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.

It is known that specific mRNAs are recruited into "RNA granules" in neuronal dendrites. RNA granules are macromolecular complexes composed mainly of mRNAs, ribosomes and RNA-binding proteins, and mediate the transport and local translation of their mRNA cargoes in response to synaptic stimulation (Figure 1). We are researching RNA granule 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.

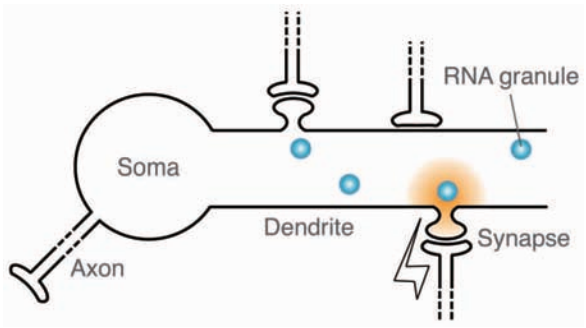

Figure 1. A model for local translation in neuronal dendrites. Specific mRNAs are recruited into RNA granules and transported to dendrites. Translation of the mRNAs is induced locally upon synaptic stimulation, which modifies local postsynapses to regulate synaptic connection and network formation.

I. Knockout mice for RNA granule proteins

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).

RNG105 knockout mice exhibit reduced dendritic synapse formation and reduced dendritic arborization, which results in poor development of neuronal networks. The knockout neonates die soon after birth due to respiratory failure, which is 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 generated RNG105 conditional knockout (cKO) mice using the Cre/loxP system. Alpha-CaMKII promoter was used to drive Cre recombinase since its promoter activity was low during embryonic stages but elevated after birth in the brain. We successfully obtained RNG105 cKO mice that grew into adults (Figure 2A). Expression of RNG105 was markedly reduced in the cerebrum, especially in the hippocampal pyramidal neurons of adult cKO mice (Figure 2B). Open field behavior testing revealed that exploratory activity of RNG105 cKO mice in a novel environment was not changed between the first and later trials, although exploratory activity of wild-type mice was reduced with increasing number of trials, suggesting that the knockout mice had some problems in being acclimated to a new environment. We are currently analyzing learning and memory in the cKO 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.

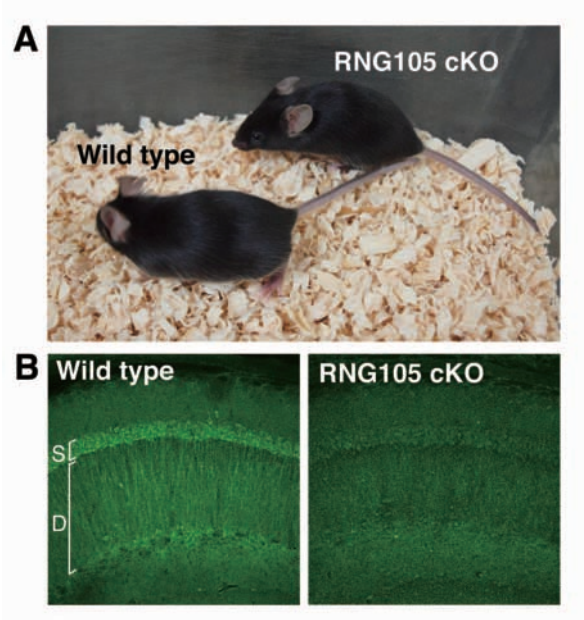

Figure 2. RNG105 conditional knockout (cKO) mice. A, 10-week-old adult wild-type and RNG105 cKO mice. B, Brain hippocampal slices from wild-type and RNG105 cKO mice were immunostained with an anti-RNG105 antibody. S, somatic layer; D, dendritic layer of hippocampal pyramidal neurons. RNG105 was reduced in pyramidal neurons of RNG105 cKO mice.

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 and virus infection 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. We previously found that RNG105 was 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 stress granules which contain mRNAs. Because knowledge about stress granules helps us in studying neuronal RNA granules, our research includes identification and characterization of molecular components of stress granules.

We have identified a novel component of stress granules, NFAR2. NFAR2 was recruited into RNG105-induced stress granules and enhanced the assembly of the stress granules (Figure 3). It is known that cellular stress activates master kinases including PKR, which induces translation repression and stress granule formation. We found that NFAR2 phosphorylation by PKR enhanced its localization to, and the formation of, stress granules. Furthermore, we found that NFAR2 was bound by its partner NF45 and antagonized its effect on stress granules in basal condition, but it overcame the antagonistic regulation by NF45 when phosphorylated by PKR. Because PKR is reportedly implicated in learning and memory, these results will provide insights into the regulatory mechanism of neuronal RNA granule formation in the brain.

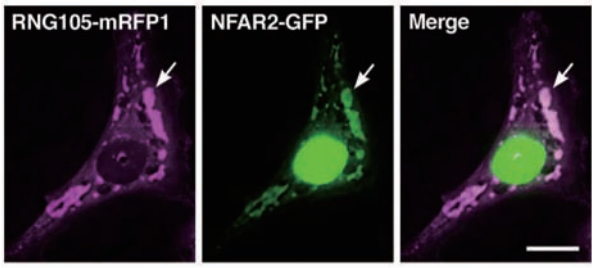

Figure 3. NFAR2 is a component of stress granules. A6 cells were co-transfected with RNG105-monomeric red fluorescent protein 1 (mRFP1) and NFAR2-green fluorescent protein (GFP). Expression of RNG105 induced the formation of cytoplasmic stress granules. NFAR2 was predominantly localized to the nucleus, and it was also recruited into and enlarged the stress granules (arrows). Scale bar, 10 μm.