relate to biological views of the Amazon biota? First, it explains why the Amazon River has an extraordinarily high diversity of endemic marine-derived species, much greater than that of the Congo or any other large river system. The introduction and isolation of such intriguing Amazon taxa as iniid dolphins, trichechid seacows, stingrays, and tilefish may now be attributed to the rise and fall of the Miocene seaway. Second, the rich terrestrial biota, for which the Amazon Basin is justly famous, is now seen to be drawn from three separate land areas (see figure). Each parcel of equatorial lowland provided an independent theater of evolutionary experimentation. Such division and coalescence conforms well to the model regarded by many evolutionists as the most productive "species pump." This new view of the rise and fall of the Amazon Sea extends the variability of the Quaternary 10 million years farther back into the Cenozoic Era in an even more dynamic scenario.

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Laminin β2 (S-Laminin): A New Player at the Synapse

Zach W. Hall

When the growth cone of a motor axon meets the surface of a myotube, the event locally transforms both cells (*1*). The motile growth cone becomes a mature nerve terminal, stably attached to the muscle cell and specialized for rapid, focal secretion of the neurotransmitter acetylcholine (ACh); the muscle cell surface accumulates receptors for ACh beneath the nerve terminal and concentrates the enzyme that metabolizes ACh, acetylcholinesterase, in the synaptic cleft. These specialized postsynaptic structures convert pulses of released ACh into brief electrical responses that trigger a muscle action potential and ultimately contraction. This complex synaptic structure induced by nerve-muscle contact is meant to last a lifetime: If either partner is damaged and replaced by regeneration, the remaining structures induce the appropriate specialization in the regenerating cell precisely at the original synaptic site.

The ability of a regenerating axon to find the site of the original synapse on a denervated muscle surface is remarkable; although the old synapse represents less than 0.1 percent of the muscle surface, virtually all new contacts form at that site. Over 15 years ago McMahan and his colleagues demonstrated that the synaptic site was marked by molecules in the synaptic basal lamina joining the two cells, and that these are sufficient to specify synaptic differentiation of either nerve or muscle during regeneration (*2*). The mechanism by which one of these markers, laminin β2, is localized to the synapse is described in a report by Sanes and his colleagues on p. 413 of this issue of *Science* (*3*).

Formation of the neuromuscular synapse, either during development or during regeneration, requires that dozens of macromolecules be concentrated at the synaptic site (*1*). These form a complex structure that extends from the cytoplasm of one cell across the synaptic gap into the cytoplasm of the other. Most of the synaptic components provided by each cell are made in the absence of the other cell; myotubes cultured without nerves, for example, make ACh receptors that spontaneously aggregate into clusters. These aneural clusters are associated with both cytoskeletal and basal lamina components that are normally part of the mature synaptic structure (*4*).

When a nerve contacts a muscle, one of the earliest steps in the assembly of the synaptic structure is the formation of a new cluster of ACh receptors at the site of contact. The synaptic receptor cluster is apparently induced at this site by agrin, a proteoglycan released by the nerve and incorporated into the synaptic basal lamina (*5*); agrin-induced ACh receptor clustering subsequently induces the accumulation of other postsynaptic components at the synapse (*6*, *7*). Agrin thus appears to be a key to postsynaptic differentiation. The recent experiments by Sanes, Merlie, and their colleagues (*3*, *8*, *9*) now focus on a second basal lamina component—the β2 chain of laminin—and examine its localization to the synapse and its role in presynaptic differentiation. An intriguing feature of their results is that the same discrete domain in the molecule appears both to localize the molecule to the synapse and to mediate its effect on nerve terminals.

