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MicroRNAs have receptor subunits in a bind

The number of glutamate receptors (GluRs) in the postsynaptic membrane is crucial to the efficacy of synaptic transmission, but the mechanisms that control receptor synthesis and localization are not well understood. Karr *et al.* have now shown that microRNAs regulate the expression levels of GluR subunits in the *Drosophila melanogaster* neuromuscular junction (NMJ).

MicroRNAs are known to be involved in the development of the nervous system and to act by suppressing gene expression. The authors used RNA interference (RNAi) to downregulate Dicer-1 (an enzyme that is essential for microRNA synthesis) in postsynaptic muscle cells in the flies. Using reverse transcription PCR and fluorescence *in situ* hybridization, two methods that allow the quantification of RNA transcripts, the authors found that there were considerably higher levels of expression of the GluR subunit isoform genes *GluRIIA* and *GluRIIB* in the NMJ of RNAi flies, indicating that these transcripts are normally repressed by microRNAs. Convergently, immunocytochemistry showed increased levels of GluRIIA and GluRIIB proteins, most of which, however, were not localized to the postsynaptic membrane. Electrophysiological investigation confirmed that the changes in protein abundance did not significantly affect total synaptic strength.

Next, the authors used finely tuned bioinformatic models to predict microRNA binding sites and identified binding sites for miR-284 in both *GluRIIA* and *GluRIIB* transcripts. In miR-284 loss-of-function mutants they found that *GluRIIB* mRNA, which has two predicted binding sites, was de-repressed more than *GluRIIA* mRNA, which has one. Furthermore, transgenic expression of miR-284 in these mutant flies restored GluRIIA and GluRIIB levels to near control levels, thereby confirming that this microRNA directly affected protein abundance, in proportion to the number of miR-284 binding sites on the transcript subtypes.

These results demonstrate that microRNAs control the levels of GluRIIA and GluRIIB subunits in the *D. melanogaster* larval NMJ. Expression of another subunit, GluRIIC, was not regulated by microRNAs. This unequal regulation of subunits suggests that microRNAs might control which subunits are available for glutamate receptor assembly, and therefore ultimately dictate glutamate receptor subunit composition and receptor properties rather than overall synapse strength. These results also link the number of microRNA binding sites to protein abundance, and further research will establish whether this concept can be generalized to other proteins.

Eleanor Beal

ORIGINAL RESEARCH PAPER Karr, J. *et al.* Regulation of glutamate receptor subunit availability by microRNAs. *J. Cell Biol.* **185**, 685–697 (2009)