may be a major laminin $\alpha$ chain of adult basal laminae.

MATERIALS AND METHODS

Degenerate primers were designed based on sequences conserved between mouse laminin $\alpha 1$ (7) and human laminin $\alpha 2$ (36). Reverse transcription-PCR was performed on RNA from postnatal day 7 mouse kidney using a GeneAmp kit (Perkin-Elmer). One pair of primers from the amino terminus of domain V (sense, 5'-GGNAGTGGYAH-T- GYTAYGGNC-3' and antisense, 5'-TRCANTGYCRRCTATDTGCC-3', where N = A/G/C/T, H = A/T/C, and D = G/A/T) produced a fragment of appropriate length (~330 base pairs). The band was excised from agarose (SeaPlaque GTG, FMC Bioproducts, Rockland, ME), reamplified with the same primers, purified with a Wizard PCR Prep kit (Promega Corp., Madison, WI), and incubated with BglI to digest laminin $\alpha 1$ products, thereby preventing their further amplification. The remaining full-length product was reamplified and ligated into the pCR II vector (Invitrogen Corp., San Diego, CA).

One resulting clone, DB2, bore an insert related to but distinct from laminins $\alpha 1$ and $\alpha 2$. The DB2 insert was labeled with $^32$P dCTP by the random primed DNA labeling kit (Boehringer Mannheim) and used to screen an adult mouse lung oligoHT+ random primed $\lambda$ ZAP II cDNA library (Stratagene, La Jolla, CA). Subsequent cDNA library screening was performed as a “walk” using selected restriction fragments of hybridizing phage to obtain overlapping clones. Clones were sequenced on an ABI 373A DNA sequencer using a Taq DyeDeoxy Terminator cycle sequencing kit (Applied Biosystems Inc., Foster City, CA). All sequences were determined from both strands. Data base homology searches were performed on the BLAST server at the National Center for Biotechnology Information (37), and sequences were compared using Genetics Computing Group programs (38).

For Northern analysis, a filter containing poly(A)-selected RNA from several adult mouse tissues (Clontech) was hybridized with a probe comprising nucleotides 7855–9361 of the $\alpha 5$ sequence. RNase protection analysis was performed as described previously (24).

RESULTS AND DISCUSSION

Molecular Cloning of Laminin $\alpha 5$—We used PCR to amplify a 334-base pair fragment from mouse kidney that encoded a novel laminin-like sequence. This fragment was used as the starting point for isolation of a series of overlapping cDNA clones that spanned >11 kb. Sequence analysis revealed that the cDNAs encoded a single open reading frame of 3610 amino acids (Fig. 1). The sequence of the predicted protein is related to but clearly distinct from those of previously reported laminin chains (7–19, 34–36). As detailed below, the novel sequence is more closely related to laminin $\alpha$ chains than to $\beta$ or $\gamma$ chains.

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Because four mammalian laminin α chains have been described to date (7, 11, 15–18), we have named the novel chain laminin α5.

The first amino acid of the deduced sequence is aspartic acid rather than methionine, indicating that the cDNAs do not reach the 5′ end of the coding sequence. Repeated efforts to obtain additional cDNAs failed. Based on homology to other laminin α chains, we believe that the mature protein contains 3630 amino acids, of which we have identified 3610.

Comparison with Other Laminin Chains—The laminin α5 chain contains eight domains, based on predicted secondary structure and homology to the laminin α1 chain (Fig. 2). Their nomenclature follows that for α1, which was in turn designed to maintain consistency with the β1 and γ1 chains (1, 7). The carboxyl-terminal half of the protein contains a large (greater than 100 kDa) globular domain (G) and an α-helical segment (I/II). The amino-terminal half contains three cysteine-rich regions predicted to form rigid, rodlike structures (IIIa, IIIb, and V) and three smaller globular regions (IVa, IVb, and VI).
Fig. 2. Relationship of a5 to other laminin chains. A, domain structure of the known laminin α chains. The names of the domains, based on accepted nomenclature (1, 7), are to the left of a5. Percent amino acid identity of individual domains of a5 with the corresponding domains of the other α chains, determined with the GAP program (38), is shown to the left of the other α chains. Numbers of EGF repeats, rounded to the nearest integer, are indicated within domains III and V. Domain structures of β1 and γ1 are shown to indicate the basis for assigning a5 to the α subfamily. aD, Drosophila A, B, relationships among mammalian and Drosophila α chains, based on sequence alignment performed by the PILEUP program (38).

Comparisons were based on domains G–IIIa, which all α chains contain. C, evolutionary scheme for vertebrate α chains, incorporating comparisons of primary sequences (from B) and predicted secondary structure (from A). In this scheme, the ancestral α gene was more similar in domain structure to a5 than to a1–4. See text for details.