Laminin β2 (or S-laminin) was originally identified with antibodies that recognize a synapse-specific component of the basal lamina (*10*); cloning revealed the protein to be homologous to the B1 (or β1) chain of laminin (*11*). Subsequent experiments showed that laminin at the synapse contains the β2 chain, presumably complexed with α and γ chains, whereas the β1 chain of laminin is confined to extrasynaptic basal lamina (*9*, *12*). In cultures of the C2 mouse muscle cell line, the two chains have a distribution analogous to that found in adult muscle: The β1 chain is widely distributed on the surface of myotubes and in the extracellular matrix, whereas the β2 chain is concentrated near the ACh receptor clusters that spontaneously arise in aneural cultures. By making chimeras of the two homologous chains and analyzing their distribution in stable C2 transfectants, Sanes and his colleagues have identified a region of 16 amino acids near the carboxyl terminal of the β2 chain that is responsible for its association with ACh receptor clusters (*9*).

How is the β2 chain localized to the ACh receptor? Although the β2 chain appears on the surface only in association with ACh receptor clusters, it is made throughout the length of the transfected myotubes. In contrast to ACh receptors, which are free to diffuse in the membrane, the β2 chains, because they are part of the extracellular matrix, are unlikely to concentrate near ACh receptor clusters by lateral migration at the cell surface. Consistent with this idea, the accumulation of another synaptic basal lamina component, acetylcholinesterase, requires protein synthesis (*6*). Basal lamina containing β2 laminin chains must thus arise either by local intracellular incorporation of the β2 chains into laminin, by local transport of β2 laminin to the surface, or by local incorporation of β2 laminin into the basal lamina (either intracellularly or on the surface). Identification of the synap-

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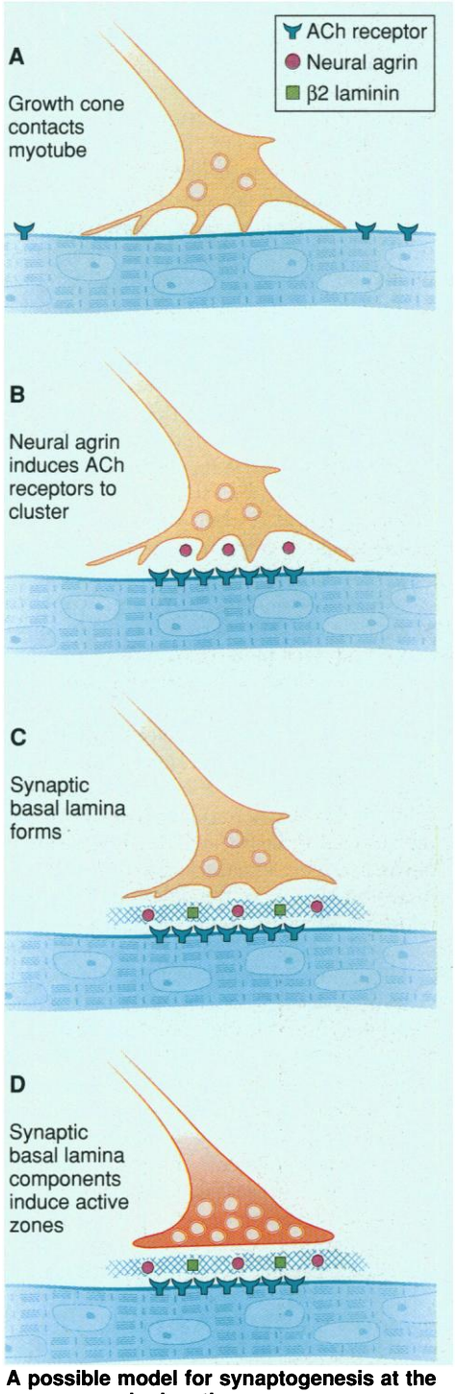

A possible model for synaptogenesis at the neuromuscular junction.

tic signals that initiate this process and of the proteins that recognize the localization domain on the β2 chain remain projects for the future.

