LABORATORY OF NEURONAL CELL BIOLOGY

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The transport of specific mRNAs and local control of translation in neuronal dendrites represent an important gene expression system that provides localized protein synthesis in dendrites at just the right time and place. It is believed that this system controls the location at which neurites will connect to each other, thereby forming neural networks. Our main interest is to understand the mechanisms and roles of mRNA transport and local translation in neuronal dendrites. We are researching factors regulating mRNA transport and local translation, their target mRNAs, and the mechanisms of localized protein synthesis using mice in order to better understand its relation to the formation of synapses and neural networks, memory, learning, and behavior.

I. Regulation of transport and local translation of dendritically-targeted mRNAs

Specific mRNAs are recruited into "RNA granules" in neuronal dendrites. RNA granules are macromolecular complexes composed mainly of mRNAs and ribosomes, and mediate the transport and local translation of their mRNA cargoes in response to synaptic stimulation (Figure 1).

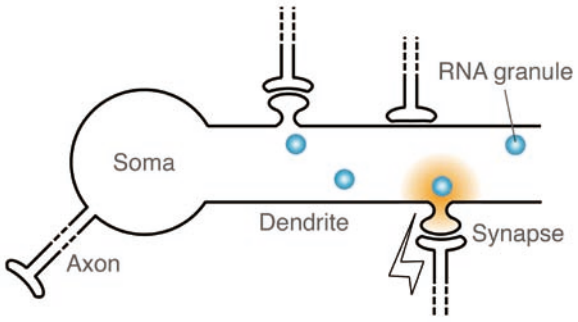

Figure 1. A model for local translation in neuronal dendrites. Specific mRNAs are recruited into RNA granules and transported to dendrites. Translation of the mRNAs is induced locally upon synaptic stimulation, which modifies local postsynapses to regulate synaptic connection and network formation.

We previously identified RNA granule protein 105 (RNG105), an RNA-binding protein, as a component of RNA granules. RNG105 is responsible for mRNA transport to dendrites, which is required for the encoded proteins to be translated and function in dendrites for proper networking of neurons (Shiina *et al*., *J. Neurosci*. **30,** 12816-12830, 2010).

To understand the regulation of transport and local translation of dendritically-targeted mRNAs, we have identified RNG105 cargo mRNAs in neurons. The cargo mRNAs, e.g., that encode Na^+/K^+ ATPase subunit isoform protein (FXYD1), are recruited to RNA granules and transported to dendrites. Furthermore, translation of the mRNAs is upregulated by stimulation of neurons with brain-derived neurotrophic factor (BDNF) which mimics synaptic stimulation (Figure 2). We are currently investigating the molecular mechanism of the mRNA transport to dendrites and BDNF-stimulated translational upregulation of the cargo mRNAs.

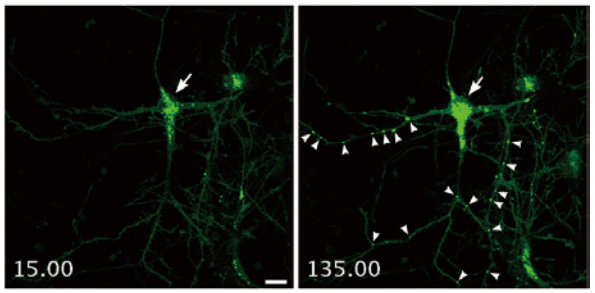

Figure 2. Translation of FXYD1-GFP is activated by BDNF in mouse cultured hippocampal neurons. Numbers indicate time (minute) after BDNF stimulation. FXYD1-GFP is increased in membrane compartments in the cell body (an arrow) and dendrites (arrowheads) after BDNF stimulation (right panel). Scale bar, 10 μm.

II. Molecular characterization of the RNA granule complex

RNA granules are abundantly formed in neurons, but not observed in many other types of cells. However, the formation of RNA granules is induced by stress such as oxidation in the cells. They are called "stress granules" and have common features with neuronal RNA granules, e.g., they are macromolecular complexes containing ribosomes and mRNAs, and repress translation of cargo mRNAs without stimulation. We previously found that RNG105 is a component of not only neuronal RNA granules but also stress granules and further that overexpression of RNG105 in fibroblastic cells induced the formation of granules which contain mRNAs.

To identify and characterize molecular components of RNA granules, we have performed mass spectrometric analysis of the RNG105-induced complex. A cultured cell line A6 transfected with RNG105-green fluorescent protein (RNG105-GFP) forms prominent granules in the cytoplasm (Figure 3). The transfected cells are subjected to immunoprecipitation with an anti-GFP antibody (Figure 3). Proteins co-precipitated with RNG105-GFP are then analyzed by mass spectroscopy, which revealed that several proteins responsible for RNA metabolism, membrane transport, posttranslational modification, etc. are contained in the complex. We are currently investigating the localization and function of the proteins in stress granules as well as in neuronal RNA granules.

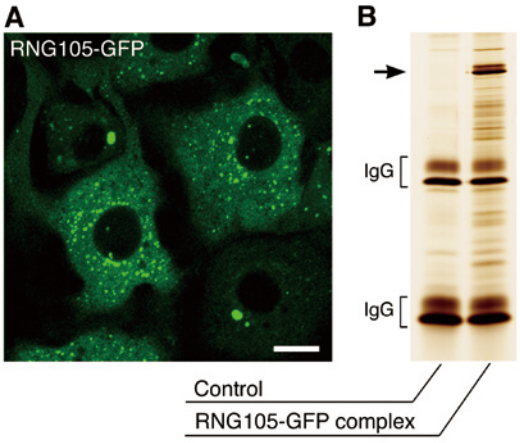

Figure 3. Identification of components of RNG105-induced granules. (A) A6 cells expressing RNG105-GFP. RNG105-GFP induced the formation of cytoplasmic granules. Scale bar, 10 μm. (B) SDS-PAGE of immunoprecipitants with an anti-GFP antibody from control A6 cells (left lane) and A6 cells expressing RNG105-GFP (right lane). The positions of RNG105-GFP (an arrow) and immunoglobulin (IgG) are indicated.

III. Knockout mice for RNA granule proteins

We previously generated RNG105 knockout mice. Neurons from the knockout mice exhibited reduced dendritic synapse formation and reduced dendritic arborization, which resulted in poor development of neuronal networks. The knockout neonates died soon after birth due to respiratory failure, which was associated with defects in fetal brainstem development. To investigate the role of RNG105 in higher brain functions, e.g., memory and learning, in adult mice, we have generated conditional RNG105 knockout mice using the Cre/loxP system. Alpha-CaMKII promoter is used to drive Cre recombinase since its promoter activity is low during embryonic stages but elevated after birth in the brain. However, even though the Cre/loxP system is used, we have not obtained adult RNG105 knockout mice. Therefore we are planning to generate drug-inducible RNG105 knockout mice.

RNG105 has one paralog, RNG140, which has RNA-binding domains highly conserved with RNG105. RNG105 and RNG140 are localized to different kinds of RNA granules and their timing of expression is also different: RNG105 is highly expressed in embryos, but RNG140 is highly expressed in adults (Shiina and Tokunaga, *J. Biol. Chem.* **285,** 24260-24269, 2010). We have obtained RNG140 knockout mice and are going to investigate the role of RNG140 in higher brain functions in adult mice.