Heptad repeats with hydrophobic residues in the first and fourth position of domain I/II; "EGF-like" repeats of ~50 amino acids each in domains IIIa, IIIb, and V; and five tandem "G" repeats of ~186 amino acids each in G.

Several features of a5 identify it as an α chain (Fig. 2A). First, it contains a G domain, which is present in all α but no β or γ chains. Second, a5, like a1 and a2, contains three sets of EGF-like repeats (IIIa, IIIb, and V) and three globular regions (IVa, IVb, and VI) in its amino-terminal half, whereas no β or γ chain has more than two of each. Third, a5 lacks the α-insert between domains I and II that characterizes β chains.

Of the four vertebrate laminin α chains characterized to date, two (a1 and a2) contain domains G–VI, whereas the other two (a3 and a4) are truncated and contain only domains G–IIIa (Fig. 2A). An alternatively spliced product of the a3 gene, a3B, which contains domains IIIb and IV, has been identified but not yet fully sequenced (12). In its domain structure, a5 is more similar to a1 and a2 than to a3 or a4, and it can therefore be classified as a "full-length" α chain. On the other hand, within the domains shared by all α chains, the laminin a5 sequence is more similar to a3 and a4 than to a1 or a2 (Fig. 2B). Thus, sequence analysis reveals an apparent discrepancy between relationships based on domain and secondary structure.

More surprising is that a5 is more similar in domain structure to the only known Drosophila A chain (aD (34, 35)) than it is to any of the vertebrate α chains. Both a5 and aD contain 11 EGF repeats in domain V, 4 in domain IIIb, and 7 in domain IIIa, whereas a1 and a2 have 4, 9, and 4 repeats in domains V, IIIb, and IIIa, respectively (Fig. 2A). Likewise, domain IVb is much larger in aD and a5 (558 and 577 amino acids) than in a1 or a2 (196 amino acids).

Together, these results suggest the evolutionary scheme diagrammed in Fig. 2C. We propose that an ancestral α gene, similar in domain structure to aD and a5, was duplicated early in the vertebrate lineage. One of the daughter genes evolved the α1/2 domain structure, perhaps by recombination (39), and was then duplicated again to generate a1 and a2. The other product of the original duplication was also reduplicated. One daughter evolved into a5 without further rearrangement, while the other suffered truncation and then duplicated yet again to generate a3 and a4. Although speculative, this scheme accounts for the otherwise puzzling findings that a5 resembles a1 and a2 in secondary structure but is most closely related to a3 and a4 in primary sequence.

Studies with synthetic peptides have provided evidence for several discrete adhesive sites within laminin α chains, although their significance in vivo remains unclear. The tripeptide RGD, which is recognized by several integrins (40), is present in a1, a3, and a4 but not in a2 or aD (7, 15, 18, 34–36, 41); it is present twice within the a5 sequence (Fig. 1). The tripeptide LRE, a major determinant of a motoneuron-selective adhesive site in β2 (42, 43), is present in a1, a3, and aD but not in a2 or a4 (7, 15, 18, 34–36, 41); it is present twice in a5. Finally, the sequence IKVAV, an adhesive site in a1 (44), is replaced by SKVKV in a5.

Expression of Laminin a5 RNA from a panel of adult mouse tissues was subjected to Northern analysis with an a5 cDNA. High levels of a5 mRNA were detected in heart, lung, and kidney (Fig. 3A). Longer exposures revealed lower but significant levels of a5 mRNA in brain, muscle, and testis (Fig. 3B). In all of these tissues, an RNA species of ~12 kb was detected; an additional RNA of 9 kb, visible only in testis, would be unable to encode full-length a5. A more sensitive RNase protection assay revealed significant levels of a5 RNA in liver, as well as in gut and skin (not shown). Moreover, RNase protection and in situ hybridization analyses indicated that a5 is expressed in many tissues by embryonic day 11. Thus, the a5 gene is widely expressed.

The broad distribution of a5 RNA in adult tissues stands in marked contrast to the restricted patterns of expression of the a1, a3, and a4 genes (16, 18, 30, 41). Laminin a2 RNA is widely distributed in adult tissues but is predominantly ex-

3 J. H. Miner, S. I. Lentz, W. D. Snider, and J. R. Sanes, manuscript in preparation.
4 J. H. Miner, unpublished results.
pressed by mesenchymal or mesodermal cells (36). Taken together, these results suggest that laminin α5 is a major α chain of adult epithelial and/or endothelial basal laminae. Moreover, it seems possible that reports of α1-like immunoreactivity in tissues such as kidney, lung, and muscle (20, 23, 25-28), which contain little α1 RNA, reflect cross-reactivity of anti-α1 antibodies with α5. We are currently preparing monospecific antibodies to evaluate this possibility.

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Fig. 3. Northern analysis of laminin α5 RNA from adult mouse tissues. Short (15 h (A)) and long (52 h (B)) exposures of a single blot (with an intensifying screen) are shown.