The International Journal of Biochemistry & Cell Biology 43 (2011) 295–298

Contents lists available at ScienceDirect

The International Journal of Biochemistry & Cell Biology

journal homepage: www.elsevier.com/locate/biocel

Molecules in focus

Muscle Specific Kinase: Organiser of synaptic membrane domains

Nazanin Ghazanfari[a], Kristine J. Fernandez[a], Yui Murata[a], Marco Morsch[a], Shyuan T. Ngo[b], Stephen W. Reddel[c], Peter G. Noakes[b], William D. Phillips[a,*]

[a] Physiology and Bosch Institute, Anderson Stuart Bldg (F13), The University of Sydney, Sydney, NSW 2006, Australia
[b] School of Biomedical Sciences, University of Queensland, St Lucia, QLD 4072, Australia
[c] Dept of Molecular Medicine, Concord Hospital, Concord, NSW 2139, Australia

ARTICLE INFO

Article history:
Received 23 September 2010
Received in revised form 15 October 2010
Accepted 15 October 2010
Available online 23 October 2010

Keywords:
MuSK
LRP4
Synapse formation
Tyrosine kinases
Agrin

ABSTRACT

Muscle Specific Kinase (MuSK) is a transmembrane tyrosine kinase vital for forming and maintaining the mammalian neuromuscular junction (NMJ: the synapse between motor nerve and skeletal muscle). MuSK expression switches on during skeletal muscle differentiation. MuSK then becomes restricted to the postsynaptic membrane of the NMJ, where it functions to cluster acetylcholine receptors (AChRs). The expression, activation and turnover of MuSK are each regulated by signals from the motor nerve terminal. MuSK forms the core of an emerging signalling complex that can be acutely activated by neural agrin (N-agrin), a heparin sulfate proteoglycan secreted from the nerve terminal. MuSK activation initiates complex intracellular signalling events that coordinate the local synthesis and assembly of synaptic proteins. The importance of MuSK as a synapse organiser is highlighted by cases of autoimmune myasthenia gravis in which MuSK autoantibodies can deplete MuSK from the postsynaptic membrane, leading to complete disassembly of the adult NMJ.

© 2010 Elsevier Ltd. All rights reserved.

1. Introduction

Muscle Specific Kinase (MuSK) is a 100 kDa transmembrane tyrosine kinase originally purified from the (synapse-rich) electric organ of the ray, *Torpedo californica* (Jennings et al., 1993). At the vertebrate neuromuscular junction (NMJ), terminals of motor axons release vesicle loads of acetylcholine onto postsynaptic acetylcholine receptors (AChR; Fig. 1A) and thereby initiate muscle contraction. Thus, effective neurotransmission depends upon tight packing of postsynaptic AChRs into AChR clusters. MuSK is essential for the stability of these AChR clusters and is concentrated within them (DeChiara et al., 1996; Kummer et al., 2006; Wu et al., 2010). N-agrin, a heparan-sulfate proteoglycan secreted from the motor nerve terminal, can initiate MuSK autophosphorylation, thereby activating MuSK (Mittaud et al., 2004). In turn, this drives diverse downstream signalling systems that reorganise the actin cytoskeleton and recruit AChR-binding scaffolding proteins such as rapsyn to cluster AChRs.

2. Structure of MuSK and the MuSK complex

The gene *Musk* is found on mouse chromosome 4 (the human orthologue is on chromosome 9). Fourteen exons give rise to several transcripts, most of which encode the full suite of polypeptide modules represented in Fig. 1B. The extracellular region consists of four immunoglobulin-like (Ig) domains and a cysteine-rich domain (C6). The cytoplasmic, juxtamembrane domain (JM), adjacent the transmembrane domain (TM), is followed by a (tyrosine kinase) catalytic domain.

