CHAPTER 1

The Neuromuscular Junction

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The neuromuscular junction (NMJ) is a highly specialised synapse between a motor neuron and a muscle fibre, which is essential for neuromuscular transmission. In this chapter, the most fundamental aspects of the NMJ organisation, structure and physiology biology, and physiology will be reviewed. This will provide the background to understanding the physiopathology and clinical manifestations of myasthenic disorders, either autoimmune (myasthenia gravis; MG) or genetic (congenital myasthenic syndromes; CMS).

ORGANISATION OF THE NEUROMUSCULAR JUNCTION

NMJs are very small structures (~20μm) compared to the length of the muscle fibres they innervate. Typically, each skeletal muscle fibre has a single NMJ where the motor axon joins the muscle. The most common classification divides the NMJ into a presynaptic terminal, a postsynaptic muscle membrane and the space that lies between, called the synaptic cleft (Fig. 1.1). The classic morphology of the NMJ in murine animal models is described as a pretzel-shaped structure. However, human NMJs are typically smaller, less complex, and more fragmented than the NMJs studied in murine animal models (Jones et al. 2017), although human NMJs exhibit a higher degree of folding than the NMJs of any other species (Slater 2017).

Presynaptic Terminal

Motor nerves travel from the spinal cord to skeletal muscles where they divide into terminal branches and subsequently form synaptic boutons that contact the muscle surface (Desaki and Uehara 1981). Synaptic buttons are small protuberances found at the terminal end of motor axons. They are filled with synaptic vesicles containing the neurotransmitter acetylcholine (ACh) ready for vesicle exocytosis upon the arrival of an action potential (Figs. 1.1 and 1.2). The nerve terminal has a complex machinery in place to allow the synthesis, exocytosis and recycling of synaptic vesicles (Lai et al. 2017). Terminal Schwann cells cover the NMJ although their role in synapse formation and maintenance is still unclear (Feng and Ko 2008).

Synaptic Cleft

The synaptic cleft is the space between the presynaptic nerve terminal and the postsynaptic muscle membrane, which is filled with a specialised form of extracellular matrix called the synaptic basal lamina (Sanes 2003). This matrix is crucial for the alignment, organisation and structural integrity of the NMJ. The main components of the basal lamina include laminins, collagens, heparan sulfate proteoglycans (muscle agrin and perlecan) and nidogens (Shi et al. 2012). It should be noted that the enzyme acetylcholinesterase (AChE), which terminates synaptic transmission by breaking down acetylcholine, is attached to the basal lamina by a collagenic tail (Ohno et al. 1998).

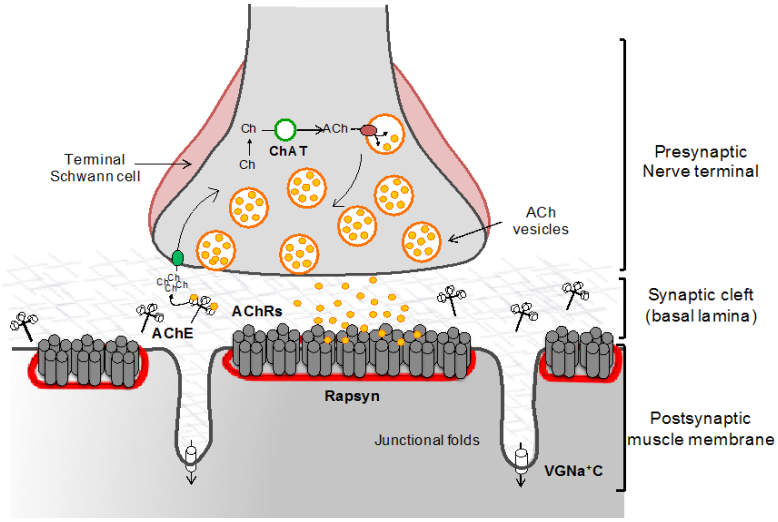

Figure 1.1 Schematic representation of the neuromuscular junction. The NMJ is divided into three different compartments. The presynaptic nerve terminal is responsible for the synthesis (ChAT) and recycling of acetylcholine and the exocytosis of synaptic vesicles. The synaptic cleft contains a specialised form of extracellular matrix called basal lamina that is essential for the structural integrity of the NMJ. This the location of the enzyme AChE, which breaks down ACh into Ch. At the postsynaptic muscle compartment, the AChRs are densely clustered at the top of the junctional folds forming a network with the intracellular anchoring protein rapsyn that stabilises AChRs (Zuber and Unwin 2013). VGNa+C located at the bottom of the junctional folds are essential in the generation and propagation of action potentials. ACh, acetylcholine; AChE, acetylcholinesterase; AChR, acetylcholine receptor; Ch, choline; ChAT, choline acetyltransferase; NMJ, neuromuscular junction; VGNa+C, voltage-gated sodium channel. A colour version of this figure can be seen in the plate section.

