Review

The role of laminins in the organization and function of neuromuscular junctions

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Abstract

The synapse between motor neurons and skeletal muscle is known as the neuromuscular junction (NMJ). Proper alignment of presynaptic and post-synaptic structures of motor neurons and muscle fibers, respectively, is essential for efficient motor control of skeletal muscles. The synaptic cleft between these two cells is filled with basal lamina. Laminins are heterotrimer extracellular matrix molecules that are key members of the basal lamina. Laminin α4, α5, and β2 chains specifically localize to NMJs, and these laminin isoforms play a critical role in maintenance of NMJs and organization of synaptic vesicle release sites known as active zones. These individual laminin chains exert their role in organizing NMJs by binding to their receptors including integrins, dystroglycan, and voltage-gated calcium channels (VGCCs). Disruption of these laminins or the laminin-receptor interaction occurs in neuromuscular diseases including Pierson syndrome and Lambert–Eaton myasthenic syndrome (LEMS). Interventions to maintain proper level of laminins and their receptor interactions may be insightful in treating neuromuscular diseases and aging related degeneration of NMJs.

Introduction

Neuromuscular junctions (NMJs) are chemical synapses located between nerve terminals and specialized sites on the post-synaptic skeletal muscle fiber plasma membrane (i.e., motor endplates). Innervation of muscle fibers by motor neurons establishes proper control of skeletal muscle contraction by the nervous system, and this innervation can become disrupted during disease and aging. Depolarization of motor neurons results in subsequent depolarization of the skeletal muscle fiber plasma membrane or sarcolemma. On the presynaptic side, depolarization of motor neurons results in synaptic vesicle fusion with the plasma membrane and exocytosis of a chemical neurotransmitter, acetylcholine (ACh), into the synaptic cleft. Synaptic vesicle release sites of the nerve terminal are well-organized structures known as active zones (see Box 1 for expanded description of active zones) [1–5]. At the endplate, the sarcolemma uniquely folds to form junctional folds, and acetylcholine receptors (AChRs) accumulate at the crest of these junctional folds to rapidly and efficiently receive ACh released from motor neurons [6–10].

During development, motor neurons must seek out and find nascent endplates making up only approximately 0.1% of the sarcolemmal surface area [11]. Individual components of the skeletal muscle fiber basement membrane help to guide the process of innervation by motor neurons, and also proper organization of pre- and post-synaptic NMJ morphology. Laminins play a large role in this process and individual laminin subunits are responsible for organizing different components of the NMJ structure [11,12]. Laminin receptors, including basal cell adhesion molecule/Lutheran blood group antigen (Bcam), dystroglycan, integrins, and voltage-gated calcium channels (VGCCs), assume different roles influenced by each laminin chain (see Box 2 for expanded description of VGCCs at NMJs) [10–20]. These interactions are disturbed during some diseases and

Box 1
Active zone organization and active zone proteins.

Active zones are a multiprotein complex accumulated at the presynaptic plasma membrane where synaptic vesicles accumulate, fuse with the plasma membrane, and release neurotransmitters into the synaptic cleft [1–5,162,204–208]. Using freeze-fracture electron microscopy, active zones were identified as two parallel arrays of 10–12 nm intramembranous particles arranged in two to four rows with each active zone containing 20 of these intramembranous particles [93,96,209,210]. The density of active zones at the presynaptic membrane of NMJ is 2.4–2.7 active zones/μm^2 in adult humans and mice [4,93,96,97], and this density is maintained during postnatal maturation periods when NMJs enlarge [175]. A collection of proteins make up the active zones (also known as the cytoskeletal matrix of the active zone (CAZ)) including Bassoon, CAST/ELKS2/Erc2, CAST2/ ELKS/Erc1, Munc13, Piccolo, Rab3 interacting protein-1/2 (RIM1/2), as well as Brunchpilot in Drosophila that is a homolog of CAST/ELKS/Erc (Fig. 3) [211–223]. These active zone proteins are involved in accumulation of synaptic vesicles to the plasma membrane and neurotransmitter release upon stimulation by calcium influx through VGCCs. It is important to note that active zone density is independent of nerve transmission. In acetylcholine transferase knockout mice, ACh cannot be properly synthesized therefore synaptic transmission is absent. However, active zone density is normal in these mice [224]. Thus, proper nerve transmission is not necessary for proper active zone organization and formation.

The active zone proteins Bassoon and Piccolo were discovered in screenings to determine structural proteins in rat brain synaptic junctions, and these two proteins share many structural similarities [212,215,219,225]. In NMJs of rodents, Bassoon and Piccolo display a punctate staining pattern by fluorescent immunohistochemistry (Fig. 4), with these puncta localizing to presynaptic active zones [63,175,189]. Mice without functional Bassoon have normal synapse formation, but impaired synaptic transmission, abnormal dendritic branches, ectopic formation of active zones, and lack proper anchoring of photoreceptor ribbon synapses to active zones with otherwise normal retinal anatomy [226,227]. Piccolo has been shown to aid in synaptic vesicle exocytosis, and when absent, synaptic vesicle trafficking is disrupted [228]. Importantly, Bassoon helps to position VGCCs near the synaptic vesicle release sites [223]. Although disruption of Bassoon reduces synaptic functionality in some regions of the central nervous system, the disruption of active zone proteins may or may not result in aberrant NMJ formation and needs to be further tested.

The role of other active zone specific proteins in the formation of NMJ active zones is not as well established. CAST family of scaffolding protein CAST2/ELKS/Erc1 along with Piccolo, but not CAST/ELKS2/Erc2, are detected at NMJs [229]. In addition, CAST/ELKS2/Erc2, similar to Bassoon, has been linked to VGCC functionality [230]. The role of CAST/ELKS2/Erc2 in active zone organization was confirmed in photoreceptor synapses and inhibitory synapses due to the loss of CAST/ELSK2α [231,232]. Munc13 has three homologous family members (Munc13-1/2/3) and has an essential role in neurotransmitter release and synaptic vesicle priming in synapses of the central nervous system [217,233,234]. Munc13-1/2 double knockout mice totally lack spontaneous and evoked synaptic transmission in excitatory and inhibitory synapses of hippocampal neurons [235], but exhibited residual amount of synaptic transmission at NMJs [236]. At the NMJ, these mice exhibit normal apposition between motor neuron terminals with endplates, and active zones with docked vesicles were detected [236,237]. Therefore, Munc family of proteins may not be essential for the structural assembly of active zones. RIMs are multi-domain proteins consisting of three isoforms (α, β, γ) that also play essential roles in connecting active zone specific proteins to active zone structures and synaptic vesicles [238–244]. For example, direct interactions of PDZ domains in RIM and N- and P/Q-type VGCCs tether these VGCCs to presynaptic active zones [244]. RIM1 interacts directly with VGCC β subunit and suppresses voltage-dependent inactivation of neuronal VGCCs [242,243]. RIM1/2 knockout mice have a reduced number of docked vesicles at active zones with reduced density of VGCCs in the calyx of Held synapse without loss of active zone density [245]. Further work is needed to elucidate the molecular mechanisms that underlie changes in active zone protein content during aging and other neuromuscular diseases.