MuSK forms the core of a multi-protein signalling complex (Fig. 1C). N-agrin does not interact directly with MuSK but rather binds low-density lipoprotein receptor-related protein 4 (LRP4; Kim et al., 2008; Zhang et al., 2008). LRP4 and MuSK interact via their respective extracellular domains. Another key player in MuSK activation is the adaptor protein downstream-of-tyrosine-kinase-7 (Dok7). Dok7 binds to a tyrosine-phosphorylated motif in the JM domain of MuSK ($NPXY_{553}$, Fig. 1B; Bergamin et al., 2010; Okada et al., 2006). The tumourous imaginal discs protein (Tid1) binds constitutively to the cytoplasmic portion of MuSK (Linnoila et al., 2008). Dishevelled (Dvl) binds the JM and kinase domains of MuSK, coupling MuSK to p21-activated kinase (PAK1; Fig. 1C; Luo et al., 2002). All of these components of the MuSK signalling complex are required to mediate AChR clustering in response to N-agrin.

* Corresponding author. Tel.: +61 2 9351 4598; fax: +61 2 9351 2058.
E-mail addresses: nazanin@physiol.usyd.edu.au (N. Ghazanfari), kfer7988@physiol.usyd.edu.au (K.J. Fernandez), ymur7519@uni.sydney.edu.au (Y. Murata), mm@physiol.usyd.edu.au (M. Morsch), s.ngo@uq.edu.au (S.T. Ngo), stephenr@med.usyd.edu.au (S.W. Reddel), p.noakes@uq.edu.au (P.G. Noakes), william.phillips@sydney.edu.au (W.D. Phillips).

1357-2725/$ – see front matter © 2010 Elsevier Ltd. All rights reserved.
doi:10.1016/j.biocel.2010.10.008

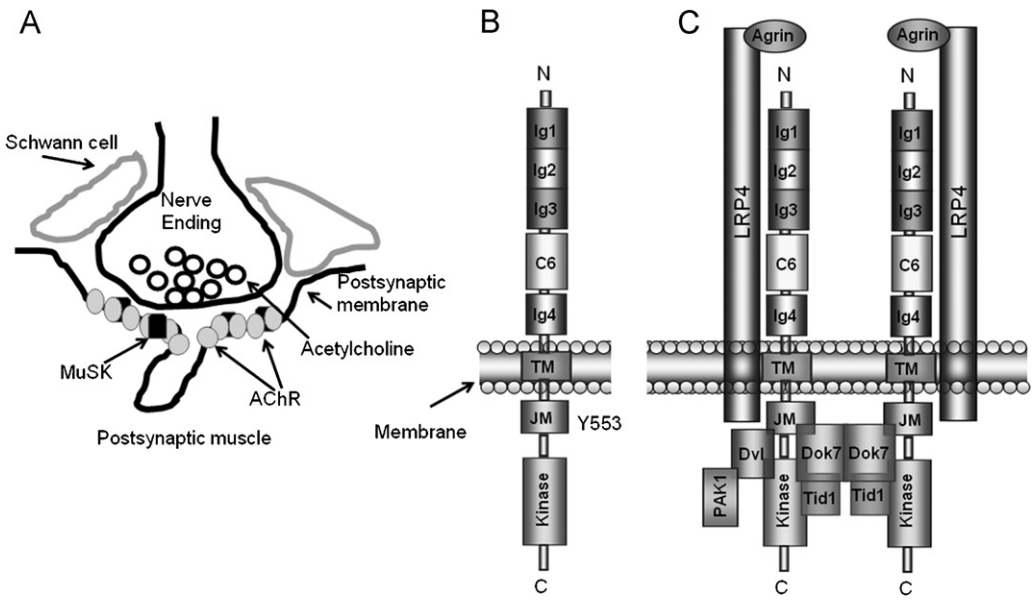

Fig. 1. Structure of the MuSK signalling complex and its synaptic context. (A) At the NMJ the nerve terminal sits in a synaptic gutter, separated from the postsynaptic (muscle) membrane by a 50 nm synaptic cleft. Schwann (glial) cells loosely enwrap the nerve terminal. AChRs are tightly packed into clusters beneath the sites of exocytosis of acetylcholine. (B) Domain structure of the ~100 kDa MuSK polypeptide (N-terminus is extracellular). (C) The assembled (dimeric) MuSK signalling complex. See text for abbreviations.