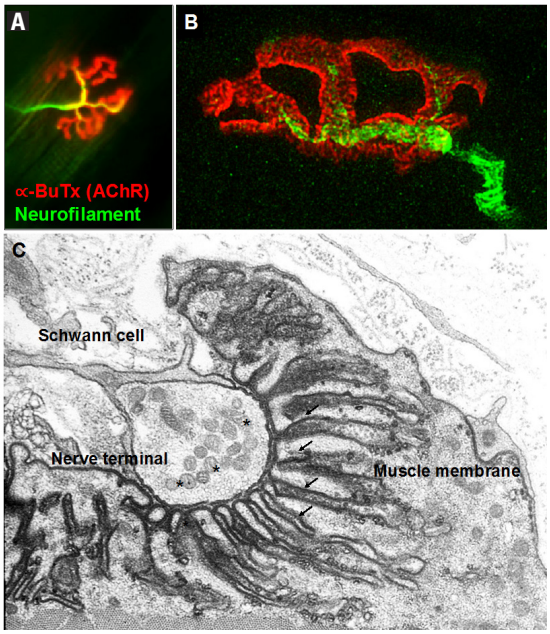

Figure 1.2 Fluorescence and electron microscopy images of the neuromuscular junction. A Fluorescence microscopy image of the NMJ showing the nerve terminal (green) innervating the muscle endplate (red) stained with fluorescently conjugated α-bungarotoxin that binds to the AChRs. **B** Super-resolution confocal microscopy image of the NMJ showing the postsynaptic muscle membrane and the junctional folds at the top of which AChRs are concentrated (image provided by Dr J. Cheung). **C** Electron microscopy image of the NMJ. The presynaptic nerve terminal is filled with synaptic vesicles containing acetylcholine (*). The postsynaptic muscle membrane exhibits a high degree of folding which extends into the muscle sarcoplasm (arrows) in order to increase the total endplate surface. The NMJ is covered by terminal Schwann cells. AChR, acetylcholine receptor; NMJ, neuromuscular junction. Adapted from Slater (2017) with permission. A colour version of this figure can be seen in the plate section.

Postsynaptic Muscle Membrane

The postsynaptic muscle membrane is a specialised structure with a high degree of folding, as shown by electron microscopy studies (De Harven and Coers 1959) (Fig. 1.2). Motor nerve terminals are embedded in the muscle in a gutter or primary cleft. Furthermore, there are a series of invaginations of the muscle membrane that extend into the sarcoplasm which are called secondary junctional folds; these increase the overall surface of the postsynaptic membrane. The acetylcholine receptors (AChRs) are clustered at high density on the crest of these folds. At the bottoms of the folds, voltage-gated Na^+ channels ($VGNa^+C$) are concentrated to facilitate the excitability of the postsynaptic membrane.

NEUROMUSCULAR JUNCTION DEVELOPMENT, MATURATION AND MAINTENANCE

During development, the axons of motor neurons are guided to innervate skeletal muscles through a process that is still not fully understood (Wu et al. 2010). In particular it is unclear whether motor neurons or muscles fibres determine the exact location of the muscle endplate. In vivo studies in mice have shown that primitive AChRs are prepatterned in the central region of the muscle (Fig. 1.3) prior to the arrival of the motor axon (Lin et al. 2001).

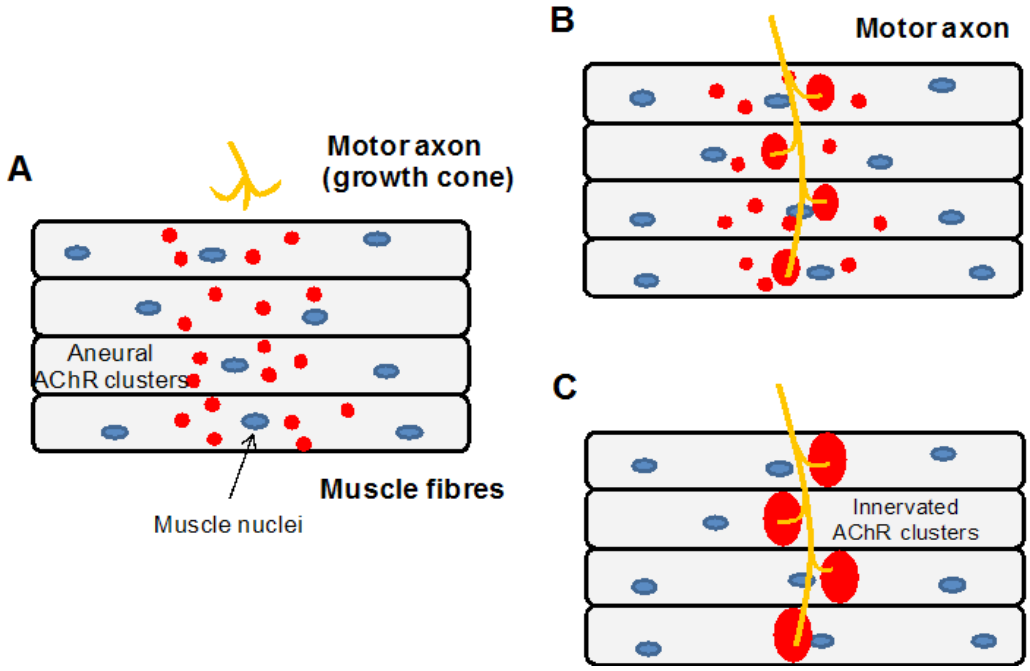

Figure 1.3 **Schematic representation of the neuromuscular junction development. A** Aneural AChR clusters are prepatterned in the midbelly of the muscle fibres prior to the arrival of the nerve terminal. **B** Upon innervation of the prepatterned clusters in the synaptic region, they become enlarged while aneural clusters located in the extrasynaptic region disappear. **C** As a result, AChR clusters are concentrated at a high density in the area underneath the nerve terminal maximising the efficiency of neuromuscular transmission. AChR, acetylcholine receptor. Adapted from Lin et al. (2001) with permission from Springer Nature. A colour version of this figure can be seen in the plate section.

It has been shown that this phenomenon also occurs in mutant animals lacking motor nerves, which suggests that prepatterning is nerve-independent (Yang et al. 2001). Subsequently, when the motor neurons innervate some of the prepatterned AChR clusters these become enlarged, while aneural AChR clusters tend to disappear. It is thought that neuronal agrin (McMahan 1990), a motor neuron-secreted proteoglycan that binds to the postsynaptic muscle membrane, drives the process by activating the LRP4-MuSK-DOK7 pathway which is crucial for the clustering of AChRs underneath the nerve terminal (Fig. 1.4).

