Molecular Heterogeneity of Basal Laminae: Isoforms of Laminin and Collagen IV at the Neuromuscular Junction and Elsewhere

Joshua R. Sanes,* Eva Engvall,‡ Ralph Butkowski,§ and Dale D. Hunter*

*Department of Anatomy and Neurobiology, Washington University School of Medicine, St. Louis, Missouri 63110; ‡La Jolla Cancer Research Foundation, La Jolla, California 92037; and §Department of Laboratory Medicine, University of Minnesota Medical School, Minneapolis, Minnesota 55455

Abstract. Laminin and collagen IV are components of most basal laminae (BLs). Recently, both have been shown to be products of multigene families. The A, B1, and B2 subunits of the laminin trimer are products of related genes, and the BL components merosin M and s-laminin are homologues of the A and B1 subunits, respectively. Similarly, five related collagen IV chains, α1(IV)-α5(IV), have been described. Here, we used a panel of subunit-specific antibodies to determine the distribution of the laminin and collagen IV isoforms in adult BLs. First, we compared synaptic and extrasynaptic portions of muscle fiber BL, in light of evidence that axonal and muscle membranes interact selectively with synaptic BL during neuromuscular regeneration. S-laminin, laminin A, and collagens α3(IV) and α4(IV) are greatly concentrated in synaptic BL; laminin B1 is apparently absent from synaptic BL; collagens α1(IV) and α2(IV) are less abundant in synaptic than extrasynaptic BL; and laminin B2 and merosin M are present at similar levels synaptically and extrasynaptically. These results reveal widespread differences between synaptic and extrasynaptic BL, and implicate several novel polypeptides as candidate mediators of neuromuscular interactions. Second, we widened our inquiry to assess the composition of several other BLs: endoneurial and perineurial BLs in intramuscular nerves, BLs associated with intramuscular vasculature, and glomerular and tubular BLs in kidney. Of eight BLs studied, at least seven have distinct compositions, and of the nine BL components tested, at least seven have distinct distributions. These results demonstrate a hitherto undescribed degree of heterogeneity among BLs.

BASAL laminae (BLs)[1] throughout the vertebrate body are thought to contain a number of common components—notably laminin, collagen IV, nidogen/entactin, and a heparan sulfate proteoglycan (reviewed in Timpl and Dziadzek, 1986; Martin and Timpl, 1987; Timpl, 1989; Inoue, 1989). These molecules are important for maintaining the structural integrity of BLs, and for mediating their attachment to other components of the extracellular matrix and to cell membranes. In addition, however, there is evidence for the existence of molecular differences among BLs, at least some of which are presumably related to tissue-specific roles that BLs play. While initial molecular analyses of BLs quite naturally focussed on their major and common constituents, recent studies have increasingly sought less abundant, tissue-specific components.

We have been interested in regional specializations of the BL that ensheathes skeletal muscle fibers. Approximately 0.1% of this BL lines the synaptic cleft that separates nerve from muscle at the neuromuscular junction; the remaining 99.9% is extrasynaptic and separates the muscle fiber membrane from endomysial connective tissue space. When motor axons regenerate to denervated muscles after nerve injury, they preferentially reinnervate the original synaptic sites, and differentiate there into nerve terminals. We found that axons also preferentially form contacts and differentiate at synaptic sites on BL sheaths from which muscle fibers have been removed, implicating components of BL in the remarkable topographic specificity of reinnervation (Sanes et al., 1978; Glicksman and Sanes, 1983). Conceptually similar experiments, in which myotubes regenerated within BL sheaths in the absence of nerves, demonstrated that synaptic sites on BL sheaths can influence the differentiation of postsynaptic structures as well (Burden et al., 1979; McMahan and Slater, 1984). Together, these experiments suggested the existence of synapse-specific components in muscle fiber BL that axons and myotubes recognize and to which they respond (reviewed in Sanes, 1989).

Immunohistochemical methods have demonstrated molecular correlates of these functional specializations. Whereas antibodies to several components of BL (laminin, collagen

D. Hunter's present address is Neuroscience Laboratories, Tufts University School of Medicine, Boston, MA 02111.

1. *Abbreviation used in this paper*: BL, basal lamina.

IV, heparan sulfate proteoglycan, and entactin) stain both synaptic and extrasynaptic BL (Sanes, 1982; Sanes and Chiu, 1983; Eldridge et al., 1986), other antibodies stain synaptic BL selectively (Sanes and Hall, 1979; Sanes and Chiu, 1983; Anderson and Fambrough, 1983; Fallon et al., 1985), and still others stain extrasynaptic BL more intensely than synaptic BL (Sanes, 1982; Sanes and Chiu, 1983). Recently, molecular cloning has revealed that one "synaptic antigen," which we call synaptic laminin or s-laminin, is a homologue of the B1 subunit of laminin (Hunter et al., 1989*a*). Laminin itself (a trimer of A, B1, and B2 subunits, all of which are homologous to each other) is known to be adhesive for neurons of many types, and to be a potent promoter of neurite outgrowth (reviewed in Sanes, 1989). We found that a recombinant fragment of s-laminin is selectively adhesive for motoneurons (Hunter et al., 1989*b*) and inhibits neurite outgrowth (Weis J., D. Hunter, and J. Sanes, unpublished data). More recently, the M subunit of merosin, a molecule present in muscle fiber BL but absent from many other BLs (Lievo and Engvall, 1988), has been found to be a homologue of the laminin A subunit (Ehrig et al., 1990). Neurons also interact with merosin (Engvall E., and M. Manthorpe, unpublished data). Thus, an intriguing possibility is that muscles use a variety of laminin-like molecules with different bioactivities to regulate axonal behavior in complex ways.

The finding that muscles contain several isoforms of laminin has motivated the two new sets of histological studies reported here. First, we examined the distribution of s-laminin, merosin M, and the laminin subunits (A, B1, and B2) in muscle and in a set of nonmuscle BLs. Specifically, we asked which subunits are enriched in or excluded from synaptic BL, and whether subunits occur in specific combinations that might suggest their mode of association. Second, we studied the distribution of another major component of BLs, collagen IV, in muscle. Here, we took advantage of recent studies on the targets of autoimmune attack in human Goodpasture syndrome; this work has culminated in the discovery of novel collagen IV-like chains called α3(IV), α4(IV), and α5(IV) to distinguish them from the previously characterized collagen chains α1(IV) and α2(IV) (Butkowski et al., 1985, 1987; Wieslander et al., 1984; Hostikka et al., 1990; reviewed in Hudson et al., 1989). Collagens composed of α1(IV) and α2(IV) chains, like laminin, are present in most BLs, while α3(IV) and α4(IV), like s-laminin and merosin, are concentrated in a limited subset of BLs (Pusey et al., 1987; Kleppel et al., 1989*a*,*b*; Butkowski et al., 1989). It was therefore natural to ask whether any of the chains of collagen IV are concentrated in or excluded from synaptic BL. Together, our results provide evidence for differences between synaptic and extrasynaptic BL, and among BLs in general, so numerous that we propose to revise the view that all BLs contain an identical set of major structural components.

Materials and Methods

Antibodies

The antibodies used in this study are listed in Table I. The locations of epitopes recognized by antibodies to laminin subunits and homologues are shown in Fig. 1. Immunoblotting analyses of antibodies that recognize rat antigens, performed as described in Hunter et al. (1989*a*), are illustrated in Fig. 2.

Table I. Antibodies Used in this Study

Antigen	Antibody designation	Antibody type	Immunogen source
S-Laminin	C1	Mouse mAb	Rat
	C4	Mouse mAb	Cow
	D5	Mouse mAb	Cow
	D7	Mouse mAb	Cow
	G1	Mouse mAb	Recombinant
	GP1	Guinea pig serum	Recombinant
	R49	Rabbit serum	Recombinant
Laminin A	4C7	Mouse mAb	Human
	11D5	Mouse mAb	Human
Laminin B1	3E5	Mouse mAb	Human
	4E10	Mouse mAb	Human
Laminin B2	2E8	Mouse mAb	Human
	D18	Mouse mAb	Rat
Merosin M	2D10	Mouse mAb	Human
	2G9	Mouse mAb	Human
	5H2	Mouse mAb	Human
Collagen α1(IV)	6	Mouse mAb	Cow
	102	Rat mAb	Human
Collagen α2(IV)	M24-1	Sheep serum	Human
Collagen α1,2(IV)	M3F7	Mouse mAb	Human
Collagen α3(IV)	975	Sheep serum	Human
	17	Mouse mAb	Cow
Collagen α4(IV)	977	Sheep serum	Human
	85	Mouse mAb	Human

Laminin. Laminin purified from the murine Engelbreth-Holm-Swarm (EHS) tumor was purchased from Miles Laboratories Inc. (Elkhart, IN) and an antiserum was prepared to it in a rabbit. A second rabbit anti-mouse EHS laminin antiserum was purchased from Polysciences, Inc. (Warrington, PA). Both antisera recognized the A and B subunits of rat tumor-derived laminin (Telios, La Jolla, CA) on immunoblots. The B1 and B2 subunits are not well resolved on these blots, but the breadth of the band indicated that the sera recognize both subunits. These sera also stained laminin B1 and/or B2 but not s-laminin in extracts of rat kidney basement membrane (Fig. 2). Previous publications describe the production and characterization of mAbs 4C7 and 11D5 to laminin A; 3E5 and 4E10 to laminin B1; and 2E8 to laminin B2 (Dillner et al., 1988; Engvall et al., 1986; Gehlsen et al., 1989; Engvall et al., 1990). A second mAb to laminin B2, D18, arose from a fusion designed to produce mAbs to s-laminin (see below); both D18 and 2E8 recognized laminin B2 but not s-laminin on immunoblots (Fig. 2).

S-Laminin. mAbs C1, C4, D5, and D7 to s-laminin, have been described previously (Sanes and Chiu, 1983; Hunter et al., 1989*a*). All four mAbs are of the IgG1 class, and all four recognize recombinant s-laminin (Hunter et al., 1989*a*; Green, T., D. D. Hunter, and J. R. Sanes, unpublished data). When large amounts of laminin were loaded onto gels, and the resulting immunoblots overdeveloped, C4, but not D5 or D7, cross-reacted faintly with the laminin B1 subunit; this cross-reaction was not detectable on immunoblots of kidney extract, presumably because of the relative paucity of laminin in these samples. In addition to these mAbs, which were raised to BL extracts, we prepared several antibodies to s-laminin fragments produced in bacteria. The pET RK36 fusion protein, comprising the COOH-terminal 20 kD of s-laminin was purified as described by Hunter et al. (1989*b*), and used to immunize a rabbit (R49) and a guinea pig (GP1). Both antisera recognized authentic s-laminin but not laminin on immunoblots (Fig. 2). A fusion protein comprising the NH_2-terminal 30% of s-laminin, encoded by the cDNA RM12 (Hunter et al., 1989*a*), was prepared in a similar fashion, and used to immunize mice, resulting in the production of mAb G1, which also recognized authentic s-laminin but not laminin on immunoblots. By using overlapping fusion proteins, we have mapped the epitope recognized by G1 to the NH_2-terminal 15% of s-laminin (T. Green and J. R. Sanes, unpublished data).