3. Expression, activation and turnover of MuSK

The MuSK gene promoter contains an E-box (CAGCTG) that is the target for the transcription factor, myogenin. This mediates the strong developmental up-regulation of MuSK as pre-muscle cells differentiate, and the subsequent down-regulation when each fiber becomes innervated (Tang et al., 2006). Each muscle fiber contains a string of nuclei, most of them distant from the NMJ. As the developing NMJ begins to evoke calcium fluxes in the muscle, MuSK expression becomes restricted to the postsynaptic membrane portion of each muscle fiber. A small group of nuclei located beneath the postsynaptic membrane continue to transcribe high levels of mRNAs for synaptic genes such as MuSK and AChR (ε- and δ-subunits). In addition to E-boxes, the promoters of these genes contain N-box elements (GTACCGGAAATA). MuSK signalling is thought to act locally via transcriptional activators of the ETS-family (Lacazette et al., 2003). These bind the N-box and so may drive ongoing transcription of MuSK and AChR in sub-synaptic nuclei.

N-agrin is the best-understood activator of MuSK (Fig. 2A). Binding of N-agrin to LRP4 enhances the LRP4–MuSK interaction and activates MuSK (Kim et al., 2008; Zhang et al., 2008). The first step in MuSK activation appears to be the autophosphorylation of Y_{553} in the $NPXY_{553}$ motif of JM (Fig. 1B; Till et al., 2002). This recruits Dok7 (via its phosphotyrosine binding domain) creating a tetramer in which a Dok7 dimer cross-links two MuSK monomers (Fig. 1C; Bergamin et al., 2010). Crystal structure of the intracellular portion of MuSK indicates that the catalytic domain is tightly auto-inhibited by an activation loop that blocks access to both the ATP- and substrate-binding sites (Till et al., 2002). Studies with the isolated intracellular portion of MuSK suggest that the autophosphorylation of Y_{553} precedes that of three other tyrosine residues (750, 754, and 755) located in the activation loop of the kinase domain (Till et al., 2002). Phosphorylation of the latter three tyrosines is thought to release autoinhibition, switching MuSK to a stable active state.

Several factors affect MuSK function. The MuSK complex has an intrinsic capacity to organise AChR clusters (Lin et al., 2001). In the absence of N-agrin, higher membrane densities of MuSK (expression level) increased AChR clustering, presumably via stochastic MuSK dimerization/activation events (Kim and Burden, 2008). Likewise the tendency of LRP4 to self-associate might contribute to basal activity of LRP4–MuSK complexes (Kim et al., 2008). In drosophila, Wnt-family glycoproteins are secreted by the motor nerve and act via postsynaptic receptors (Frizzled and Derailed) to regulate NMJ formation (Wu et al., 2010). Mouse spinal cord and muscles express Wnt11. In zebrafish the homologous Wnt was reported to bind directly to the C6 domain of MuSK. Thus, while details remain uncertain, Wnts secreted by mammalian nerve and/or muscle might represent an additional modulator of MuSK signalling. Casein kinase 2 (CK2) phosphorylates serine residues in the kinase insert of MuSK, thereby enhancing AChR clustering (Cheusova et al., 2006). As with the actions of Wnt ligands, the precise mechanism by which serine phosphorylation by CK2 influences MuSK signalling remains to be determined.

MuSK activation and turnover both involve a regulated internalisation process (Fig. 2C). Rodent and human MuSK share a carboxyl-terminus (-VXV) that facilitates binding of the E3 ubiquitin ligase, PDZRN3. When muscle cells are exposed to N-agrin, MuSK becomes a substrate for PDZRN3 (Lu et al., 2007). Overexpression of PDZRN3 in heterologous cells reduced the surface expression of co-transfected MuSK, while down-regulation of endogenous PDZRN3 in muscle cells increased surface levels of MuSK. Hence, N-agrin-induced ubiquitination leads to internalisation of MuSK (Fig. 2C pathway 11). Others demonstrated that N-ethylmalemide Sensitive Factor (NSF) was needed for the N-agrin-induced activation and internalisation of MuSK (Zhu et al., 2008). MuSK activation and internalisation appear to be coupled (Fig. 2C pathway 13). N-agrin also triggers binding of MuSK to caveolin-3 (Hezel et al., 2010). It is not certain yet whether caveolae serve as the vehicle for internalisation of activated MuSK. Nor do we know whether internalised MuSK is subsequently recycled to the plasma membrane or targeted for degradation.