On the other hand, aneural clusters not stabilised by agrin signalling are dispersed by a negative signal. This pathway is believed to be driven by the release of acetylcholine by the nerve terminal through a cyclin-dependent kinase 5 (CdK5) mechanism (Lin et al. 2005) linked to the interaction of the intracellular anchoring protein rapsyn and the calcium-dependent protease calpain (Chen et al. 2007).

Disruption of MuSK signalling postnatally is also deleterious for the NMJ structure and function (Barik et al. 2014; Tezuka et al. 2014). Therefore, these mechanisms are not only crucial for the development and maturation of the NMJ but for its maintenance during adulthood. Autoantibodies against MuSK cause autoimmune MuSK myasthenia gravis (Hoch et al. 2001). On the other hand, mutations within the clustering pathway, especially in the *DOK7* gene, are responsible for a meaningful proportion of congenital myasthenic syndromes (Beeson et al. 2006).

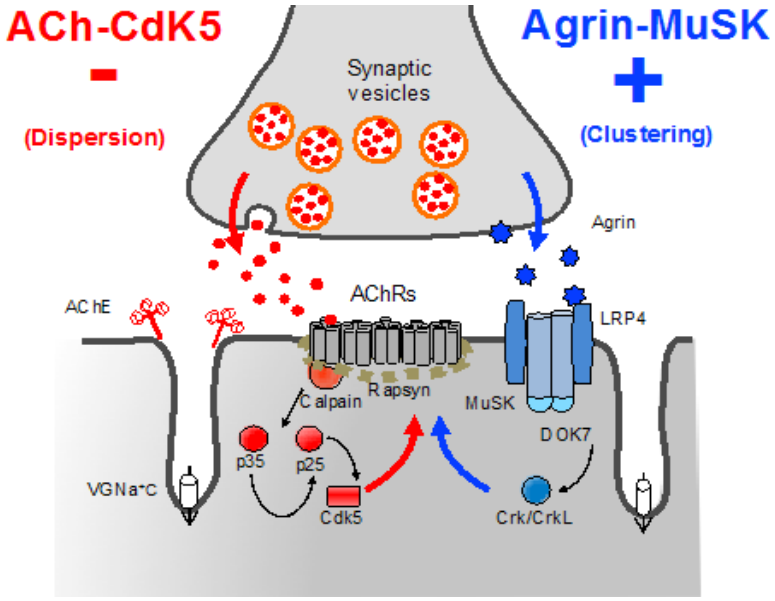

Figure 1.4 Schematic representation the neuromuscular junction and the acetylcholine receptor clustering and dispersal pathways. Upon the release of agrin by the nerve terminal, agrin binds to LRP4 resulting in MuSK dimerisation and activation (Kim et al. 2008). This leads to the recruitment of DOK7 (Okada et al. 2006), a muscle-specific cytoplas- mic adaptor of MuSK that further stimulates MuSK activation propagating the signal downstream which results in the clustering of the AChRs by the cytoplasmic anchoring protein rapsyn. By contrast, ACh is believed to disperse AChR clusters through a cyclin-dependent kinase (CdK5) mechanism linked to the interaction of rapsyn and the calcium-dependent protease calpain (Chen et al. 2007). Calpain activity promotes the cleavage of p35 to p25 (Patrick et al. 1999), an activator of CdK5. Rapsyn is believed to stabilise AChR clusters by suppressing calpain activity. ACh, acetylcholine; AChR, acetylcholine receptor; LRP4, low-density lipoprotein receptor 4; MuSK, muscle-specific kinase; VGNa+C, voltage-gated Na+ channel. Adapted from Rodriguez Cruz et al. (2018) with permission. A colour version of this figure can be seen in the plate section.

Schwann cells

Schwann cells are the main glial cells of the peripheral nervous system (PNS) and can be subdivided into two types: myelinating and non-myelinating. In contrast to myelinating Schwann cells, which ensheath axons to allow efficient electrical impulse propagation, significantly less is known about non-myelinating Schwann cells. There are three types of non-myelinating Schwann cells: Remak SCs, specialised sensory transducers (such as Pacinian corpuscles), and tSCs, which differ in structure and location. tSCs (also known as perisynaptic Schwann cells or teloglia), are non-myelinating Schwann cells that reside at the NMJ and play a vital role in the growth and maintenance of the synapse. They are integral contributors to NMJ function and recovery, and show numerous pathological changes. An improved understanding of tSCs may help decipher the pathogenesis of disease and provide new therapeutic targets for the many patients who suffer from peripheral nerve injuries and neuromuscular diseases (Santosa et al. 2018). A study by Barik et al. (2016) also highlights that Schwann cells are not only required for NMJ formation, but also necessary for its maintenance; and postsynaptic function and structure appear to be more sensitive to Schwann cell ablation.

THE PHYSIOLOGY OF NEUROMUSCULAR TRANSMISSION

Synthesis and recycling of acetylcholine

Acetylcholine is synthesised by the enzyme choline acetyltransferase (ChAT) (Nachmansohn and Machado 1943) from acetyl coenzyme A (AcCoA) and choline. Subsequently, acetylcholine is packed into synaptic vesicles thanks to the vesicular acetylcholine transporter (VAChT) (Roghani et al. 1994). Pools of synaptic vesicles accumulate in the presynaptic terminal near release sites, termed active zones (Südhof 2012), ready to be released upon the arrival of an action potential (Fig. 1.5). The necessary choline for the synthesis of