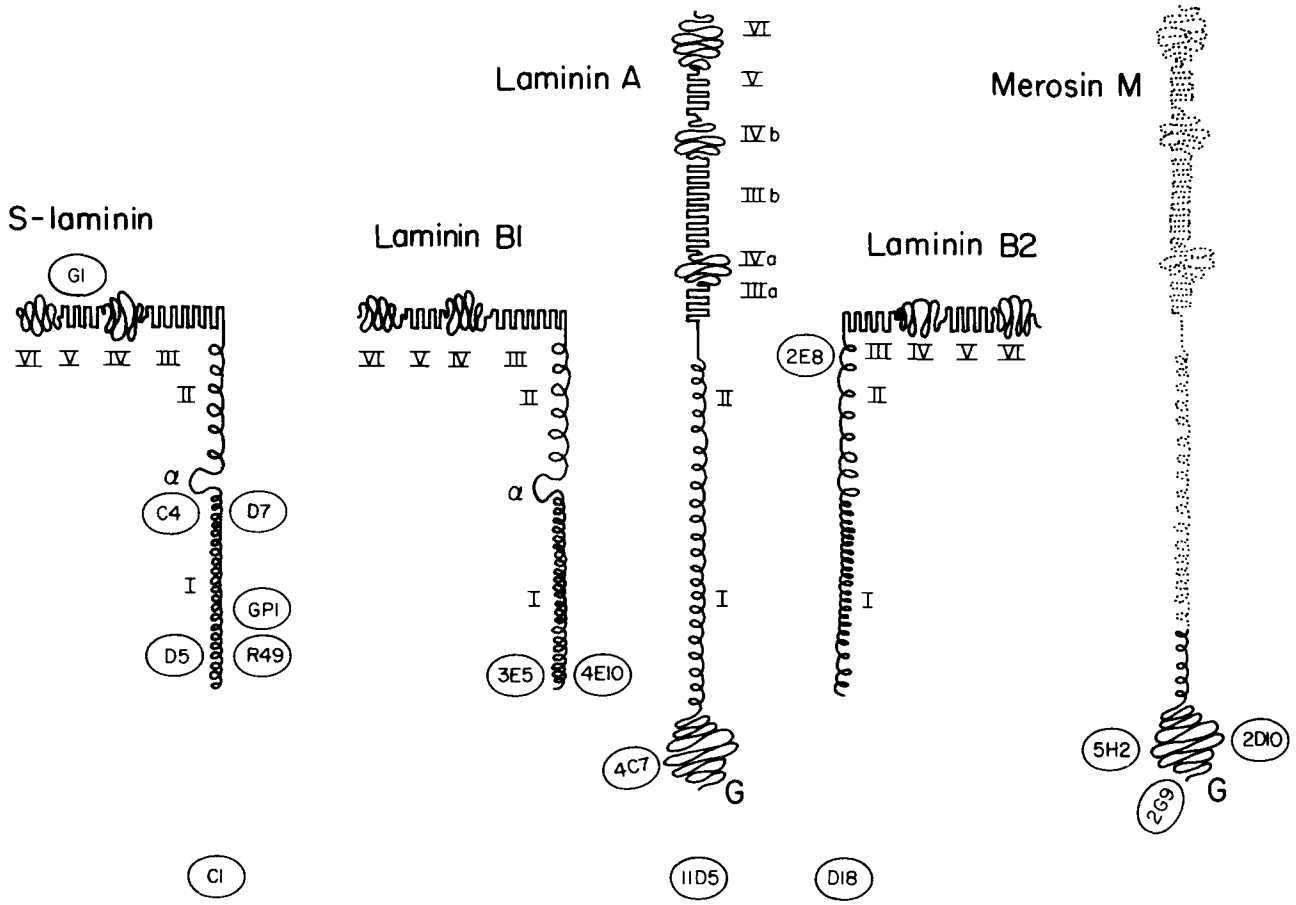


Figure 1. Location of epitopes recognized by antibodies to laminin subunits and homologues. Domain structures of the polypeptides are from Sasaki et al. (1987, 1988), Hunter et al. (1989*a*), and Ehrig et al. (1990). Dashed line denotes region of merosin M that has not been sequenced. Characterization of the antibodies is described in Materials and Methods. Antibodies whose epitopes have not been mapped are shown beneath the appropriate subunit.

Merosin. Three mAbs to human merosin M, 2G9, 2D10, and 5H2 were produced in mice. All three antibodies are IgGs, and all react with a COOH-terminal fragment of merosin M (Leivo and Engvall, 1988; Leivo et al., 1989).

Collagens IV. Antisera to the NC1 fragments of human collagen chains α2(IV) (serum No. M24-1), α3(IV) (serum No. 975), and α4(IV) (serum No. 977), were raised in sheep as described by Butkowski et al. (1989) and affinity purified on nitrocellulose-bound antigen (Robinson et al., 1988). The following mAbs to NC1 fragments of collagen IV chains were also used: mAb 6, mouse anti-bovine α1(IV); mAb 102, rat anti-human α1(IV); mAb 17, mouse anti-bovine α3(IV); and mAb 85, mouse anti-human α4(IV) (Kleppel et al., 1989*a*; Saus et al., 1988). Finally, we used mAb M3F7 (ICN Immunochemicals, Lisle, IL), which binds to the triple helical domain of collagen α1 and α2 chains (Foellmer et al., 1983).

Second Antibodies. Fluorescein-conjugated goat antibodies to mouse,

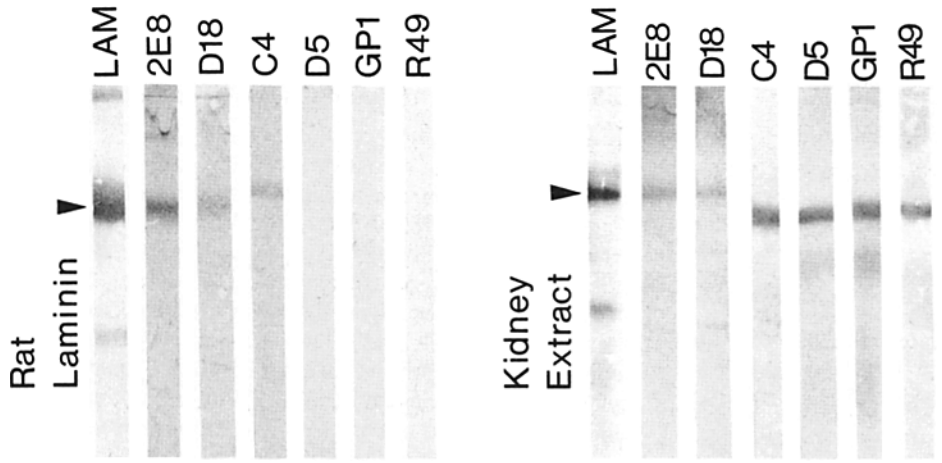


Figure 2. Immunoblot analysis of antibodies that recognize rat laminin or s-laminin. Purified rat laminin (*left*) or a BL-rich extract of adult rat kidney (*right*; see Hunter et al., 1989*a*) was submitted to gel electrophoresis under reducing conditions, and blotted as described in Hunter et al. (1989*a*, *b*). The antibodies used, and the subunits they recognize, are enumerated in Materials and Methods. The position of a 200-kD standard (myosin) is indicated by arrowheads.

Table II. Staining of Basal Laminae by Antibodies to Laminin Subunits and Homologues

			Staining							
			Muscle fibers		Nerve trunks		Blood vessels		Kidney	
Chain	Antibody	Species	Synaptic	Extrasynaptic	Perineurial	Endoneurial	Arterial	Capillary	Glomerular	Tubular
S-laminin	C1	Rat	+	−	+	−	+	−	+	−
		Human	+	−	+	−	+	−	+	−
	C4	Rat	+	−	+	−	+	−	+	−
		Rabbit	+	−	+	−	+	−	+	−
		Guinea Pig	+	−	+	−	+	−	+	−
		Human	+	±	+	−	+	−	+	−
	D5	Rat	+	±	+	±	+	−	+	−
		Rabbit	+	±	+	±	+	−	+	−
		Guinea Pig	+	±	+	±	+	−	+	−
	D7	Rat	+	−	+	−	+	+	+	−
		Rabbit	+	−	+	−	+	+	+	−
		Guinea Pig	+	−	+	−	+	+	+	−
Laminin A	4C7	Rabbit	+	−	±	−	+	?	+	+
		Guinea Pig	+	−	±	−	+	?	+	+
		Human	+	−	±	−	+	?	+	+
	11D5	Human	+	−	±	−	+	?	+	+
Laminin B1	4E10	Rabbit	−	+	−	+	−	?	−	+
		Human	−	+	−	+	−	?	−	+
	3E5	Human	−	+	−	+	−	?	−	+
Laminin B2	2E8	Rat	+	+	+	+	+	+	+	+
		Guinea Pig	+	+	+	+	+	+	+	+
		Human	+	+	+	+	+	+	+	+
	D18	Rat	+	+	+	+	+	+	+	+
		Guinea Pig	+	+	+	+	+	+	+	+
		Human	+	+	+	+	+	+	+	+
Merosin M	2G9	Rabbit	+	+	−	+	−	−	−	−
		Human	+	+	−	+	−	−	−	−
	2D10	Rabbit	+	+	−	+	−	−	−	−
		Human	+	+	−	+	−	−	−	−
	5H2	Rabbit	+	+	−	+	−	−	−	−
		Human	+	+	−	+	−	−	−	−

All antibodies were tested on sections of rat, rabbit, guinea pig, and human kidney and muscle. Species in which staining was observed are listed. +, clear, consistent staining. ±, dim but unequivocal staining. −, negative or equivocal staining. ? indicates variability among sections.

rabbit, rat, and sheep IgG were purchased from Boehringer Mannheim Diagnostics, Inc. (Indianapolis, IN) and Sigma Chemical Co. (St. Louis, MO).