4. Biological function of MuSK

MuSK plays a central coordinating role in the formation of the NMJ during embryonic development. Mouse embryos lacking

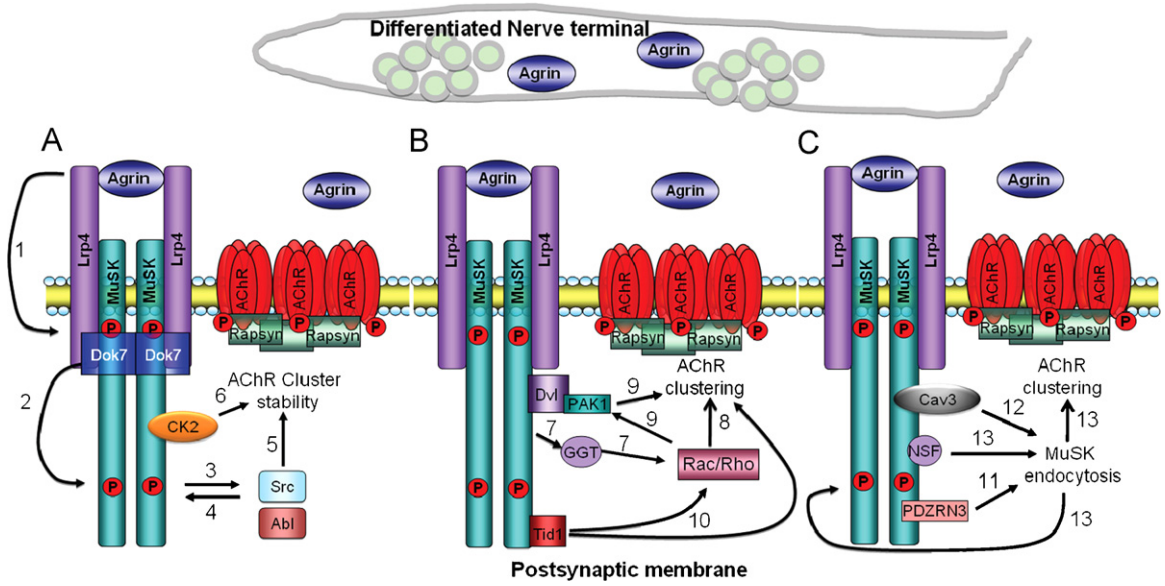

Fig. 2. Activation of the MuSK complex drives AChR clustering. (A–C) Aspects of MuSK signalling initiated by the ligand, N-agrin. Numbered arrows represent signalling interactions between components of the postsynaptic MuSK protein complex and downstream effectors of AChR clustering. (A) (1) Binding of N-agrin to LRP4–MuSK leads to autophosphorylation of Y_{553}, thereby recruiting a dimer of Dok7. (2) Formation of a stable MuSK-Dok7 (2 + 2) tetramer probably favours further autophosphorylation of tyrosines within the activation loop, disinhibiting the catalytic domain. (3) This, in turn, activates non-receptor tyrosine kinases such as Abl and Src. (4) These kinases can serve as positive feedback for MuSK activation. (5) Abl and Src are important for stabilising nascent AChR clusters. Stabilisation involves tyrosine phosphorylation of the AChR β-subunit. This recruits rapsyn linking AChR to the cytoskeleton. (6) Casein kinase 2 (CK2) phosphorylates serine residues on MuSK, favouring AChR cluster stability. (B) Reorganisation of the cortical cytoskeleton is critical for formation of AChR clusters. (7) MuSK activates geranylgeranyltransferase I (GGT) which, in turn is needed to activate the small GTPase, Rho. (8) Successive activation of Rac then Rho leads to the assembly of AChR microaggregates and large clusters respectively. (9) Activated Rac acts via PAK I kinase to facilitate actin cytoskeleton-reorganisation and AChR clustering. (10) Tid1 may act via small GTPases (such as Rac) and/or via heat shock proteins (such as Hsp70) to drive AChR clustering. (C) Internalisation is coupled to MuSK activation. (11) Activation leads to ubiquitination of MuSK by the E3 ligase PDZRN3. (12) Activated MuSK is recruited to caveolae by binding to caveolin3. (13) NSF-dependent internalisation of MuSK is coupled to full activation of MuSK and AChR clustering. See text for abbreviations and citations.

