## 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.

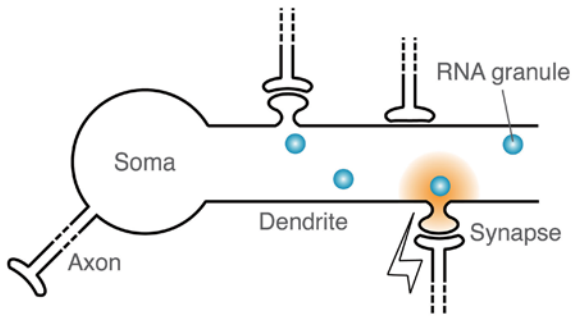


Figure 1. A model for local translation in neuronal dendrites. Specific mRNAs are recruited into RNA granules and transported to dendrites. Translation of the mRNAs is induced locally upon synaptic stimulation, which modifies local postsynapses to regulate synaptic connection and network formation.

### I. Analyses of RNG105 conditional knockout mice

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 (Shiina *et al*., J. Neurosci. *30,* 12816-12830, 2010).

To investigate the role of RNG105 in higher brain functions, we have generated RNG105 conditional knockout (cKO) mice using the alpha-CaMKII-Cre/loxP system. Expression of RNG105 was markedly reduced in the cerebrum, especially in the hippocampal pyramidal neurons of adult cKO mice (Figure 2A). To investigate comprehensively the changes in the dendritic localization of mRNAs in RNG105 cKO neurons, we dissected brain slices to isolate dendritic areas of hippocampal CA1 pyramidal neurons (Figure 2B). Quantitative RT-PCR experiments confirmed that dendritic mRNAs, but not somatic mRNAs, were concentrated in the isolated dendrite fragments, which indicated that dendrites of hippocampal neurons were successfully isolated. We are going to compare the mRNAs contained in the dendrite fragments between wild-type and RNG105 cKO mice using next-generation sequencing to understand the effect of RNG105 on mRNA transport to neuronal dendrites in adult mouse brains.

We further analyzed behavior of RNG105 cKO mice. Open field behavior tests 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 cKO mice had some problems in being acclimated to a new environment. Passive avoidance test is one of the learning and memory tests, in which mice receive an electric foot shock in a room and thereafter they remember the situation and do not enter the room for more than several days. In this test, RNG105 cKO mice did not enter the room at 5 minutes after they received a foot shock, but entered the room after 24 hours. These results suggested that RNG105 cKO mice acquired short-term, but not long-term memory, which is consistent with a model that dendritic mRNA transport and local translation are key bases for the conversion of short-tem memory to long-term memory.

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.

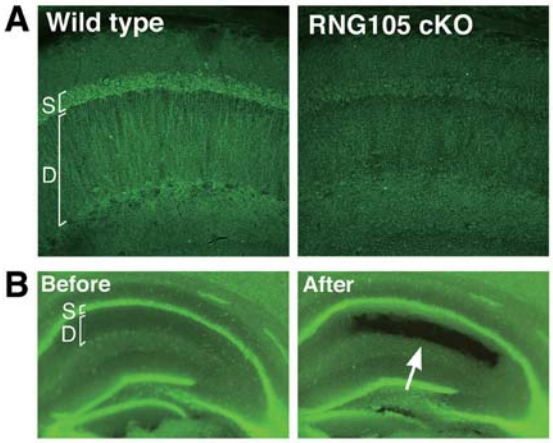


Figure 2. (A) Brain CA1 region of hippocampal slices from wild-type and RNG105 cKO mice were immunostained with an anti-RNG105 antibody. S, somatic layer; D, dendritic layer of hippocampal pyramidal neurons. RNG105 was reduced in pyramidal neurons of RNG105 cKO mice. (B) A hippocampal slice from a wild-type mouse stained with YO-PRO1 to visualize nuclei. Left and right panels are before and after the isolation of the dendritic area of CA1 pyramidal neurons. An arrow indicates the dendritic area removed by dissection.

## II. Molecular mechanism of RNA granule assembly and disassembly

We used proteomic analyses to identify proteins associated with RNG105. RNG105 fused to green fluorescence protein (GFP) was expressed in cultured A6 cells and immunoprecipitated with an anti-GFP antibody, and the immunoprecipitates were analyzed by mass spectrometry. Among the identified proteins, we focused on nuclear factor 45 (NF45) and its binding partner, nuclear factor associated with dsRNA 2 (NFAR2). Expression of NFAR2 in cells enhanced the assembly of RNG105-containing RNA granules, whereas expression of NF45 disassembled the RNA granules (Figure 3).

NFAR1 and NFAR2 are splice variants and their different properties from one another have been unclear. We have found that NFAR2, but not NFAR1, has the ability to localize with and enhance the assembly of RNG105-containing RNA granules through NFAR2-specific GQSY domain (Figure 3). The GQSY domain interacted with RNG105-containing messenger ribonucleoprotein (mRNP) complexes and was structurally and functionally similar to the low complexity (LC) sequence domain of FUS/TLS, which is known to drive RNA granule assembly.

Another domain of NFAR2, DZF domain, was dispensable for the interaction with the RNG105 mRNP complexes, but involved in positive and negative regulation of RNA granule assembly by being phosphorylated by PKR, a master kinase inducing RNA granule assembly, and by association with NF45, respectively (Figure 3).

Our results suggest a model that NFAR2 functions as a connector of RNG105 mRNP complexes through its multivalent domains, i.e., the GQSY domain and the DZF domain, in the assembly of RNA granules. The connector function may be enhanced by phosphorylation by PKR and blocked by NF45 binding. We are going to elucidate the roles of NFAR2 and NF45 in neurons, including their relation to neurodegeneration, because defective regulation of RNA granule assembly by LC sequence domain-containing proteins such as FUS/TLS and TDP-43 is recently suggested to be associated with neurodegenerative disease such as amyotrophic lateral sclerosis (ALS) and frontotemporal lobar degeneration (FTLD).

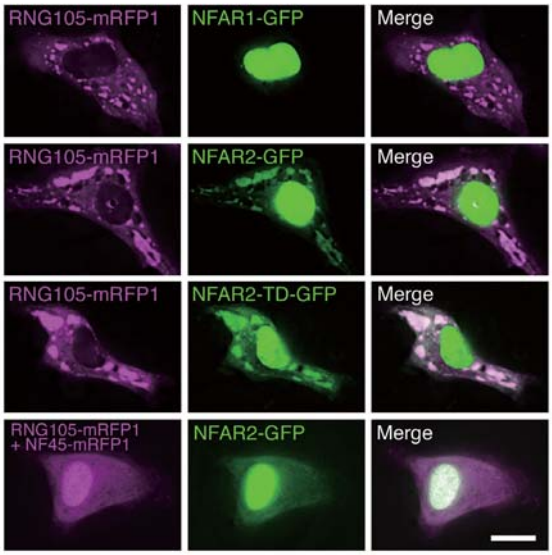


Figure 3. Effects of NFAR2, its phosphorylation by PKR, and NF45 on the assembly of RNG105-containing RNA granules. A6 cells were co-transfected with RNG105-monomeric red fluorescent protein 1 (mRFP1) and NFAR1-GFP, NFAR2-GFP, phosphomimetic NFAR2 at PKR phosphorylation sites (NFAR2-TD-GFP), or NFAR2-GFP plus NF45-mRFP1. NFAR2 was predominantly localized to the nucleus, and also co-localized to and enlarged RNG105-containg RNA granules. RNA granules were much more increased in size by phosphomimetic NFAR2, but disassembled by co-expression of NF45. Scale bar, 10 μm.