Histology

Adult female Holtzman rats and Hartley guinea pigs were obtained from Sasco, Inc. (Omaha, NE); and New Zealand white rabbits from Boswell's Bunny Farm (Pacific, MO). Rat muscles were denervated by cutting the phrenic nerve intrathoracically or the sciatic nerve in midthigh, both in ether-anesthetized animals. The lower leg muscles of a rabbit were denervated by cutting the sciatic nerve of a halothane-anesthetized animal in midthigh. Incisions were closed with surgical staples and tissues were collected 10 d after surgery.

Diagnostic biopsies of human muscles were provided by Kenneth Kaiser and Michael Brooke, Department of Neurology, Washington University Medical Center, St. Louis, MO. Studies reported here used sections from 4-, 16-, 33-, and 71-yr-old males and 2- and 4-yr-old females. These biopsies had been obtained for evaluation of muscle weakness, but they revealed no histological or histochemical abnormalities upon examination by Dr. Brooke. No age-related differences among specimens were observed with the antibodies used. Pieces of human kidney, obtained at autopsy, were provided by Dr. Eric Simon, Department of Medicine, Washington University Medical Center.

Tissues were sectioned and stained as described in Covault and Sanes (1986). Briefly, unfixed specimens were frozen in liquid N_2-cooled isopentane and cross sectioned at 2–6 μm in a cryostat. Sections were incubated with antibody (~1 h), washed, reincubated with fluorescein-second antibody (~1 h), washed again, mounted in glycerol-para-phenylenediamine, and observed with epifluorescent illumination. Rhodamine-α-bungarotoxin was mixed with the second antibody, to mark synaptic sites in sections of muscle.

Results

Laminin Isoforms at the Neuromuscular Junction

S-Laminin. We showed previously that mAbs C1, C4, D5, and D7, all of which recognize s-laminin, stain synaptic BL more intensely than extrasynaptic BL on rat muscle fibers (Sanes and Chiu, 1983; Hunter et al., 1989*a*). We have now extended this study in two ways. First, we stained cryostat sections of rabbit, guinea pig, and human muscle with the anti-s-laminin mAbs. Synaptic sites were identified by co-staining with rhodamine-α-bungarotoxin, which binds to acetylcholine receptors concentrated in the postsynaptic membrane (see Sanes and Hall, 1979; Covault and Sanes, 1986). At least two mAbs cross reacted with each species tested. In each case, s-laminin-like immunoreactivity was

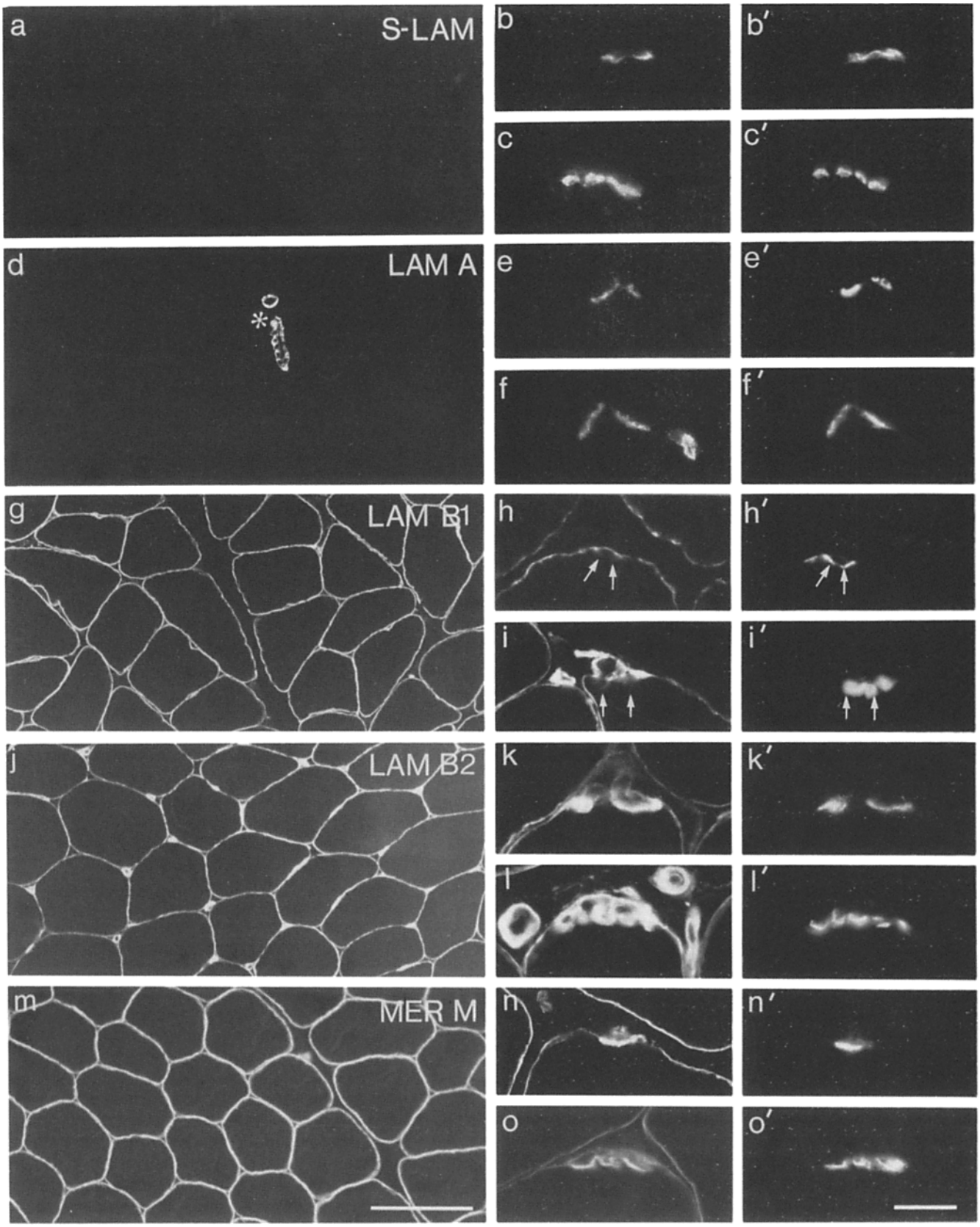


Figure 3. Staining of synaptic and extrasynaptic BL of skeletal muscle fibers by antibodies to s-laminin (*a–c*), laminin A (*d–f*), laminin B1 (*g–i*), laminin B2 (*j–l*), and merosin M (*m–o*). Left panels show, at low magnification, that extrasynaptic BL is stained by antibodies to B1, B2, and merosin but not by antibodies to s-laminin or A. Right panels show, at higher magnification, staining of synaptic sites, identified by counterstaining with rhodamine-α-bungarotoxin (*b–o* are photographed with fluorescein optics; *b'–o'* are the same fields photographed with rhodamine optics). S-Laminin and laminin A are concentrated at synaptic sites, B1 is absent from synaptic sites, and B2 and merosin are present both synaptically and extrasynaptically. Asterisk in *d* indicates a laminin A-positive blood vessel (see Fig. 6). Antibodies used and species stained are: (*a* and *b*) D7, rabbit; (*c*) C1, human; 1 (*d* and *e*) 4C7, rabbit; (*f*) 4C7, guinea pig; (*g* and *h*) 4E10, rabbit; (*i*) 3E5, human; (*i* and *k*) D18, human; (*l*) 2E8, human; (*m*) 2D10, human; (*n*) 2D10, rabbit; (*o*) 2G9, rabbit. Bars: (*m*) 50 μm; (*o'*) 20 μm.

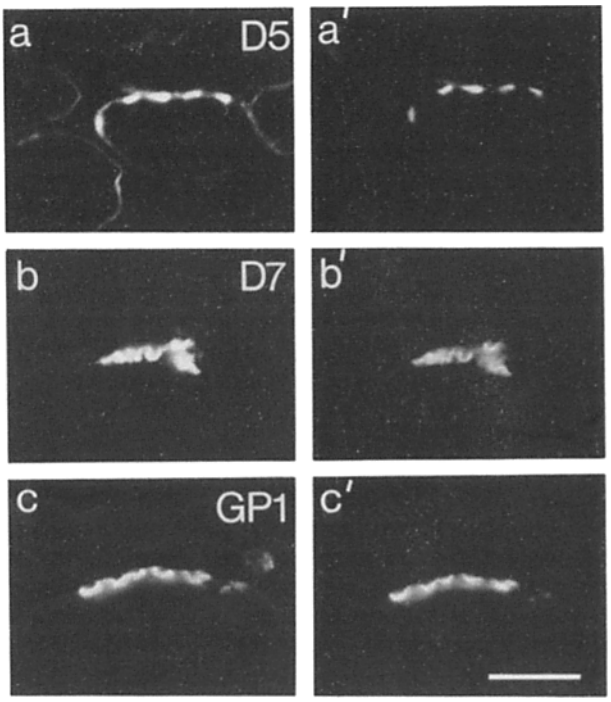


Figure 4. Synaptic sites in rat muscle are selectively stained by monoclonal (*a*, D5; *b*, D7) and polyclonal (*c*, GP-1) antibodies to s-laminin. However, mAb D5 also stains extrasynaptic BL faintly. *a'–c'* are same fields as *a–c*, counterstained with rhodamine-α-bungarotoxin. Bar, 20 μm.

concentrated at synaptic sites (Table II and Fig. 3, *a–c*). Thus, the synaptic localization of s-laminin is not peculiar to rat, but is apparently a general feature of mammalian muscle.

Second, we tested several new antibodies to s-laminin. Although our original set of mAbs all stained synaptic BL more intensely than extrasynaptic BL, two (C4 and D5) stained extrasynaptic BL to a limited extent, while the other two (C1 and D7) did not (Fig. 4, *a* and *b*). Some differences among antibodies were also seen in nonmuscle tissues (see below). These differences could reflect the presence of cross-reactive molecules in some BLs, selective exposure or masking of some epitopes (e.g., by variable glycosylation), or variations in primary structure (e.g., as a consequence of alternative splicing). To address these possibilities, we prepared polyclonal antisera to a COOH-terminal fragment in a rabbit and a guinea pig, reasoning that they would recognize a broad array of epitopes. Both antisera recognized s-laminin but not laminin on immunoblots (Fig. 2), and both stained synaptic sites far more intensely than extrasynaptic areas on cryostat sections of muscle (Fig. 4 *c*). In addition, because the polyclonal antisera and at least three of the four mAbs (the C1-binding site has not been localized) recognize epitopes located on the COOH-terminal 40% of the molecule (Fig. 1), we tested a mAb, G1, that recognizes an epitope on the NH_2-terminal 15% of s-laminin (Fig. 1 and see Materials and Methods). Like the other antibodies to s-laminin, G1 stained synaptic sites intensely and extrasynaptic areas weakly if at all (not shown). Together, these immunohistochemical results all support our conclusion that s-laminin is concentrated at synaptic sites.