MuSK fail to form stable NMJs. The paralysed embryos die just before birth (DeChiara et al., 1996). MuSK is essential for targeting AChRs, acetylcholinesterase and a variety of other postsynaptic proteins to the developing synapse (Kummer et al., 2006). During embryonic development, AChR clusters form on immature muscle fibers prior to, and independent of, the motor nerves (Harris, 1981; Lin et al., 2001). These 'prepatterned' AChR clusters are restricted to the central part of the fiber where MuSK is most highly expressed and where nerves will shortly innervate.

N-agrin-MuSK signalling appears necessary to stabilise these embryonic synapses. When an actin promoter was used to artificially drive high levels of MuSK expression throughout the embryonic muscle fiber, additional AChR clusters appeared in more distal parts of the fibre (Kim and Burden, 2008). Some of these AChR clusters became innervated, suggesting that a local high density (and/or activity) of MuSK is sufficient to prefigure the site of synapse formation on the target muscle fiber. Studies in zebrafish embryos suggest MuSK may also play a role in guiding growing motor axons (Wu et al., 2010).

The signalling pathways through which MuSK acts to build an AChR-rich postsynaptic membrane remain to be fully defined (Fig. 2). However, assembly of AChRs into AChR clusters requires the small GTPases Rac and Rho (Weston et al., 2003). When N-agrin is added to muscle cells, MuSK activation and tyrosine phosphorylation of geranylgeranyltransferase I leads to activation of Rac I and formation of tiny AChR micro-aggregates (Fig. 2B pathways 7–8; Luo et al., 2003). Rac activation is followed by the activation of Rho, which is thought to act via PAK I to consolidate constellations of AChR micro-aggregates into large (>10 μm diameter) AChR clusters (Fig. 2B pathway 9; Luo et al., 2002). The cytoplasmic tyrosine kinases Abl and Src are also activated by MuSK. They play overlapping roles in sustaining MuSK activation and fostering tyrosine phosphorylation of the AChR β-subunit (Fig. 2A pathways 3–5; Mittaud et al., 2004). Tyrosine phosphorylation of the AChR β-subunit (Y_{390}), recruits the adaptor protein, rapsyn, thereby stabilising the AChR cluster (Borges et al., 2008).

Positive feedback cycles characterise MuSK-mediated membrane domain construction. For example, Abl kinase acts back to bolster MuSK phosphorylation (Fig. 2A pathways 3–4). Through such local, positive feedback pathways small MuSK–AChR clusters tend to grow into bigger membrane domains, recruiting more MuSK, rapsyn and AChR. Other systems act to counter the growth of AChR clusters. MuSK activation initiates a negative-feedback pathway involving the tyrosine phosphatase, Shp2 (Qian et al., 2008). Furthermore, acetylcholine released by the nascent nerve terminal acts via postsynaptic calpain and cyclin-dependent kinase 5 (CDK5) to drive disassembly of AChR clusters (Kummer et al., 2006). The N-agrin-MuSK-rapsyn system acts locally to inhibit this disassembly pathway, by sequestering calpain (Chen et al., 2007). Thus, the growth, homeostasis and remodelling of the immature AChR cluster appears to depend upon the balance of local positive and negative signalling pathways.

MuSK is also expressed in mammalian brain and sperm. To test the significance of MuSK in central neurons, antisense oligonucleotide was injected into the hippocampal regions of the rat brain (Garcia-Osta et al., 2006). Suppression of MuSK expression in the hippocampi led to abnormalities in memory consolidation: the process through which new memories become stable. The mid-piece region of sperm also expresses a truncated form of MuSK, adjacent to N-agrin, rapsyn and (neuronal type) α7-AChR immunostaining (Kumar et al., 2006). It is tempting to predict that MuSK will be found to help organise specialised membrane domains at excitatory central synapses and in the mid-piece of sperm.