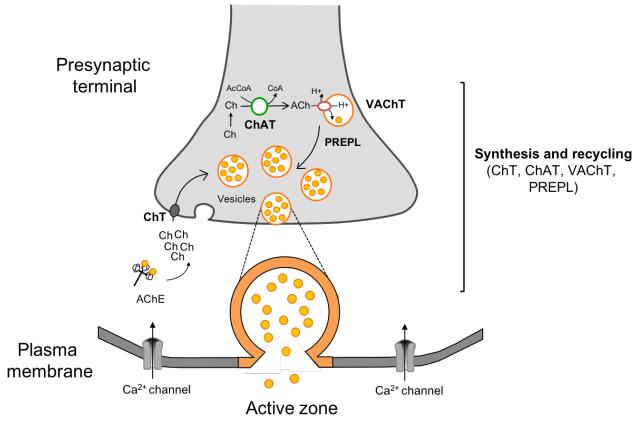

Figure 1.5 Schematic representation of the nerve terminal and the main molecules involved in the synthesis and recycling of acetylcholine. In the synaptic cleft, AChE breaks down acetylcholine (ACh) into acetate and Ch, which is uptaken by the ChT to the presynaptic terminal. The enzyme ChAT catalyses the synthesis of ACh from AcCoA and choline, and the vesicular acetylcholine transporter VAChT loads ACh into synaptic vesicles. *PREPL* encodes a protein thought to be involved in the trafficking of VAChT. Adapted from Ma et al. (2013). AcCoA, acetyl coenzyme A; ACh, acetylcholine; AChE, acetylcholinesterase; AChR, acetylcholine receptor; Ch, choline; ChAT, choline acetyltransferase; ChT, sodium-dependent high-affinity choline transporter 1; NMJ, neuromuscular junction; VAChT, vesicular acetylcholine transporter; VGNa+C, voltage-gated sodium channel. A colour version of this figure can be seen in the plate section.

acetylcholine is uptaken from the synaptic cleft thanks to the high-affinity choline transporter 1 (ChT) after breakdown of acetylcholine. Mutations in the genes encoding ChAT, VAChT and ChT result in a group of congenital myasthenic syndromes characterised by episodic apnoeas (Ohno et al. 2001; Bauché et al. 2016). Antibodies against the voltage-gated calcium channels located in the presynaptic terminal are responsible for Lambert–Eaton myasthenic syndrome (LEMS) (Eaton and Lambert 1957).

Release of Synaptic Vesicles at the Presynaptic Terminal

Synaptic vesicles loaded with ACh need to dock and be primed near release sites, termed active zones (Südhof 2012). Upon the arrival of an action potential, voltage-dependent Ca^{2+} channels open and the influx of Ca^{2+} cause the fusion of vesicles to the plasma membrane through the Soluble N-ethylmaleimide-sensitive factor Attachment protein REceptor (SNARE) complex and associated proteins (Baker and Hughson 2016) (Fig. 6).

Binding of Acetylcholine to the Acetylcholine Receptor

The adult nicotinic AChR is a pentameric complex composed of four different transmembrane subunits (2 α, β, δ and ε–subunits) (Karlin 2002). Acetylcholine released by the nerve terminal binds to the

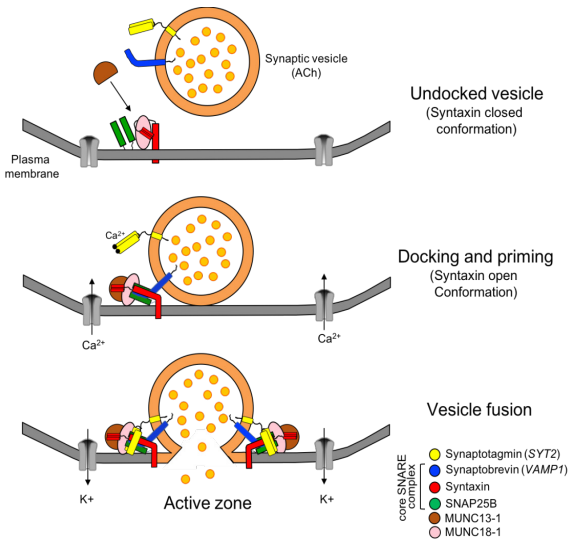

Figure 1.6 Schematic representation of synaptic vesicles at the active zones of presynaptic terminals. The influx of Ca^{2+} following an action potential causes synaptic vesicles to fuse to the plasma membrane through SNARE proteins (synaptobrevin, syntaxin and SNAP25B) releasing the neurotransmitter acetylcholine to the synaptic cleft (Chen and Scheller 2001). The calcium current persists until the membrane potential is returned to normal by outward fluxes of potassium. Munc18-1 is a chaperone that binds to a self-inhibited "closed" conformation of syntaxin-1B and to SNARE complexes (Lai et al. 2017). Munc13-1, is believed to be involved in synaptic vesicle priming prior to vesicle fusion by catalysing the transition of syntaxin 1B from a closed configuration with Munc18-1 into an open state ready to form SNARE complexes and fuse rapidly. The core SNARE complex, a 4-α helix structure formed by the synaptic vesicle-associated synaptobrevin (v-SNARE) and the presynaptic SNAP25B and syntaxin 1B (t-SNARES) proteins, brings the vesicle and plasma membranes together. Finally, calcium-bound Synaptotagmin (a vesicle Ca^{2+} sensor) binds to the SNARE complex causing vesicle fusion and exocytosis. Adapted from Ma et al. (2013). Mutations in *SYT2* (Whittaker et al. 2015), *VAMP1* (Salpietro et al. 2017), *SNAP25B* (Shen et al. 2014) and *MUNC13-1* cause presynaptic CMS. A colour version of this figure can be seen in the plate section.