Laminin. Polyclonal antisera to laminin stain both synaptic and extrasynaptic portions of muscle fiber BL (Sanes, 1982). However, these antisera recognize all three of the subunits contained in the tumor-derived laminin to which they were raised, A, B1, and B2 (Fig. 2). One cannot, therefore, conclude that any individual subunit is present both synaptically and extrasynaptically. We therefore tested a panel of mAbs that recognize individual subunits. Because direct biochemical identification of the subunits is not feasible in situ, we used two mAbs to each subunit and stained muscle from several species to enhance our confidence in the immunohistochemical results. In fact, staining of all species with both antibodies yielded consistent results for each subunit.

Laminin A is concentrated at synaptic sites and is not detectable in extrasynaptic areas; staining is precisely coincident with that of rhodamine-α-bungarotoxin, and clearly reflects the selective association of this subunit with synaptic BL (Fig. 3, *d–f* and Table II). In contrast, laminin B1 is concentrated in extrasynaptic areas and is present at considerably lower levels, if at all, at synaptic sites (Fig. 3, *g–i*). The close apposition of B1-positive Schwann cell BL to synaptic BL makes it difficult to determine whether or not some synaptic sites are faintly B1 positive in human and rabbit muscle. However, staining by the anti-B1 mAbs used here is strikingly similar to that previously seen in rat muscle with the anti-laminin mAbs C21 and C22. These were shown to stain extrasynaptic but not synaptic BL by both light and electron microscopic immunohistochemical methods (Sanes and Chiu, 1983; Chiu and Sanes, 1984; Gatchalian, C., A. Y. Chiu, and J. R. Sanes. 1985. *J. Cell Biol.* 101[No. 5, Pt. 2]:89a[Abstr.]). MAbs C21 and C22 do not react with denatured laminin on immunoblots, and have therefore not been assigned to an individual subunit; however, in light of the present results, we suggest that they recognize a B1-dependent epitope. Finally, antibodies to laminin B2 stained both synaptic and extrasynaptic BL, although synaptic sites were slightly more intensely stained than extrasynaptic areas (Fig. 3, *j–l*). The enhanced staining of synaptic sites probably reflects the presence of struts of BL that extend into invaginations of postsynaptic membrane called junctional folds (e.g., Sanes et al., 1978). Because junctional folds are present only at synaptic sites there is two to threefold more BL per unit circumference in synaptic areas than extrasynaptically (see Sanes and Chiu, 1983, for details). Thus, laminin B2 is probably present at similar levels in synaptic and extrasynaptic BL.

Merosin. Three mAbs were available that recognize merosin M, a novel homologue of laminin A, in human and rabbit tissue (Fig. 1 and Table I). Previous work has established that merosin is present in extrasynaptic areas of skeletal muscle fibers (Lievo and Engvall, 1988), but we did not know whether it was also present in synaptic areas. All antibodies to merosin M clearly stained both synaptic and extrasynaptic areas of both human and rabbit muscle fibers (Table II and Fig. 3, *m–o*). In general, synaptic areas were stained somewhat more intensely than extrasynaptic areas. However, as for laminin B2 (see above), the difference presumably reflects the greater quantity of BL at these sites. In any event, it is clear that merosin M is present throughout muscle fiber BL.

Laminin Isoforms in Nonmuscle Cells

Sections of skeletal muscle generally contain structures

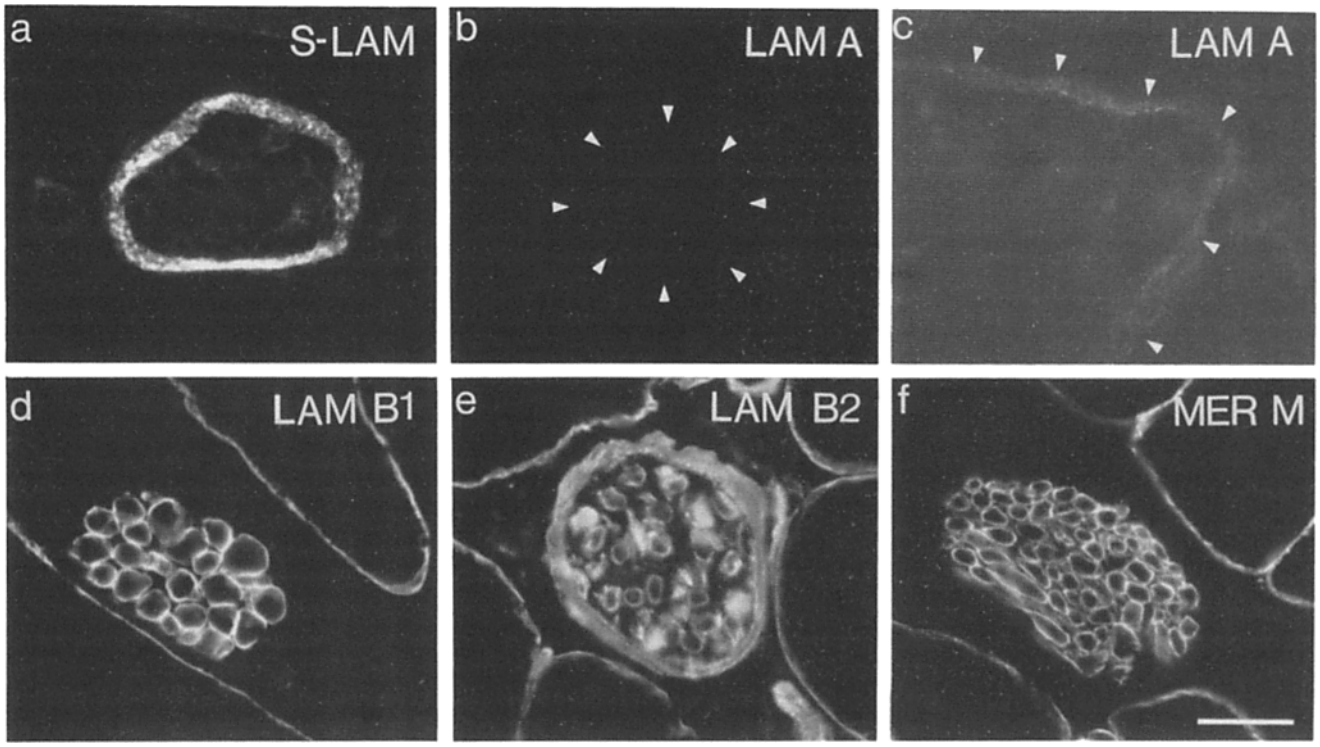


Figure 5. Distribution of laminin subunits and homologues in intramuscular nerve. (*a*) Anti-s-laminin stains perineurial BL more intensely than endoneurial BL (C4, rabbit). (*b*) Anti-laminin A stains nerve poorly (4C7, rabbit); outline of nerve indicated by arrowheads. However, faint staining of perineurial BL by anti-laminin A is consistently detectable and can be seen with long photographic exposure (*c*). (*d*) Anti-laminin B1 stains endoneurial but not perineurial BL (4E10, rabbit). (*e*) Anti-laminin B2 stains both endoneurial and perineurial BL (2E8, human). (*f*) Anti-merosin stains endoneurial but not perineurial BL (2G9, rabbit). Bar, 20 μm.

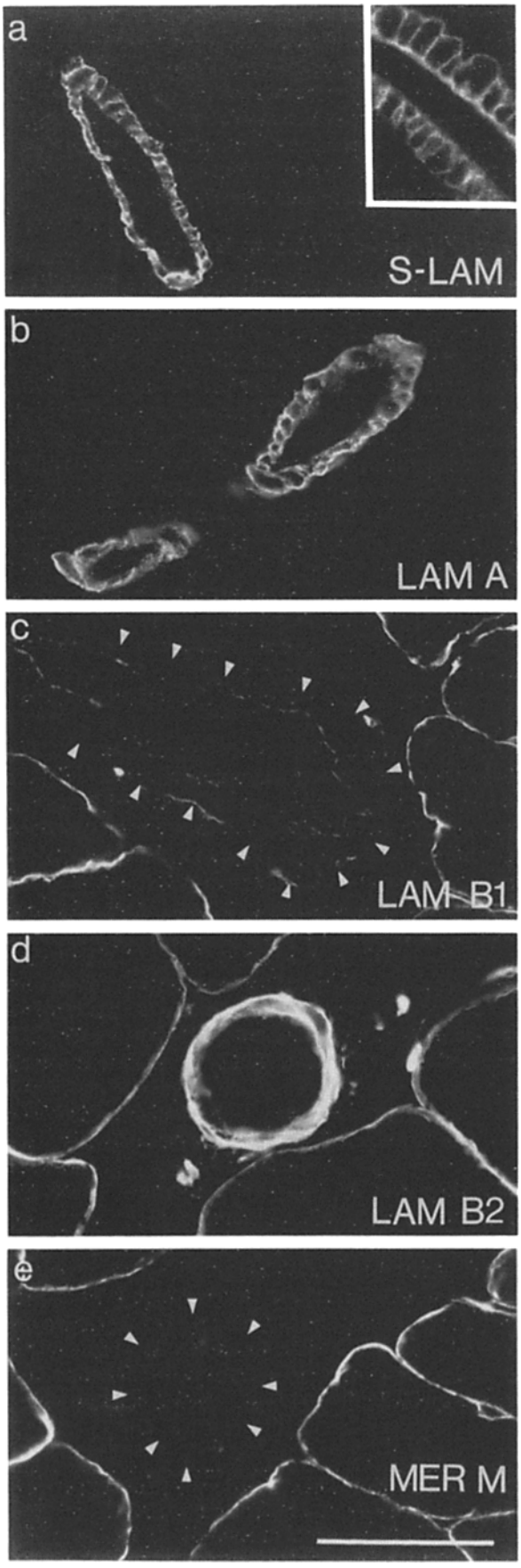
other than muscle fibers, including intramuscular nerve branches. Because our work is motivated by an interest in molecules that axons encounter, we routinely determined whether or not nerve-associated BLs were stained by each antibody. In addition, we assessed the staining of intramuscular blood vessels, which frequently accompany nerve branches. Finally, because we previously found a more restricted distribution of s-laminin than of laminin in kidney (Sanes and Chiu, 1983; Hunter et al., 1989*a*), we tested the subunit-specific mAbs on sections of kidney.

