SYNAPSE FORMATION

The missing link

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Synapse formation requires a complex interchange of signals to efficiently juxtapose presynaptic release sites with specialized postsynaptic sites. Neuronal agrin, a factor released from motor neurons, induces rapid phosphorylation of MuSK, a transmembrane tyrosine kinase of skeletal muscle cells. Phosphorylated MuSK is important for the postsynaptic clustering of acetylcholine receptors (AChRs) at neuromuscular junctions. As agrin and MuSK do not interact directly, it has remained unknown how agrin conveys the signal to MuSK. Now, two publications identify the long-elusive postsynaptic receptor

for agrin as LRP4, a member of the family of low-density lipoprotein receptors, and demonstrate that LRP4 and MuSK associate.

In a screen to identify developmentally relevant genes, mutations in *Lrp4* were neonatally lethal and resulted in mice that did not form neuromuscular junctions. The defects in these mice were similar to those seen in mice carrying mutations in *Musk*, making it conceivable that LRP4 has a role in the agrin–MuSK signalling pathway.

First, Zhang *et al.* used immunostaining to show that LRP4 is specifically expressed in myotubes, where it colocalizes with AChRs at the neuromuscular junction *in vivo*, and not in immature myoblasts. Kim *et al.* demonstrated that agrin failed to stimulate the phosphorylation of MuSK in cultured muscle cells from *Lrp4*-mutant mice. Similarly, Zhang *et al.* showed that inhibition of LRP4 expression in cultured myotubes by RNA interference resulted in decreased agrin binding, MuSK phosphorylation and AChR clustering.

In binding studies with recombinant proteins or cell-based attachment assays, both groups showed that LRP4 binds specifically to neuronal agrin. Furthermore, neuronal agrin stimulated the phosphorylation of MuSK in the non-muscle cell lines BaF3 and HEK293 only when MuSK and LRP4 were co-expressed. Together, these results suggest that LRP4 is the functional receptor for agrin.

To test whether LRP4 and MuSK interact directly, Zhang *et al.* used recombinant, extracellular domains

of MuSK and LRP4 and performed immunoprecipitation studies in the absence or presence of agrin. They showed that MuSK and LRP4 associated in the absence of agrin, but that agrin enhanced this association. Kim *et al.* observed that BaF3 cells expressing LRP4 self-aggregated in culture. MuSK-expressing cells did not self-aggregate, but co-aggregated with LRP4-expressing cells. Moreover, MuSK was co-immunoprecipitated with LRP4 from cell homogenates when LRP4 and MuSK were co-expressed. Furthermore, Zhang *et al.* showed that the LRP4–MuSK complex was detectable in mouse-muscle homogenates, suggesting that LRP4 and MuSK associate *in vitro* and *in vivo*.

The interaction of LRP4 with MuSK suggests that LRP4 may regulate MuSK activity. Both papers showed that MuSK was more active when co-expressed with LRP4 alone. This agrin-independent activation of MuSK by LRP4 may be a mechanism that mediates pre-patterning of muscle fibres.

These papers identify LRP4 as the elusive skeletal-muscle receptor for neuronal agrin. In addition to being expressed at synapses at the neuromuscular junction, agrin, LRP4 and MuSK are expressed at central synapses, raising the possibility that an analogous mechanism applies to synapse formation in the CNS.

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ORIGINAL RESEARCH PAPERS Kim, N. *et al.* Lrp4 is a receptor for agrin and forms a complex with MuSK. *Cell* **135**, 334–342 (2008) | Zhang, B. *et al.* Lrp4 serves as a coreceptor of agrin. *Neuron* **60**, 285–297 (2008)