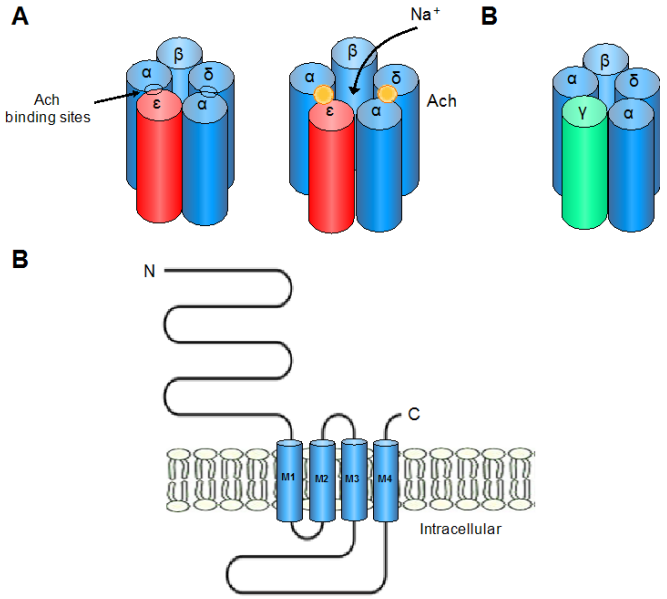

Figure 1.7 **Schematic representation of the adult and fetal acetylcholine receptors. A** The AChR is made up of five subunits arranged around a central pore. The binding of acetylcholine to the ACh binding sites results in a conformational change that allows the influx of sodium into the muscle and the trigger of an EPP. The fetal AChR has a γ-subunit (green) as opposed to the adult AChR, where the γ-subunit is replaced by an ε-subunit (red). **B** Each AChR subunit is composed of an extracellular domain, four transmembrane domains (M1–M4) and a large cytoplasmic loop that links M3 and M4. ACh, acetylcholine; AChR, acetylcholine receptor; EPP, endplate potential. A colour version of this figure can be seen in the plate section.

acetylcholine binding site located at the α and either δ and ε-subunits interfaces of the AChR (Fig. 7). Upon acetylcholine binding, the AChR subunits undergo a conformational change to open the channel creating a pore (Miyazawa et al. 2003). This event allows the influx of positively charged ions to move across the channel generating a change in the membrane potential that triggers an endplate potential (EPP).

Autoantibodies against the muscle AChR nicotinic receptor cause myasthenia gravis, the most common disorder of neuromuscular transmission (Lindstrom et al. 1976). The AChR antibodies are mostly targeted against the main immunogenic region located in the α-subunit. They cause disease by different pathogenic mechanisms such as complement activation, cross-linking and internalisation of AChRs (Le Panse and Berrih-Aknin 2013). Interestingly, there are patients with autoantibodies against the fetal γ-subunit of the AChR, which is present prenatally but substituted by the ε-subunit before birth, at approximately 33 weeks (AChR γ-to-ε switch) (Missias et al. 1996). As a result, these antibodies have no effect in adults but they can cause neonatal myasthenia (Vernet-Der Garabedian et al. 1994) or arthrogryposis multiplex congenita (Oskoui et al. 2008) via maternal transfer in the fetus.

Mutations in the genes encoding any of the adult AChR subunits can cause AChR deficiency. This is one of the most common congenital myasthenic syndrome (Finlayson 2013), which is characterised by reduced AChR numbers at the endplate (10–30% of normal values) (Vincent et al. 1993). Most of the mutations occur in the ε-subunits and result in protein truncation or loss of essential residues for AChR assembly or function (Abicht et al. 2016). It is believed that incorporation of the fetal AChR γ-subunit into the AChR pentamer enables individuals with null ε-subunits alleles to survive (Cossins et al. 2004). Mutations in the AChR subunits can also alter ion channel function leading to prolonged openings (slow

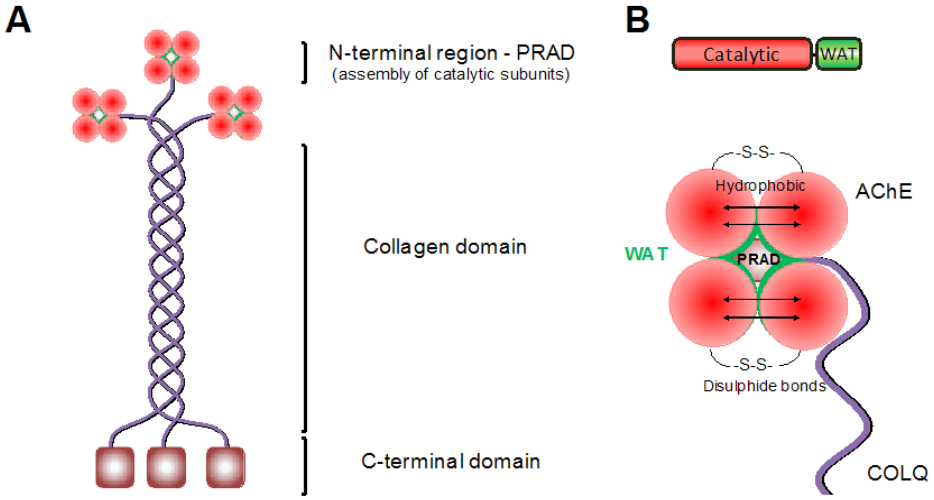

Figure 1.8 **Schematic representation of acetylcholinesterase and its collagenic-like tail. A** ColQ is composed of a N-terminal domain with a Proline Rich Attachment Domain (PRAD) that binds to the catalytic subunits of AChE, a triple-helix collagenic domain, and a C-terminal domain. **B** AChE is composed of a catalytic domain followed by a WAT (tryptophan [W] amphiphilic tetramerisation) domain at the C-terminal region. The association between catalytic subunits is primarily driven by the interaction between WAT domains (green colour) and PRAD (Chen et al. 2011). Additional forces include hydrophobic interactions and disulphide bonds. AChE, acetylcholinesterase. A colour version of this figure can be seen in the plate section.

channel syndrome; SCS) (Ohno et al. 1995) or abbreviated openings (fast channel syndrome; FCS) (Sine et al. 2003).

The Margin of Safety of Neuromuscular Transmission

The depolarisation of the postsynaptic membrane that follows the binding of acetylcholine to the AChR generates an endplate potential (EPP), which is greater than the threshold needed to activate the $Na_v1.4$ voltage-gated sodium channels that help trigger an action potential (Engel et al. 2015). Under different stresses or conditions, such as the myasthenic disorders, this margin can be affected leading to abnormal neuromuscular transmission and fatigable muscle weakness (Wood and Slater 2001). Finally, the action potential spreads from the motor endplate to the rest of the muscle sarcolemma generating the contraction of the muscle.

