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Molecules in focus

Muscle Specific Kinase: Organiser of synaptic membrane domains

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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.

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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.

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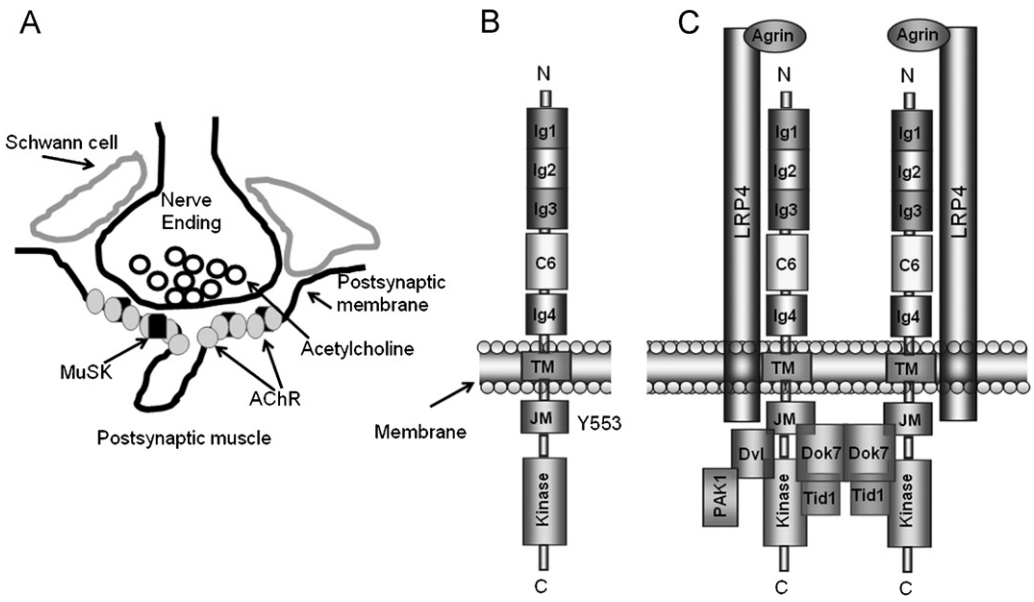

Fig. 1. Structure of the MuSK signalling complex and its synaptic context. (A) At the NMJ the nerve terminal sits in a synaptic gutter, separated from the postsynaptic (muscle) membrane by a 50 nm synaptic cleft. Schwann (glial) cells loosely enwrap the nerve terminal. AChRs are tightly packed into clusters 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

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