Nerve. In peripheral nerves, each axon is ensheathed by myelinating or nonmyelinating Schwann cells, and each axon–Schwann cell unit is surrounded by a BL called the endoneurial BL. Fascicles of axon–Schwann cell units are encircled by a multilamellar cellular sheath, called the perineurium, which also bears BL. Antibodies to s-laminin stained perineurial BL far more intensely than endoneurial BL (Fig. 5 *a*). In contrast, antibodies to laminin B1 and to merosin M stained endoneurial BL but not perineurial BL (Fig. 5, *d* and *f*). Antibodies to laminin B2 stained both endoneurial and perineurial BL with similar intensity (Fig. 5 *e*), whereas antibodies to laminin A did not stain either BL intensely (Fig. 5 *b*). At high magnification, however, weak staining of perineurial BL by anti-laminin A was detectable (Fig. 5 *c*). While considerably weaker than staining of end-plates or arterioles (see below) in the same sections, the staining of perineurium by antibodies to laminin A was consistent and specific.

Blood Vessels. The arterial vessels that accompany intramuscular nerves provided further evidence that laminin subunits are differentially distributed. All antibodies to s-laminin and to laminin A stained the BL of small arteries and arterioles intensely (Fig. 6, *a* and *b*), describing a pattern which suggested that smooth muscle BL (see Fawcett, 1986) is immunoreactive (Fig. 6 *a*, *inset*). These vessels were consistently the most intensely s-laminin- and laminin-A–immunoreactive structures in sections of muscle. Antibodies to laminin B2 also stained arterial BL (Fig. 6 *d*). In contrast, antibodies to laminin B1 and to merosin M failed to stain arterial vessels in any species tested (Fig. 6, *c* and *e* and Table II).

The intramuscular vasculature consists of capillaries and venules as well as arterial vessels. Capillary BL was stained by both antibodies to laminin B2, but not by any of the antibodies to merosin M (Fig. 3, *j* and *m*). Interestingly just one of the mAbs to s-laminin, D7, stained capillary BL, and antibodies to laminin A and B1 stained capillary BL in some sections but not in others (Table II). Possible explanations for this variability include the presence of other laminin-like subunits with which some mAbs react, real interindividual variation in the composition of capillary BL, and the existence of multiple subtypes of capillaries. In general, the BL of capillaries and of venules exhibited similar patterns of immunoreactivity in any given individual or species.

Kidney. Early evidence of the site-restricted expression of laminin isoforms was the staining of glomerular but not tubular BL by antibodies now known to recognize s-laminin (Sanes and Chiu, 1983). In addition, several mAbs to laminin



have been reported to stain tubular and glomerular BL differentially (Wan et al., 1984; Leu et al., 1986; Horikoshi et al., 1988; Abrahamson et al., 1989; Gatchalian, C., A. Y. Chiu, and J. R. Sanes. 1985. *J. Cell Biol.* 101[No. 5, Pt. 2]:89a[Abstr.]). In view of these results, and new results on collagen isoforms presented below, we compared the distribution of the laminin subunits to that of s-laminin in kidney. In rabbit, guinea pig, and human, as in rat, antibodies to s-laminin stained glomerular BL far more intensely than tubular BL (Fig. 7 *a* and Table II). In contrast, antibodies to laminin B1 stained tubular BL but did not react detectably with glomerular BL; glomerular mesangium was weakly laminin-B1-positive in some species (Fig. 7 *c*). This pattern is similar to that seen previously with several mAbs to laminin (Sanes and Chiu, 1983; Leu et al., 1986; Abrahamson et al., 1989), some of which we now believe to recognize B1 (4E10, C21, and C22; see above). Antibodies to laminin B2 stained both glomerular and tubular BL (Fig. 7 *d*), while antibodies to merosin stained neither (Fig. 7 *e*). Finally, anti-laminin A stained both glomerular and tubular BL (Fig. 7 *b*), although the staining was fainter than that of synaptic sites or blood vessels. In a previous study, only a subset of kidney BLs were reported to be stained by an antibody to the E3 fragment of laminin A (Ekblom et al., 1990); we have no explanation for the discrepancy, but note that low levels of laminin A mRNA have been found in both tubules and glomeruli by in situ hybridization (Laurie et al., 1989). Thus, in kidney as in muscle, different BLs contain different combinations of laminin-related proteins.

Laminin Isoforms in Denervated Nerve and Muscle

After nerve injury, axons regenerate through denervated nerve trunks and into denervated muscles, where they form new neuromuscular junctions. The axons frequently grow along BLs, and evidence obtained both in vivo and in vitro indicates that laminin is an important promoter of their extension (reviewed in Sanes, 1989). It was therefore of interest to test the possibility that denervation affected the distribution of one or more laminin-like proteins. Accordingly, we stained sections of 10-d denervated rat and rabbit muscle and nerve with the panel of antibodies described above. Diaphragm and soleus muscles were studied in rat, soleus and extensor digitorum longus in rabbit, and intramuscular nerve branches and sciatic nerves in both species. In all cases, similar results were obtained: denervation had no consistent or major effect on the staining of synaptic, extrasynaptic, endoneurial, or perineurial BL by any antibody (Fig. 8; see also Sanes et al., 1986). Thus, although effects of denervation on levels of laminin synthesis might not be detected by

Figure 6. Distribution of laminin subunits and homologues in intramuscular arterioles. (*a*) Anti-s-laminin (D7, rabbit). (*b*) Anti-laminin A (4C7, rabbit). (*c*) Anti-laminin B1 (4E10, rabbit). (*d*) Anti-laminin B2 (2E8, human). (*e*) Anti-merosin (2G9, rabbit). Arteriole BL is s-laminin, laminin A, and laminin B2 positive, but laminin B1 and merosin negative. Arrowheads indicate outlines of arterioles in *c* and *e*; faint staining of vessel in *c* is autofluorescence of the internal elastic lamina. Muscle fibers are stained in *c–e*. Inset shows loop-like staining, which presumably corresponds to smooth muscle BL, and is characteristic of laminin A as well as s-laminin. Bar: (*a–e*) 50 μm; (*inset*) 30 μm.

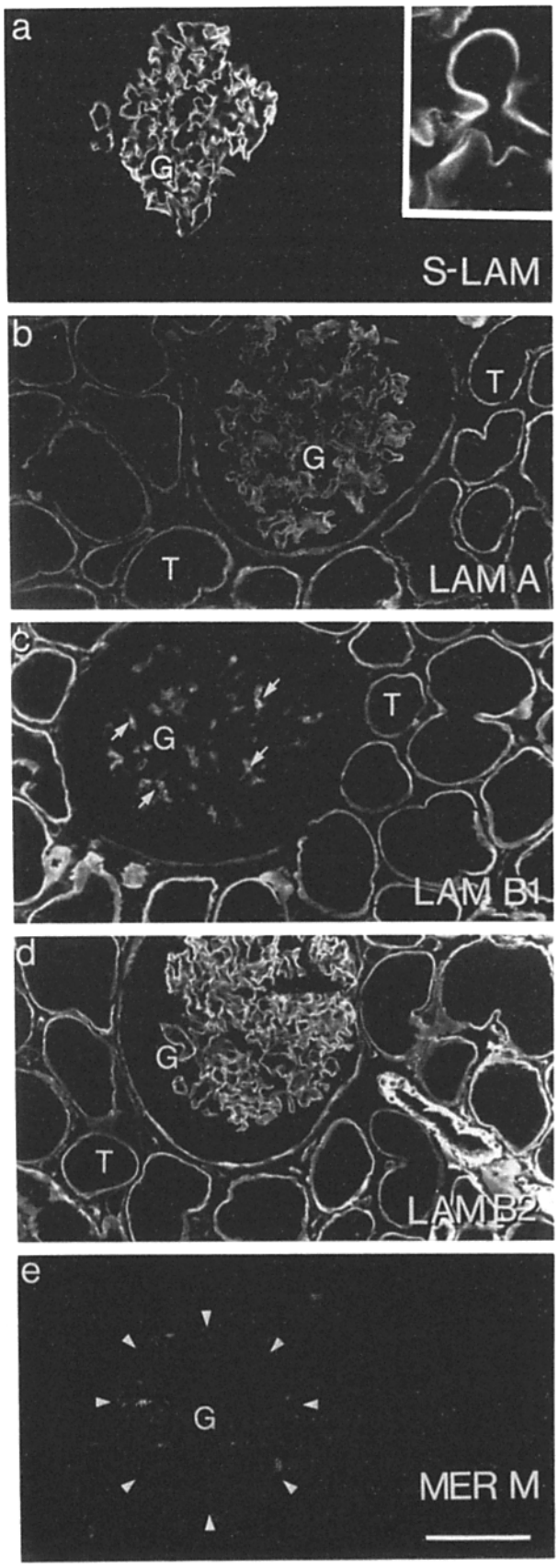


Figure 7. Distribution of laminin subunits and homologues in kidney BLs. (*a*) Anti-s-laminin (D5, rabbit). (*b*) Anti-laminin A (4C7, human). (*c*) Anti-laminin B1 (3E5, human). (*d*) Anti-laminin B2 (2E8, human). (*e*) Anti-merosin M (2D10, human). Glomerular (*G*) BL is stained by antibodies to s-laminin, laminin A, and laminin B2; inset shows glomerular BL stained by mAb C4 at higher power. Tubular (*T*) BL is stained by antibodies to laminin A, B1, and B2. Anti-laminin B1 also stains glomerular mesangium (*arrows* in *c*). Arrowheads outline glomerulus in *e* which appears faintly merosin M positive. Bar: (*a–e*) 50 μm; (*inset*) 20 μm.

our methods, it does not appear that changes in the distribution of these molecules affect the ability of nerve to promote axon outgrowth or of denervated muscle to accept synapses. Particularly noteworthy is the lack of effect of denervation on the apparent abundance of laminin A: this subunit is thought to bear a neurite outgrowth-promoting site (Tashiro et al., 1989), but is not detectable in endoneurial BL along which axons preferentially regenerate (Sanes, 1989).