Breakdown of Acetylcholine by Acetylcholinesterase

Acetylcholinesterase (AChE) helps to terminate synaptic transmission by breaking down acetylcholine. AChE is linked to the synaptic basal lamina by a collagenic-like tail, also known as ColQ (Fig. 1.8).

THE N-GLYCOSYLATION PATHWAY OF PROTEINS

The *N*-linked glycosylation pathway is a ubiquitous process in eukaryote cells defined by the sequential attachment of sugar moieties to the lipid dolichol, which is then transferred to an asparagine residue in a protein (Fig. 9). Next generation sequencing has aided the discovery of an unexpected relationship between

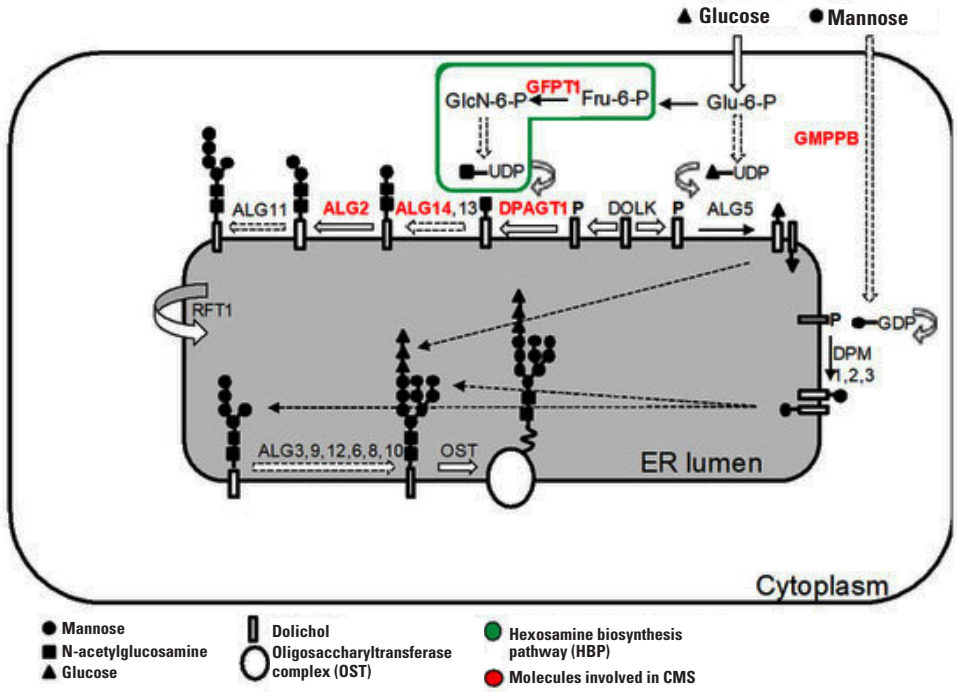

Figure 1.9 Simplified representation of the *N*-glycosylation pathway of proteins. The *N*-linked glycosylation of proteins takes place in the endoplasmic reticulum. It starts with the assembly of the core glycan (*N*-acetylglucosamine, glucose and mannose) on the lipid dolichol. A series of cytosolic glycosyltransferases proceed to dolichol glycosylation on the cytoplasmic face of the endoplasmic reticulum: GFPT1 synthesises UDP-GlcNAc (uridine diphosphate *N*-acetylglucosamine); DPAGT1 and the ALG13/14 complex are involved in adding the first and second *N*-acetylglucosamine to dolichol. Additional sugar residues are added by ALG2 and other enzymes until the resulting product is flipped into the endoplasmic reticulum lumen by RFT1. Inside the endoplasmic reticulum lumen, sugar moieties are incorporated until the glycan is transferred to asparagine residues of nascent proteins by the multimeric oligosaccharyl transferase complex (OST) that subsequently will be modified inside the endoplasmic reticulum and Golgi. DOLK, dolichol kinase; DPM, dolichol-phosphate mannose synthase; Fru-6-P, fructose-6-phosphate; GlcN-6-P, glucosamine-6-phosphate; Glu-6-P, glucose-6-phosphate; GMPPB, GDP-mannose pyrophosphorylase B. Adapted from Rodríguez Cruz (2016) with permission from BMJ Publishing Group Limited. A colour version of this figure can be seen in the plate section.

glycosylation defects in the early stages of the *N*-glycosylation pathway and myasthenic disorders (Senderek et al. 2011; Belaya et al. 2012, 2015). This highlights that genes with no defined function in neuromuscular transmission can also impair the communication between the nerve and the muscle. The reasons why, in certain cases, defects in a ubiquitous process result in dysfunction largely restricted to the NMJ is unclear.

Glycosylation of AChR subunits is required for the correct assembly of AChR pentamers and for efficient export to the cell surface (Gehle et al. 1997) and thus abnormal glycosylation results in reduced AChRs at the muscle endplates, which is most likely the primary mechanisms leading to impaired neuromuscular transmission (Zoltowska et al. 2013). Patients with mutations in the glycosylation genes constitute a distinctive clinical group where muscle weakness is often confined to the limb girdles, and classic myasthenic manifestations – such as ptosis, ophthalmoplegia or facial weakness – are not present (Finlayson et al. 2013; Rodríguez Cruz et al. 2016). Concomitant myopathy is often present, which makes it a progressive condition over time.

REFERENCES

Abicht A, Müller JS, Lochmüller H (2016) *Congenital myasthenic syndromes.* In: Adam MP, Ardinger HH, Pagon RA, et al. (Eds), *Gene Reviews®*. Seattle, WA: University of Washington.