What does the concentrated β2 laminin do at the synapse? Two recent papers from the Sanes and Merlie laboratories indicate that it helps mediate presynaptic differentiation and may direct regenerating axons to find the original synaptic site. The first observations (*8*) reveal the consequences of synaptogenesis without the β2 chain. In a β2 knockout mouse synapses form, but are simpler and lack presynaptic specialization. Synaptic vesicles are evenly distributed in the nerve terminal, rather than being concentrated near the site of nerve-muscle contact, and the number of active zones, the site of rapid transmitter release by exocytosis, is drastically reduced. The second report shows that β2 laminin may serve as a stop signal to ingrowing motor axons (*7*). When cultured on laminin as a substrate, motor neurons extend neurites. When cultured on the β2 chain, they adhere but do not extend neurites; moreover, in mixtures, β2 laminin inhibits neurite growth of motor neurons (but not of other neuronal types). When motor neurons are cultured on alternating strips of laminin and the β2 chain, neurites extend on the laminin surface but stop at or slightly beyond the laminin-β2 border. These experiments led Sanes and his colleagues to the idea that the β2 chain may help attach resident terminals to the postsynaptic surface during synaptogenesis. During regeneration, neurites growing on the laminin-rich extrasynaptic surface of muscle fibers would stop at the synapse site. β2 chains could thus trap and confine growing terminals to the appropriate place.

Competition experiments with synthetic peptides derived from the β2 laminin sequence identify a site that appears to mediate neurite adhesion and inhibition of neurite extension. The tripeptide LRE, a sequence that occurs three times in the β2 laminin chain (and is also found in several other components of the synaptic basal lamina but is not in the β1 chain), is an effective inhibitor of both neurite adhesion and of neurite extension on a laminin substrate (*9*, *13*). The inhibition appears to be specific for motorneurons, as neurite growth of dorsal root ganglion cells and PC12 cells on laminin is unaffected by the peptide. Remarkably, LRE is part of the 16-amino-acid sequence responsible for synaptic localization of the β2 laminin. Two ideas flow from this observation: LRE may be part of a general signal targeting a variety of components to the synaptic basal lamina, and the resulting concentration of LRE sites at the synapse could identify it to the growth cones of motor axons.

Present knowledge of the two components of the synaptic basal lamina—agrin and laminin β2—suggests a simplified narrative of basal lamina signaling during synaptic differentiation (see the figure). First, agrin secreted from the growth cones of ingrowing motor axons causes ACh receptors to cluster at sites of nerve-muscle contact. Second, the muscle fiber forms a synaptic basal lamina in association with the ACh receptor clusters by the local accumulation of laminin β2, along with other components of the extracellular matrix (acetylcholinesterase, heparin sulfate proteoglycan, and muscle agrin). Third, laminin β2 and possibly other synaptic basal lamina components induce synaptic vesicle accumulation and the formation of active zones in the presynaptic terminals. According to this scheme, laminin β2 may be a key link in the signaling pathway that assures the precise registration of presynaptic and postsynaptic structures.

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John Merlie (1945–1995)

On 27 May, John Merlie suddenly and unexpectedly died of a heart attack. The news was doubly shocking, because John was in midstride of what many thought the most important work of an already distinguished career. Merlie was a pioneer in the use of molecular methods to study the synapse. In a series of experiments that began in the laboratory of Jean-Pierre Changeux, he and his colleagues defined the pathways by which the subunits of the nicotinic acetylcholine receptor subunits are synthesized, folded, assembled, and transported to the surface. This work stands as a model for understanding how oligomeric ion channels are synthesized and how their synthesis is regulated. Later, he turned to problems of synaptogenesis, and once again set the pace for others to follow. He cloned rapsyn and demonstrated its role in acetylcoline receptor clustering and, with Josh Sanes, gave vivid demonstration to the idea that nuclei at the neuromuscular junction differentially express mRNAs for synaptic proteins. Most recently, he and Sanes had embarked on an ambitious program using knockout mice to define the role of synaptic proteins. This work, much of it still in progress and eagerly awaited, promises to raise our understanding of synaptic differentiation by a quantum jump. Unassuming and modest, John never sought the limelight, but let his work speak for itself. With its eloquent voice, he has left an indelible mark on our field.