Collagen IV Isoforms in Human Muscle

In human kidney, the collagen α3(IV), α4(IV), and α5(IV) chains are concentrated in glomerular BL, as is s-laminin, although at least α3(IV) and α4(IV), unlike s-laminin, are also present in a subset of tubular BLs (Fig. 9 *a*; Kleppel et al., 1989*a*,*b*; Butkowski et al., 1989; Hostikka et al., 1990). In contrast, the α1(IV) and α2(IV) chains are present in all tubular and glomerular BL, as is laminin B2 (Fig. 9 *b*). Struck by these parallels, we examined the distribution of the type IV collagen chains in skeletal muscle. To this end, we stained tissues with antibodies specific for the α1(IV), α2(IV), α3(IV), and α4(IV) chains (Table I); discovery of the α5(IV) chain (Hostikka et al., 1990) was reported after completion of this work. Preliminary tests showed that the antibodies to α3(IV) and α4(IV) either did not stain rabbit, rat, hamster, guinea pig, or mouse kidney or, in a few instances, did stain but did not distinguish glomerular from tubular BL. We therefore limited this study to human muscle.

Monoclonal antibody 17, which is specific for α3(IV), selectively stained synaptic sites in human muscle (Figs. 9 *d* and 10 *a*). Extrasynaptic BL, as well as arterial, capillary, endoneurial, and perineurial BL were not detectably stained by this antibody, even after prolonged incubation (Fig. 9, *d* and *g*). In muscle spindles, motor endplates were mAb 17-positive, whereas the extrasynaptic surfaces of intrafusal fibers and spindle capsules (which are s-laminin-positive; Sanes and Chiu, 1983) were negative (Fig. 9 *h*). Similar results were obtained with polyclonal anti-α3(IV) (antiserum 975; Fig. 10 *b*), although staining by the antiserum was less intense than that obtained with mAb 17. Thus, α3(IV)-like immunoreactivity is concentrated at synaptic sites in human muscle.

In several instances, antibodies to the collagen α3(IV) or α4(IV) chains have been found to stain tissue only after sections were incubated with urea in acid (e.g., Butkowski et al., 1989; Kleppel et al., 1989*a*; Thorner et al., 1989); this treatment denatures the collagen chain to expose an epitope that is buried in the native molecule (Wieslander et al., 1985). Although mAb 17 and antiserum 975 stained synaptic sites and glomerular BL without denaturation, it was possible that additional deposits of α3(IV) were present extrasynaptically but masked in the sections. We therefore treated sections of kidney and muscle with 6 M urea, 100 mM glycine, pH 3.2 (Kleppel et al., 1989*a*) before staining

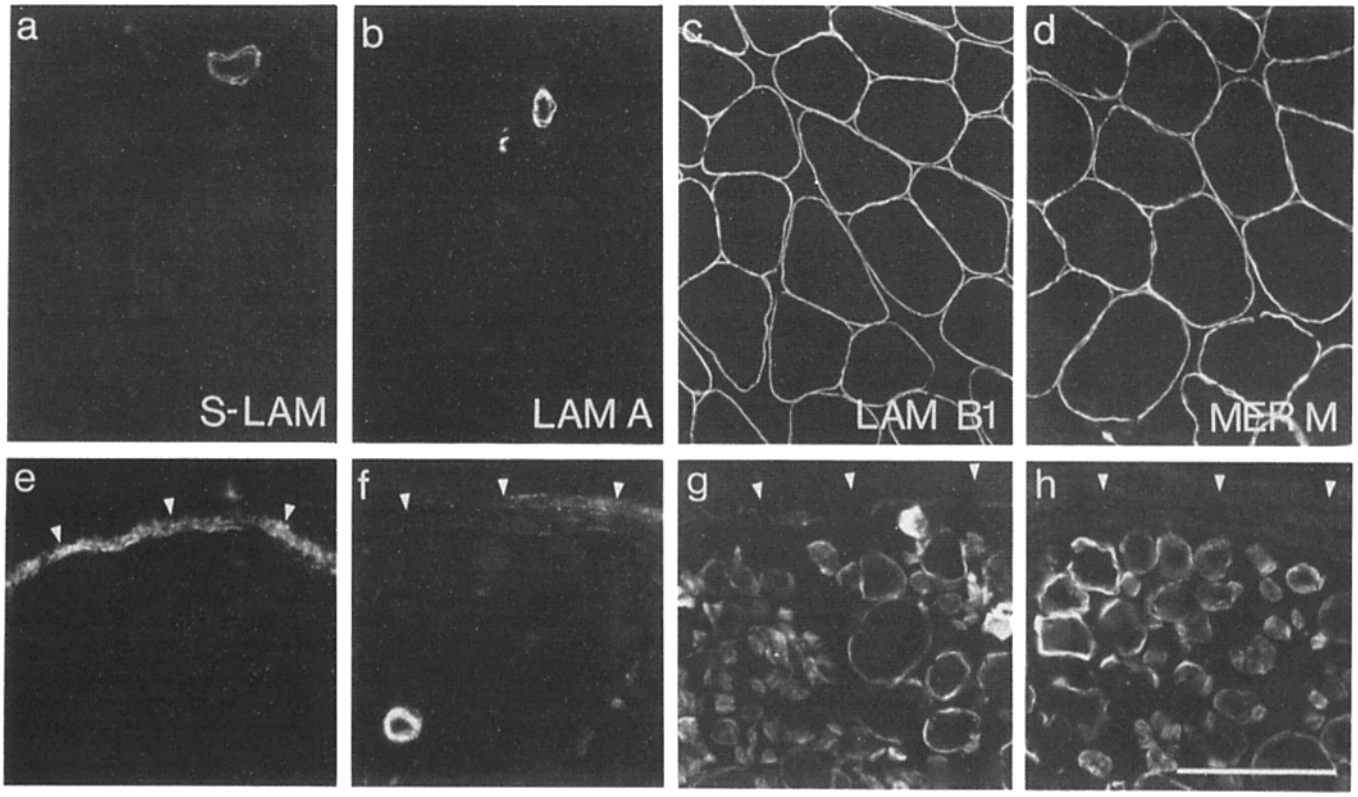


Figure 8. Distribution of laminin subunits and homologues in denervated rabbit muscle (*a–d*) and sciatic nerve (*e–h*). (*a*) Anti-s-laminin (D7). (*b* and *f*) Anti-laminin A (4C7). (*c* and *g*) Anti-laminin B1 (4E10). (*d*) Anti-merosin (2D10). (*e*) Anti-s-laminin (C4). (*h*) Anti-merosin (2G9). Perineureum is arrowed in *e–h*. Small blood vessels are visible in *a*, *b*, and *f*. Denervation has no obvious effect on the distribution of these antigens; compare with Figs. 3 and 5. Bar: (*a–d*) 100 μm; (*e–h*) 50 μm.

them with mAb 17. Treated sections were more intensely stained than native sections, but the specificity of staining was unaffected by denaturation, both in kidney (not shown) and in muscle (Fig. 10 *c*). In muscle, acid-urea treatment abolished reactivity of acetylcholine receptors with rhodamine-α-bungarotoxin (Fig. 10 *c*), but mAb 17–stained synaptic sites were readily identifiable by their size, shape, and arrangement. Furthermore, the enhanced clarity of staining after such treatment made it possible to discern fine striations corresponding to the short struts of BL that penetrate junctional folds in the postsynaptic membrane (Fig. 10 *d*; see Sanes, 1982 and Sanes and Chiu, 1983 for electron microscopic immunohistochemistry with antibodies to other BL antigens). Thus, although ultrastructural localization was infeasible in the present study, there is little doubt that antibodies to α3(IV) stain synaptic BL.

mAb 85, which recognizes the α4(IV) chain, stained untreated sections of kidney and muscle poorly. After treatment with acid-urea, however, mAb 85 stained glomerular BL and a subset of tubular BLs intensely in kidney (not shown) and synaptic BL intensely in muscle (Fig. 10 *e*). Similar results were obtained with serum 977, a polyclonal antiserum to collagen α4(IV). In both tissues, the distribution of α4(IV) was identical to that of α3(IV). This result is consistent with the suggestion that the α3(IV) and α4(IV) chains occur together within a single triple helical collagen molecule (Hudson et al., 1989).

Antibodies to collagen chains α1(IV) (mAbs 6 and 102) and α2(IV) (serum M24-1) and to the α1, α2 trimer (mAb M3F7) stained both glomerular and tubular BLs in kidney (Fig. 9 *c*) and both synaptic and extrasynaptic BL in muscle (Fig. 10 *f*). Capillary and arterial BLs in muscle were also stained by these antibodies (Fig. 9 *c*), as were endoneurial and perineurial BLs in nerves (Fig. 9 *e*) and intrafusal fiber and capsular BLs in muscle spindles (Fig. 9 *f*). Thus, the collagen IV chains α1(IV) and α2(IV) appear to be common constituents of all BLs.

On the other hand, the antibodies to α1(IV) and α2(IV) did reveal subtle differences among BLs. As previously reported (Butkowski et al., 1989; Kleppel et al., 1989*a*), regions of the glomerular BL that are rich in α3(IV) and α4(IV) chains are relatively poor in α1(IV) and α2(IV) (Fig. 9, *a* and *b*). Similarly, synaptic BL, which is α3(IV)- and α4(IV)-rich, was less intensely stained by antibodies to α1(IV) and α2(IV) than neighboring stretches of extrasynaptic BL (Fig. 10 *f*). Although this difference was not striking, it is particularly significant in light of the fact that there is two- to threefold more BL at synaptic sites than extrasynaptically (see above, and Fig. 3, *k–o*). Furthermore, the difference was seen with antibodies to the globular domains of both α1(IV) and α2(IV). In addition, extrasynaptic BL was stained more intensely than synaptic BL by mAb M3F7, which binds to the triple helical region of the α1,α2 trimer, distant from the globular domain (Fig. 10 *a*). This result decreases the likelihood that the dim staining of synaptic sites merely reflects local masking of determinants in the globular domain. Thus,