Baker RW, Hughson FM (2016) Chaperoning SNARE assembly and disassembly. *Nat Rev Mol Cell Biol* **17:** 465–479.

Barik A, Lu Y, Sathyamurthy A et al. (2014) LRP4 is critical for neuromuscular junction maintenance. *J Neurosci* **34:** 13892–13905.

Barik A, Li L, Sathyamurthy A, Xiong WC, Mei L (2016) Schwann cells in neuromuscular junction formation and maintenance. *J Neurosci* **36:** 9770–9781.

Bauché S, O'Regan S, Azuma Y et al. (2016) Impaired presynaptic high-affinity choline transporter causes a congenital myasthenic syndrome with episodic apnea. *Am J Hum Genet* **99:** 753–761.

Beeson D, Higuchi O, Palace J et al. (2006) Dok-7 mutations underlie neuromuscular junction synaptopathy. *Science* **313:** 1975–1978.

Belaya K, Finlayson S, Slater CR et al. (2012) Mutations in DPAGT1 cause a limb-girdle congenital myasthenic syndrome with tubular aggregates. *Am J Hum Genet* **91:** 193–201.

Belaya K, Rodríguez Cruz PM, Liu WW et al. (2015) Mutations in GMPPB cause congenital myasthenic syndrome and bridge myasthenic disorders with dystroglycanopathies. *Brain* **138:** 2493–2504.

Chen F, Qian L, Yang ZH et al. (2007) Rapsyn interaction with calpain stabilizes AChR clusters at the neuromuscular junction. *Neuron* **55:** 247–260.

Chen YA, Scheller RH (2001) SNARE-mediated membrane fusion. *Nat Rev Mol Cell Biol* **2:** 98–106.

Chen VP, Luk WK, Chan WK et al. (2011) Molecular assembly and biosynthesis of acetylcholinesterase in brain and muscle: the roles of t-peptide, FHB domain, and N-linked glycosylation. *Front Mol Neurosci* **4:** 36.

Cossins J, Webster R, Maxwell S et al. (2004) A mouse model of AChR deficiency syndrome with a phenotype reflecting the human condition. *Hum Mol Genet* **13:** 2947–2957.

Desaki J, Uehara Y (1981) The overall morphology of neuromuscular junctions as revealed by scanning electron microscopy. *J Neurocytol* **10:** 101–110.

Eaton LM, Lambert EH (1957) Electromyography and electric stimulation of nerves in diseases of motor unit; observations on myasthenic syndrome associated with malignant tumors. *J Am Med Assoc* **163:** 1117–1124.

Engel AG, Shen XM, Selcen D, Sine SM (2015) Congenital myasthenic syndromes: pathogenesis, diagnosis, and treatment. *Lancet Neurol* **14:** 420–434.

Feng Z, Ko C-P (2008) The role of glial cells in the formation and maintenance of the neuromuscular junction. *Ann N Y Acad Sci* **1132:** 19–28.

Finlayson S, Beeson D, Palace J (2013) Congenital myasthenic syndromes: an update. *Pract Neurol* **13:** 80–91.

Finlayson S, Palace J, Belaya K et al. (2013) Clinical features of congenital myasthenic syndrome due to mutations in DPAGT1. *J Neurol Neurosurg Psychiatry* **84:** 1119–1125.

Gehle VM, Walcott EC, Nishizaki T, Sumikawa K (1997) N-glycosylation at the conserved sites ensures the expression of properly folded functional ACh receptors. *Brain Res Mol Brain Res* **45:** 219–229.

De Harven E, Coers C (1959) Electron microscope study of the human neuromuscular junction. *J Biophys Biochem Cytol* **6:** 7–10.

Hoch W, McConville J, Helms S, Newsom-Davis J, Melms A, Vincent A (2001) Auto-antibodies to the receptor tyrosine kinase MuSK in patients with myasthenia gravis without acetylcholine receptor antibodies. *Nat Med* **7:** 365–368.

Jones RA, Harrison C, Eaton SL et al. (2017) Cellular and molecular anatomy of the human neuromuscular junction. *Cell Rep* **21:** 2348–2356.

Karlin A (2002) Emerging structure of the nicotinic acetylcholine receptors. *Nat Rev Neurosci* **3:** 102–114.

Kim N, Stiegler AL, Cameron TO et al. (2008) Lrp4 is a receptor for Agrin and forms a complex with MuSK. *Cell* **135:** 334–342.

Lai Y, Choi UB, Leitz J et al. (2017) Molecular mechanisms of synaptic vesicle priming by Munc13 and Munc18. *Neuron* **95:** 591–607.e10.

Le Panse R, Berrih-Aknin S (2013) Autoimmune myasthenia gravis: autoantibody mechanisms and new developments on immune regulation. *Curr Opin Neurol* **26:** 569–576.

Lin W, Burgess RW, Dominguez B, Pfaff SL, Sanes JR, Lee KF (2001) Distinct roles of nerve and muscle in postsynaptic differentiation of the neuromuscular synapse. *Nature* **410:** 1057–1064.

Lin W, Dominguez B, Yang J et al. (2005) Neurotransmitter acetylcholine negatively regulates neuromuscular synapse formation by a Cdk5-dependent mechanism. *Neuron* **46:** 569–579.

Lindstrom JM, Seybold ME, Lennon VA, Whittingham S, Duane DD (1976) Antibody to acetylcholine receptor in myasthenia gravis. Prevalence, clinical correlates, and diagnostic value. *Neurology* **26:** 1054–1059.

Ma C, Su L, Seven AB, Xu Y, Rizo J (2013) Reconstitution of the vital functions of Munc18 and Munc13 in neurotransmitter release. *Science* **339:** 421–425.

McMahan UJ (1990) The agrin hypothesis. *Cold Spring Harb Symp Quant Biol* **55:** 407–418.