5. Congenital and autoimmune diseases act on the MuSK system

The NMJ is severely impaired, leading to muscle weakness, in rare congenital myasthenic syndromes (CMS). Some CMS cases are caused by mutations in either rapsyn, Dok7 or MuSK. Patients with mutant MuSK displayed anticipated deficiencies in postsynaptic AChR clustering and presynaptic nerve terminal differentiation (Chevessier et al., 2004). Slightly less rare (~1:100,000), are cases of autoimmune myasthenia gravis caused by antibodies against MuSK. When anti-MuSK-positive patient IgG was injected into mice it depleted MuSK from the postsynaptic membrane (Cole et al., 2010). This led to disassembly of postsynaptic AChR clusters, retraction of the nerve terminal and fatiguing muscle weakness. Muscle weakness resulting from loss of NMJs is a common feature of amyotrophic lateral sclerosis (a motor neuron disease), and normal old-age (sarcopenia). Better understanding of the molecular physiology of the MuSK system may aid the development of new strategies to reduce NMJ loss and muscle weakness.

Acknowledgments

Many laboratories have contributed to our understanding of MuSK and its role in synapse formation. We apologise to colleagues that due to limits on length and citation number we have only touched on recent advances and have been forced to omit many original citations. We thank Dr Steve Assinder for helpful feedback on the manuscript. The authors' work was supported by project grants from MDA (4172) and NHMRC Australia (570930).

References

Bergamin E, Hallock PT, Burden SJ, Hubbard SR. The cytoplasmic adaptor protein Dok7 activates the receptor tyrosine kinase MuSK via dimerization. Mol Cell 2010;39:100–9.

Borges L, Yechikov S, Lee YI, Rudell JB, Friese MB, Burden SJ, et al. Identification of a motif in the acetylcholine receptor β-subunit whose phosphorylation regulates rapsyn association and postsynaptic receptor localization. J Neurosci 2008;28:11468–76.

Chen F, Qian L, Yang Z-H, Huang Y, Ngo ST, Ruan N-J, et al. Rapsyn interaction with calpain stabilizes AChR clusters at the neuromuscular junction. Neuron 2007;55:247–60.

Cheusova T, Khan MA, Schubert SW, Gavin A-C, Buchou T, Jacob G, et al. Casein kinase 2-dependent serine phosphorylation of MuSK regulates acetylcholine receptor aggregation at the neuromuscular junction. Genes Dev 2006;20:1800–16.

Chevessier F, Faraut B, Ravel-Chapuis A, Richard P, Gaudon K, Bauche S, et al. MUSK, a new target for mutations causing congenital myasthenic syndrome. Hum Mol Genet 2004;13:3229–40.

Cole RN, Ghazanfari N, Ngo ST, Gervasio OL, Reddel SW, Phillips WD. Patient autoantibodies deplete postsynaptic Muscle Specific Kinase leading to disassembly of the ACh receptor scaffold and myasthenia gravis in mice. J Physiol (Lond) 2010;588(17):3217–29.

DeChiara TM, Bowen DC, Valenzuela DM, Simmons MV, Poueymirou WT, Thomas S, et al. The receptor tyrosine kinase MuSK is required for neuromuscular junction formation in vivo. Cell 1996;85:501–12.

Garcia-Osta A, Tsokas P, Pollonini G, Landau EM, Blitzer R, Alberini CM. MuSK expressed in the brain mediates cholinergic responses, synaptic plasticity, and memory formation. J Neurosci 2006;26:7919–32.

Harris AJ. Embryonic growth and innervation of rat skeletal muscles III. neural regulation of junctional and extrajunctional acetylcholine receptors. Philos Trans R Soc Lond B 1981;293:287–314.

Hezel M, de Groat WC, Galbiati F. Caveolin-3 promotes nicotinic acetylcholine receptor clustering and regulates neuromuscular junction activity. Mol Biol Cell 2010;21:302–10.