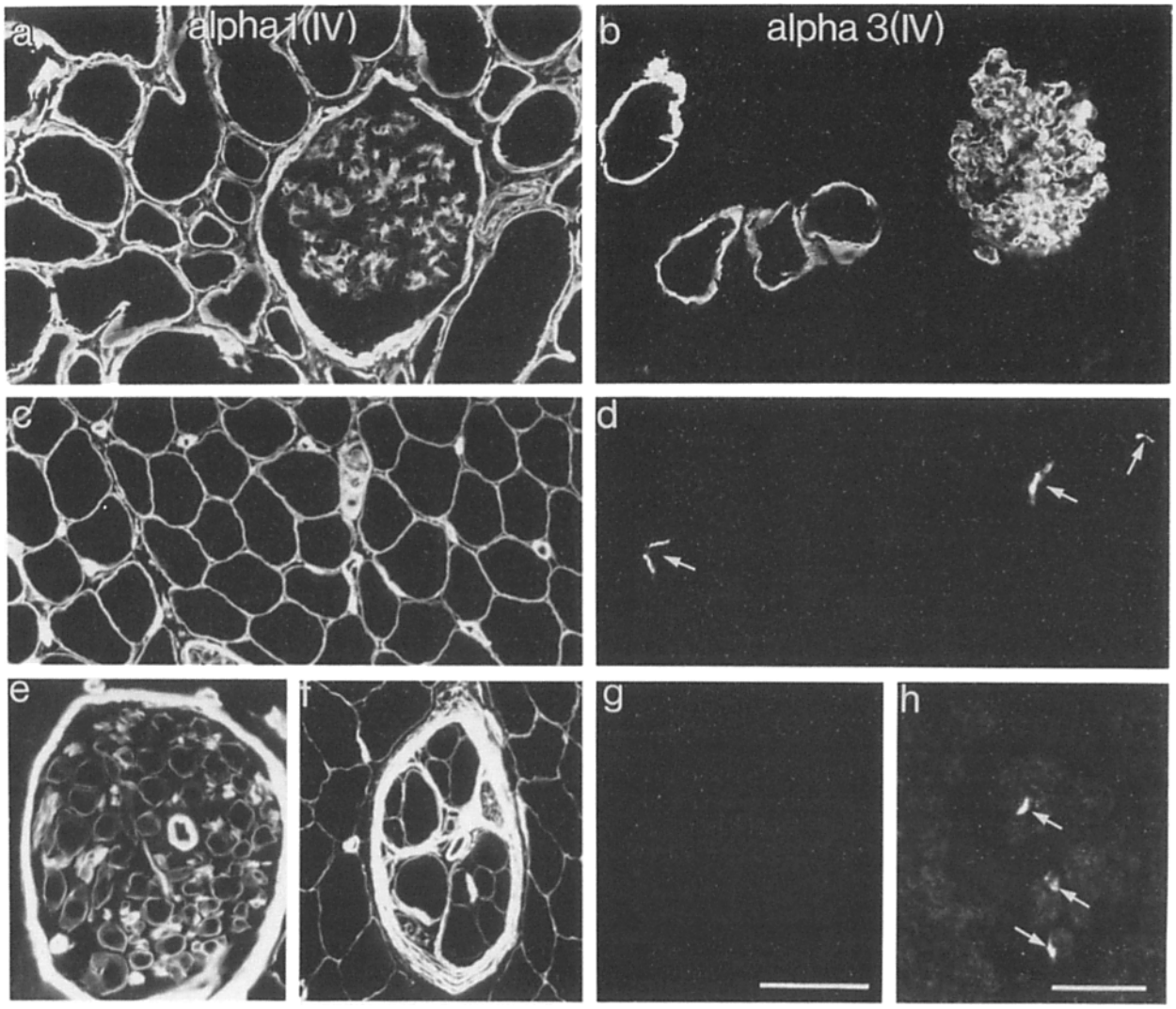


Figure 9. Distribution of collagen IV chains in human kidney and muscle. Anti-α1(IV) (mAb 102) stain both glomerular and tubular BLs in kidney (*a*), muscle fiber and vascular BLs in muscle (*c*), endoneurial and perineurial BLs in nerve (*e*), and capsular and intrafusal fiber BLs in muscle spindles (*f*). In contrast, anti-α3(IV) (mAb 17) stains glomerular BL and a limited subset of tubules in kidney (*b*), no BLs except synaptic BL in muscle (*d*), and muscle spindles (*h*), and no BLs in nerve (*g*). Identity of arrowed synaptic sites in *d* and *h* was confirmed by counter staining with rhodamine α-bungarotoxin. Sections in *d*, *g*, and *h* are serial to those in *c*, *e*, and *f*, respectively. Bar in *g*: (*a–e*, and *g*) 50 μm; bar in *h*: (*f* and *h*) 20 μm.

the α1(IV) and α2(IV) chains appear to be less abundant in synaptic than extrasynaptic BL.

Discussion

We have used immunohistochemical methods to map the distribution of laminin- and collagen IV–related proteins in the BLs of muscle and kidney. Our results, which are summarized in Table III, provide new insights into the composition of BLs in general and the molecular architecture of synaptic BL in particular.

Heterogeneity of BLs

Although BLs have been subjected to biochemical study for many years (Kefalides, 1970), their analysis was long complicated by the extreme insolubility of adult BLs in aqueous solutions and by the extensive covalent cross-linking of their components. A major advance, therefore, was the discovery of tumor cell lines that secrete large amounts of matrix material in relatively soluble form. Laminin, collagen IV, entactin/nidogen, and BL-associated heparan sulfate proteoglycans were all discovered and have been characterized most extensively using such cells (Timpl and Dziadek, 1986; Timpl, 1989). With rare exceptions, antibodies to these tumor-derived molecules stained all BLs in fetal and adult mammals (Laurie et al., 1983; Inoue, 1989). Accordingly, a prevailing view arose that most BLs share many molecular features.

At the same time, it has been generally acknowledged that BLs are heterogeneous to some degree. Both biochemical (Kefalides, 1970) and immunohistochemical (Pierce and Nakane, 1967) comparisons originally pointed to this con-

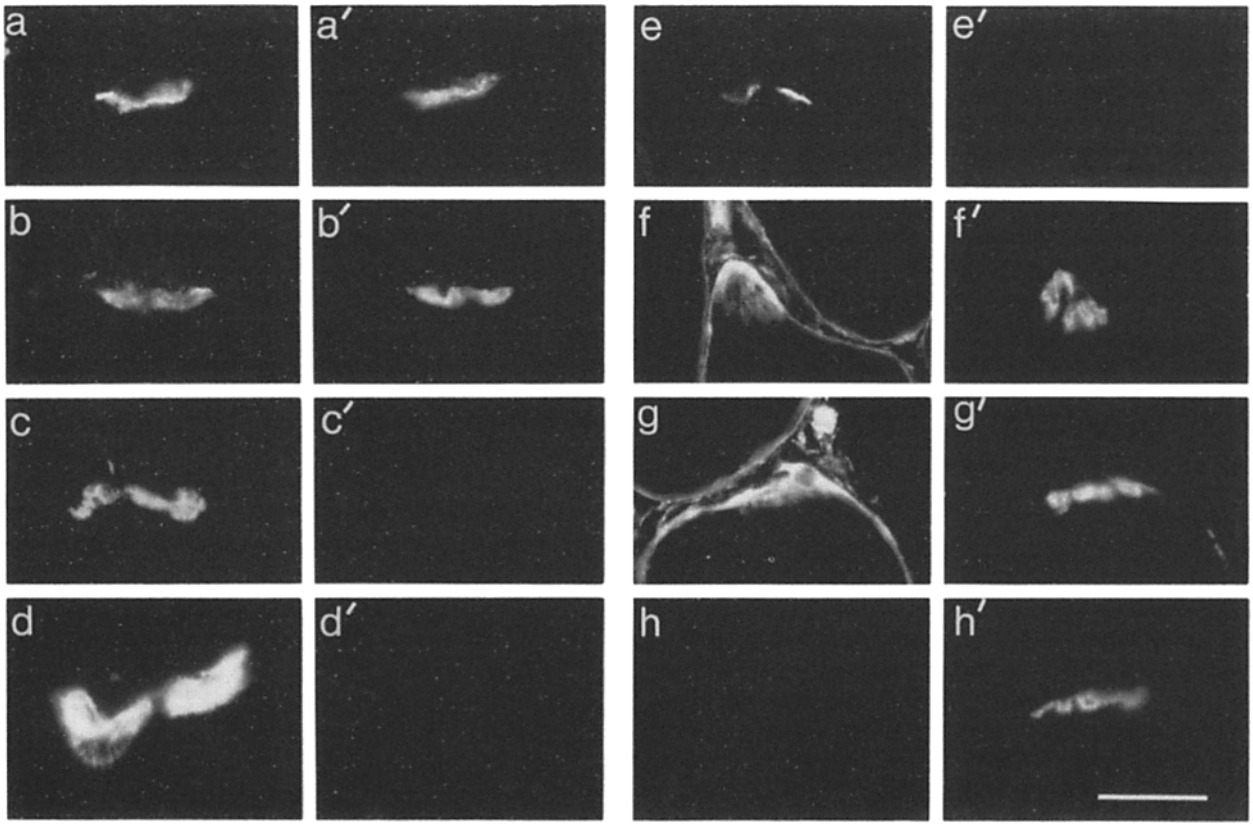


Figure 10. Presence of collagen IV chains at synaptic sites in human muscle. (*a*, *c*, and *d*) Anti-α3(IV) mAb 17. (*b*) Anti-α3 (IV) serum 975. (*e*) Anti-α4(IV) mAb 85. (*f*) Anti-α1(IV) mAb 6. (*g*) Anti-α1,2(IV) mAb M3F7. (*h*) No first antibody. (*a′–h′*) Same fields as *a–h*, counterstained with rhodamine-α-bungarotoxin. Sections in *c–e* were treated with acid-urea, which abolishes reactivity with bungarotoxin. Struts of BL that enter junctional folds are visible in *d*. Bar: (*d*) 10 μm; (*a–c*, *e–h*, and *a′–h′*) 20 μm.

clusion. However, difficulties of solubilizing BLs, and of isolating any but a few atypically thick BLs in pure form, complicated this interpretation. More recently, mAbs have been produced in several laboratories that stain only a subset of BLs in immunohistochemical assays (e.g., Sanes and Chiu, 1983; Fitch and Linsenmeyer, 1983; Hessle et al., 1984; Wan et al., 1984; Leu et al., 1986; Pusey et al., 1987; Horikoshi et al., 1988; Kleppel et al., 1989*a*). In many of these cases, however, it has been difficult to determine whether an absence of immunoreactivity indicates absence of the antigen or merely reflects masking of the epitope in question. Indeed, differential access has been proposed as an explanation for differential staining in some cases (e.g., Leu et al., 1986; Horikoshi et al., 1988). Nonetheless, at least some molecular differences among BLs (e.g., the differences between synaptic and extrasynaptic regions of muscle fiber BL, which provided the starting point for the present study) are well established.