Missias AC, Chu GC, Klocke BJ, Sanes JR, Merlie JP (1996) Maturation of the acetylcholine receptor in skeletal muscle: regulation of the AChR γ-to-∈ switch. *Dev Biol* **179:** 223–238.

Miyazawa A, Fujiyoshi Y, Unwin N (2003) Structure and gating mechanism of the acetylcholine receptor pore. *Nature* **423:** 949–955.

Nachmansohn D, Machado A (1943) The formation of acetylcholine. A new enzyme: "choline acetylase". *J Neurophysiol* **5:** 397–403.

Ohno K, Hutchinson DO, Milone M et al. (1995) Congenital myasthenic syndrome caused by prolonged acetylcholine receptor channel openings due to a mutation in the M2 domain of the ∈ subunit. *Proc Natl Acad Sci USA* **92:** 758–762.

Ohno K, Brengman J, Tsujino A, Engel AG (1998) Human endplate acetylcholinesterase deficiency caused by mutations in the collagen-like tail subunit (ColQ) of the asymmetric enzyme. *Proc Natl Acad Sci USA* **95:** 9654–9659.

Ohno K, Tsujino A, Brengman JM et al. (2001) Choline acetyltransferase mutations cause myasthenic syndrome associated with episodic apnea in humans. *Proc Natl Acad Sci USA* **98:** 2017–2022.

Okada K, Inoue A, Okada M et al. (2006) The muscle protein Dok-7 is essential for neuromuscular synaptogenesis. *Science* **312:** 1802–1805.

Oskoui M, Jacobson L, Chung WK et al. (2008) Fetal acetylcholine receptor inactivation syndrome and maternal myasthenia gravis. *Neurology* **71:** 2010–2012.

Patrick GN, Zukerberg L, Nikolic M, de la Monte S, Dikkes P, Tsai L-H (1999) Conversion of p35 to p25 deregulates Cdk5 activity and promotes neurodegeneration. *Nature* **402:** 615–622.

Rodríguez Cruz PM, Belaya K, Basiri K et al. (2016) Clinical features of the myasthenic syndrome arising from mu- tations in GMPPB. *J Neurol Neurosurg Psychiatry* **87:** 802–809.

Rodríguez Cruz PM, Palace J, Beeson D (2018) The Neuromuscular junction and wide heterogeneity of congenital myasthenic syndromes. *Int. J. Mol*. Sci. **19:** 1677.

Roghani A, Feldman J, Kohan SA et al. (1994) Molecular cloning of a putative vesicular transporter for acetylcholine. *Proc Natl Acad Sci USA* **91:** 10620–10624.

Salpietro V, Lin W, Delle Vedove A et al. (2017) Homozygous mutations in VAMP1 cause a presynaptic congenital myasthenic syndrome. *Ann Neurol* **81:** 597–603.

Sanes JR (2003) The basement membrane/basal lamina of skeletal muscle. *J Biol Chem* **278:** 12601–12604.

Santosa KB, Keane AM, Jablonka-Shariff A, Vannucci B, Snyder-Warwick AK (2018) Clinical relevance of terminal Schwann cells: an overlooked component of the neuromuscular junction. *J Neurosci Res* **96:** 1125–1135.

Senderek J, Müller JS, Dusl M et al. (2011) Hexosamine biosynthetic pathway mutations cause neuromuscular transmission defect. *Am J Hum Genet* **88:** 162–172.

Shen XM, Selcen D, Brengman J, Engel AG (2014) Mutant SNAP25B causes myasthenia, cortical hyperexcitability, ataxia, and intellectual disability. *Neurology* **83:** 2247–2255.

Shi L, Fu AKY, Ip NY (2012) Molecular mechanisms underlying maturation and maintenance of the vertebrate neuromuscular junction. *Trends Neurosci* **35:** 441–453.

Sine SM, Wang HL, Ohno K, Shen XM, Lee WY, Engel AG (2003) Mechanistic diversity underlying fast channel congenital myasthenic syndromes. *Ann N Y Acad Sci* **998:** 128–137.

Slater CR (2017) The structure of human neuromuscular junctions: some unanswered molecular questions. *Int J Mol Sci* **18:** E2183.

Südhof TC (2012) The presynaptic active zone. *Neuron* **75:** 11–25.

Tezuka T, Inoue A, Hoshi T et al. (2014) The MuSK activator agrin has a separate role essential for postnatal maintenance of neuromuscular synapses. *Proc Natl Acad Sci USA* **111:** 16556–16561.

Vernet-der Garabedian B, Lacokova M, Eymard B et al. (1994) Association of neonatal myasthenia gravis with antibodies against the fetal acetylcholine receptor. *J Clin Invest* **94:** 555–559.

Vincent A, Newsom-Davis J, Wray D et al. (1993) Clinical and experimental observations in patients with congenital myasthenic syndromes. *Ann N Y Acad Sc* **681:** 451–460.

Whittaker RG, Herrmann DN, Bansagi B et al. (2015) Electrophysiologic features of SYT2 mutations causing a treatable neuromuscular syndrome. *Neurology* **85:** 1964–1971.

Wood SJ, Slater CR (2001) Safety factor at the neuromuscular junction. *Prog Neurobiol* **64:** 393–429.

Wu H, Xiong WC, Mei L (2010) To build a synapse: signaling pathways in neuromuscular junction assembly. *Development* **137:** 1017–1033.

Yang X, Arber S, William C et al. (2001) Patterning of muscle acetylcholine receptor gene expression in the absence of motor innervation. *Neuron* **30:** 399–410.

Zoltowska K, Webster R, Finlayson S et al. (2013) Mutations in GFPT1 that underlie limb-girdle congenital myasthenic syndrome result in reduced cell-surface expression of muscle AChR. *Hum Mol Genet* **22:** 2905–2913.

Zuber B, Unwin N (2013) Structure and superorganization of acetylcholine receptor-rapsyn complexes. *Proc Natl Acad Sci USA* **110:** 10622–10627.