Functional analysis of UNC-18 and UNC-13 in neurotransmitter release and its transcriptional regulation by novel protein F09G2.9

Summary
At chemical synapses, neurotransmitter release is a highly complicated process and regulated by several protein-protein interactions. The *Caenorhabditis elegans* unc-18, unc-13 and unc-64 genes are required for normal synaptic transmission. The UNC-18 protein binds to the unc-64 gene product *C.elegans* syntaxin. I analyzed the mechanisms of displacement of UNC-18 from syntaxin. UNC-13 transiently interacts with the UNC-18-syntaxin, resulting in rapid displacement of UNC-18 from the complex. I found that UNC-13 contributes to the modulation of the interaction between UNC-18 and syntaxin.

To understand how expression of unc-18 is regulated in neuronal cells, we analyzed unc-18 promoter region. Through deletion analyses, we identified the minimal regulatory 19-bp sequence element of 250 bp upstream from the ATG start codon. Using this sequence as a probe, we identified proteins bound in expression library screening. One of these proteins, F09G2.9 contains AT hook motif. In order to know the functional significance in unc-18 expression, we used the feeding-RNAi method. In F09G2.9 (RNAi) animals, the expression of unc-18 was suppressed. We confirmed the downregulation of UNC-18 protein in F09G2.9(RNAi) animals by immuno-precipitation using anti-UNC-18 antibody and western blot analysis, suggesting that F09G2.9 positively regulates the unc-18 gene expression. A F09G2.9::GFP transgene with 0.7 kb of upstream sequence was expressed in head neurons, ventral nerve code and tail neurons, whose expression is similar to that of unc-18::GFP. To examine the protein property F09G2.9 was expressed in *E.coli* as GST fusion protein. GST-C terminal fragment could bind to double strand and sense strand probes in electrophoretic mobility shift assay.

In learning assay using NaCl and starvation, F09G2.9 (RNAi) animals showed the abnormal response toward NaCl after conditioning, indicating the important function of F09G2.9 in executing the associative learning behaviors.