Table III. Occurrence of Laminin and Collagen IV Isoforms in Various BLs

	Laminins					Collagens IV			
	A	B1	B2	mer M	S-Laminin	α1	α2	α3	α4
Muscle fiber									
Synaptic	+	−	+	+	+	±	±	+	+
Extrasynaptic	−	+	+	+	−	+	+	−	−
Nerve									
Endoneurial	−	+	+	+	−	+	+	−	−
Perineurial	±	−	+	−	+	+	+	−	−
Kidney									
Distal tubular	+	+	+	−	−	+	+	+	+
Proximal tubular	+	+	+	−	−	+	+	−	−
Glomerular	+	−	+	−	+	±	±	+	+
Arterial	+	−	+	−	+	+	+	−	−

Our results confirm and extend these observations. Of the eight BLs studied in detail, at least seven have distinct compositions, and of the nine BL components tested, at least seven have distinct distributions (see Table III). To some extent these conclusions are subject to caveats noted above: absence of immunoreactivity might not reflect absence of antigen, and presence of immunoreactivity could result from the presence of a related antigen. However, we obtained consistent results with at least two antibodies to each protein and (for the laminins) in at least two different species. Thus, it is likely that our results accurately reflect the distribution of the antigens. Furthermore, because we focussed on isoforms of major structural components of BLs, we believe that the heterogeneity observed reflects major and important molecular differences among BLs.

Thus, we now conclude that each BL contains some laminin-like and collagen IV-like molecules, but that these vary from one BL to another, and are members of multigene

families. In this regard, BLs are analogous to the sarcomeres of muscle fibers. Each fiber contains myosin heavy and light chains, actin, tropomyosin, and so on. However, the early belief that each component is unique has been supplanted by the realization that each is actually a group of homologous proteins encoded by a family of related genes (for example, see Emerson et al., 1986). The contractile properties of individual muscle fibers reflect the particular isoforms of which its sarcomeres are composed. Likewise, the laminins and collagens IV may play similar roles in all BLs, but the unique properties of each BL may be determined by its isotype.

Laminins and Collagens IV

Initially, the laminin of authentic BLs was presumed to be identical to tumor-derived laminin: a trimer of A, B1, and B2 subunits, all of which are homologous and similar in domain structure (Sasaki et al., 1987, 1988; reviewed in Timpl, 1989; see Fig. 1). Early evidence for laminin isoforms was the apparent absence of the A chain from laminins produced by several cell types (Timpl, 1989); however, this observation was controversial because A chains are known to be more protease labile than B chains (Rao et al., 1982). More direct evidence for isoforms is the purification of variant laminins (Paulsson and Saladin, 1989; Ehrig et al., 1990, and references therein), and our demonstration that s-laminin and merosin M are homologues of laminin B1 and A, respectively (Hunter et al., 1989*a*; Ehrig et al., 1990).

Given the existence of variant laminins and novel isoforms, the question of how individual polypeptides are combined into oligomers becomes an important one. Our results suggest some possible rules of assembly. First, each BL we studied contains either laminin B1 or s-laminin; none contains both. This pattern is consistent with the possibility that these polypeptides can substitute for each other in a laminin-like trimer. Second, six of the BLs contain only laminin A, and two contain only merosin; only synaptic BL contains both. As for s-laminin and B1, these observations are consistent with the notion that each laminin-like molecule contains a heavy chain (A or M). Third, laminin B2 is present in all of the BLs studied, suggesting that it is a common subunit of all laminin oligomers. Thus, it is possible that structural considerations favor the assembly of laminin monomers into heterotrimers of A-like, B1-like, and B2-like subunits, with diversity arising from the shuffling of homologues in a common structure.

New data may require revision of this scheme. Other laminin-like subunits may exist (e.g., in capillaries or perineurium) and, as noted above, some laminins may lack a large (A-like) subunit. In addition, our immunohistochemical results only show coexistence of subunits in a BL, not in an individual molecule. Better evidence on subunit assembly can be obtained by analyzing complexes immunoprecipitated by the subunit-specific antibodies described herein. Initial results of such experiments are described elsewhere (Engvall et al., 1990).

The differential distribution of the collagen IV chains also suggests combinations in which they may occur. The α1(IV) and α2(IV) chains are known to occur together in a triple helical molecule ($[\alpha1(IV)]_2[\alpha2(IV)]_1$), and their genes share a common regulatory element (Timpl, 1989). Our results, showing a widespread codistribution of α1(IV) and α2(IV), are consistent with their presence in the same molecule. The α3(IV) and α4(IV) chains have not yet been isolated in native form, and their mode of association has therefore been inferred only from the structure of multimers of their COOH-terminal globular (NC1) domains (Hudson et al., 1989). Our results are those expected if the α3(IV) and α4(IV) chains occur together within a single triple helical molecule.

The Synaptic Cleft of the Neuromuscular Junction

Our interest in regional specialization of BLs stems from evidence that the BL in the synaptic cleft of the neuromuscular junction plays important roles in the formation and maintenance of that synapse. These include specifying the precise reinnervation of original synaptic sites during regeneration (Sanes et al., 1978), triggering the differentiation of regenerating axons into nerve terminals (Glicksman and Sanes, 1983), mediating the adhesion of nerve to muscle (Sanes et al., 1978), and organizing the differentiation of the postsynaptic membrane (Burden et al., 1979). Several molecules have been identified that are concentrated in synaptic BL, including a collagen-tailed form of acetylcholinesterase (McMahan et al., 1978; Sanes and Hall, 1979; Massoulie and Bon, 1982), a heparan sulfate proteoglycan (Anderson and Fambrough, 1983), agrin (McMahan and Wallace, 1989), an *N*-acetylgalactosamine-terminated glycoconjugate (Scott et al., 1988), and s-laminin (Hunter et al., 1989*a*). Agrin has been implicated in the induction of postsynaptic structures (McMahan and Wallace, 1989), and s-laminin bears a motoneuron-selective adhesive site (Hunter et al., 1989*b*). However, the molecular basis of synaptic organization remains poorly understood.

In this context, it is intriguing that the present study has provided evidence for three novel synapse-concentrated polypeptides in BL (laminin A and collagen chains α3(IV) and α4(IV)), for one polypeptide that is excluded from synaptic BL (laminin B1), and for two polypeptides that are depleted from synaptic BL (the α1(IV) and α2(IV) chains). All of these proteins will now need to be considered as candidate mediators of the functional differences between synaptic and extrasynaptic BL. Some specific suggestions along these lines are considered below. More generally, the notion that synaptic and extrasynaptic BL differ in fundamental ways encourages the view that the structure of synaptic BL as a whole may be responsible for its functional properties.

Neuronal Interactions with Laminin and Collagen

The laminin A, B1, B2 trimer is adhesive to neurons of many types, and is a potent promoter of neurite outgrowth (reviewed in Sanes, 1989). A defined site on s-laminin is selectively adhesive for motoneurons, and inhibits neurite outgrowth on laminin (Hunter et al., 1989*b*; and unpublished data). Merosin is adhesive for at least some neurons (Engvall, E., and M. Manthorpe, unpublished data). Thus, although studies of native molecules remain to be completed, it seems likely that the isoforms of laminin are all neuroactive but differ in their specificity and effects.

Individual subunits bear several potential active sites that may mediate some of these effects. These include: a potential integrin-binding site, RGD, in laminin A (Sasaki et al., 1988); a motoneuron-selective adhesion site, LRE, in s-lam-

inin (Hunter et al., 1989*b*); two other occurrences of LRE in s-laminin, two in B2, and one in A, but none in B1 (Hunter et al., 1989*b*); neurite outgrowth-promoting sites in A (Tashiro et al., 1989) and B2 (Liesi et al., 1989); and at least two discrete sites in B1 that bind nonneural cells (Graf et al., 1987; Charonis et al., 1988). The variations among BLs that we have documented here imply that the BLs in peripheral nerve and muscle contain different combinations of these sites. For example, synaptic sites would bear a high density of LRE's in s-laminin and laminin A, whereas endoneurial and extrasynaptic BL substitute LRE-less B1 for s-laminin and merosin M for A. Similarly, the cell-binding sites in laminin B1 are present in endoneurial and extrasynaptic BLs but absent from the synapse. Thus, the variable arrangements of laminin isoforms in the BLs that regenerating axons encounter may serve to display different combinations of active sites to axons at different points along their path, thus influencing axonal growth and differentiation in complex ways.

Similar considerations may apply to the collagens IV. Conventional collagen IV (the [α1(IV)]$_2$ [α2(IV)] trimer) is adhesive for and promotes neurite outgrowth from some types of neurons (Sanes, 1989). Collagens have also been suggested to influence clustering of acetylcholine receptors in muscle membranes (Sanes, 1989). It is tempting to speculate that the α3(IV) and α4(IV) chains have unique and/or cell type-specific activities that could influence nerve or muscle at synaptic sites. Direct tests of this notion await isolation and characterization of the molecule(s) containing the α3(IV) and α4(IV) chains.

Goodpasture Syndrome and Alport Nephritis

Patients with Goodpasture syndrome, an autoimmune glomerulonephritis, produce antibodies that selectively stain glomerular BL in normal human kidneys. The α3(IV) chain of collagen IV was discovered as the major target of autoantibodies in such patients, and has therefore been identified as the "Goodpasture antigen" (Butkowski et al., 1985, 1987; Saus et al., 1988; reviewed in Hudson et al., 1989). Subsequently, α3(IV) has been implicated in the etiology of Alport nephritis, an X-linked disease often accompanied by deafness: α3(IV) is undetectable in patients with Alport's, and several results suggest that the Alport locus is linked to the structural genes for α3(IV), for α4(IV), or for the newly discovered α5(IV) chain (Kleppel et al., 1987; Hostikka et al., 1990; reviewed in Hudson et al., 1989; but see Kastan et al., 1989; Kleppel et al., 1989*b*).

In light of these intriguing pathological observations, it will be of obvious interest to study the neuromuscular junctions of patients with Goodpasture and Alport nephritis. Although no major neuromuscular symptoms have been detected in such individuals, subtle abnormalities have not been sought. If the Alport mutation deletes a specific component of synaptic BL, one could then ask what other components are disordered and what developmental and structural abnormalities, if any, result. The obvious difficulty of performing such analyses with human material may be mitigated by the recent characterization of a canine model of X-linked Alport syndrome in which, as in humans, α3(IV) is apparently lacking and fatal nephritis results (Thorner et al., 1987, 1989).

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