Jennings CGB, Dyer SM, Burden SJ. Muscle-specific trk-related receptor with a kringle domain defines a distinct class of receptor tyrosine kinases. Proc Natl Acad Sci USA 1993;90:2895–9.

Kim N, Burden SJ. MuSK controls where motor axons grow and form synapses. Nat Neurosci 2008;11:19–27.

Kim N, Stiegler AL, Cameron TO, Hallock PT, Gomez AM, Huang JH, et al. Lrp4 is a receptor for agrin and forms a complex with MuSK. Cell 2008;135:334–42.

Kumar P, Ferns MJ, Meizel S. Identification of agrinSN isoform and muscle-specific receptor tyrosine kinase in sperm. Biochem Biophys Res Commun 2006;342:522–8.

Kummer TT, Misgeld T, Sanes JR. Assembly of the postsynaptic membrane at the neuromuscular junction: paradigm lost. Curr Opin Neurobiol 2006;16:74–82.

Lacazette E, Le Calvez S, Gajendran N, Brenner HR. A novel pathway for MuSK to induce key genes in neuromuscular synapse formation. J Cell Biol 2003;161:727–36.

Lin W, Burgess RW, Dominguez B, Pfaff SL, Sanes JR, Lee K-F. Distinct roles of nerve and muscle in postsynaptic differentiation of the neuromuscular synapse. Nature 2001;410:1057–64.

Linnoila J, Wang Y, Yao Y, Wang ZZ. A mammalian homologue of Drosophila tumorous imaginal discs, TID1, mediates agrin signaling at the neuromuscular junction. Neuron 2008;60:625–41.

Lu Z, Je H-S, Young P, Gross J, Lu B, Feng G. Regulation of synaptic growth and maturation by a synapse-associated E3 ubiquitin ligase at the neuromuscular junction. J Cell Biol 2007;177:1077–89.

Luo ZG, Wang Q, Zhou JZ, Wang J, Luo Z, Liu M, et al. Regulation of AChR clustering by dishevelled interacting with MuSK and PAK1. Neuron 2002;35:489–505.

Luo ZG, Je H-S, Wang Q, Yang F, Dobbins C, Yang Z-H, et al. Implication of geranylgeranylyltransferase I in synapse formation. Neuron 2003;40:703–17.

Mittaud P, Camilleri AA, Willmann R, Ern-Vogtli S, Burden SJ, Fuhrer C. A single pulse of agrin triggers a pathway that acts to cluster acetylcholine receptors. Mol Cell Biol 2004;24:7841–54.

Okada K, Inoue A, Okada M, Murata Y, Kakuta S, Jigami T, et al. The muscle protein Dok-7 is essential for neuromuscular synaptogenesis. Science 2006;312:1802–5.

Qian YK, Chan AWS, Madhavan R, Peng HB. The function of Shp2 tyrosine phosphatase in the dispersal of acetylcholine receptor clusters. BMC Neurosci 2008;9:70.

Tang H, Veldman MB, Goldman D. Characterization of a muscle-specific enhancer in human MuSK promoter reveals the essential role of myogenin in controlling activity-dependent gene regulation. J Biol Chem 2006;281:3943–53.

Till JH, Becerra M, Watty A, Lu Y, Ma Y, Neubert TA, et al. Crystal structure of the MuSK tyrosine kinase insights into receptor autoregulation. Structure 2002;10:1187–96.

Weston C, Gordon C, Teressa G, Hod E, Ren X-D, Prives J. Cooperative regulation by Rac and Rho of agrin-induced acetylcholine receptor clustering in muscle cells. J Biol Chem 2003;278:6450–5.

Wu H, Xiong WC, Mei L. To build a synapse: signaling pathways in neuromuscular junction assembly. Development 2010;137:1017–33.

Zhang B, Luo S, Wang Q, Suzuki T, Xiong WC, Mei L. LRP4 serves as a coreceptor of agrin neuron 2008;60:285–97.

Zhu D, Yang Z, Luo Z, Luo S, Mei L, Xiong WC. Muscle-specific receptor tyrosine kinase endocytosis in acetylcholine receptor clustering in response to agrin. J Neurosci 2008;28:1688–96.