CDB SEMINAR

Toshihiro Sassa

Division of Clinical laboratory science, Department of Physical Information, Kanazawa University

Thursday, June 15

16:00~17:00 A7F Conference Room

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.

Host:
Kiyoji Nishiwaki
Cell Migration, CDB
nishiwak@cdb.riken.jp
Tel:078-306-3264
(ext:1745)