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 dendritic protein synthesis 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.

Specific mRNAs are recruited into "RNA granules" and transported to 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 mechanism 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.

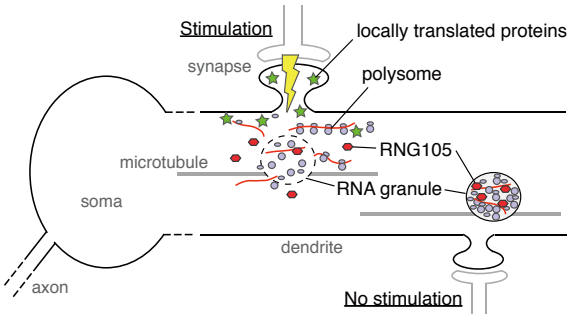

Figure 1. A model for local translation in neuronal dendrites. Specific mRNAs are recruited into RNA granules and transported to dendrites. Translation of the mRNAs is induced locally upon synaptic stimulation, which modifies local postsynapses to regulate synaptic connection and network formation.

I. Dendritic mRNA localization and learning and memory in RNG105 conditional knockout mice

We previously identified RNA granule protein 105 (RNG105)/caprin1, 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 that is associated with defects in fetal brainstem development (Shiina *et al*., J. Neurosci. 30, 12816-12830, 2010).

To investigate the role of RNG105 in the adult mouse brain, we have generated RNG105 conditional knockout (cKO) mice in which the *rng105* gene was disrupted in the brain after birth. Using RNG105 cKO adult mice, we performed comprehensive analysis of dendritic localization of mRNAs. Brain slices were dissected to isolate somatic and dendritic layers of hippocampal CA1 neurons, and from each layer, RNA was purified and analyzed by deep sequencing. Comparison of mRNAs from dendritic and somatic layers identified mRNAs concentrated in dendrites and mRNAs concentrated in the soma (Figure 2). The difference in the mRNA composition between dendrites and soma was relatively small in RNG105 cKO mice compared to control mice (Figure 2), which suggested that the dendritic mRNA composition in RNG105 cKO neurons changed toward the somatic type, i.e., dendritic localization of specific mRNAs was reduced in RNG105 cKO mice. Gene ontology analysis revealed that dendritically enriched mRNAs included small G protein regulators and translation regulators, which were reduced in dendrites of RNG105 cKO mice. We are going to investigate whether proteins encoded by these mRNAs are involved in dendritic synapse function and neuronal network formation.

We further analyzed behavior of RNG105 cKO mice. 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 the foot shock, but entered the room after 24 hours. We performed another learning and memory test, Morris water maze. In this test, mice learn the location of a hidden platform that allows the mice to escape from water. After several days of training, control mice learned the platform location and escape on the platform faster than before the training. However, the training did not shorten the latency of RNG105 cKO mice to

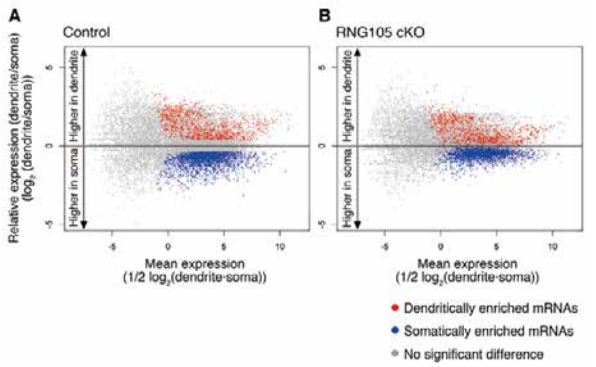

Figure 2. Comparison of mRNA composition between dendrite and soma of hippocampal CA1 neurons. (A) RNAs from triplicate samples were analyzed with Cufflinks. The graph shows MA plot between dendrite RNAs and soma RNAs from control neurons. The x-axis shows average $\log_2$ expression of dendrite RNAs and soma RNAs and the y-axis shows $\log_2$ fold change of dendrite RNAs vs. soma RNAs. Gray, all RNAs; red, mRNAs significantly concentrated in dendrites; blue, mRNAs significantly concentrated in soma. (B) MA plot between dendrite RNAs and soma RNAs from RNG105 cKO neurons. The same mRNAs are colored the same as in A.

find the platform. These results suggested that RNG105 cKO mice had deficits in learning and memory.

RNG105/caprin1 has one paralog, RNG140/caprin2, 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 examined RNG140 mRNA expression by qRT-PCR. RNG140 mRNA was most highly expressed in the brain and significantly reduced in the brain of RNG140 knockout mice. We are going to investigate the role of RNG140 in higher brain functions in adult mice.

II. RNA granule assembly and disassembly modulated by NFAR2 and NF45

We used proteomic analyses to identify proteins associated with RNG105. Among the identified proteins, we focused on nuclear factor associated with dsRNA 2 (NFAR2) and its binding partner, nuclear factor 45 (NF45). NFAR2 co-localized with and enhanced the assembly of RNG105-containing RNA granules, whereas NF45 induced the disassembly of RNA granules (Figure 3).

NFAR2 has a GQSY domain that is structurally and functionally similar to the low complexity (LC) sequence domain of FUS/TLS, which is known to drive RNA granule assembly. We have found that the GQSY domain of NFAR2 has the ability to interact with RNG105-containing messenger ribonucleoprotein (mRNP) complexes and enhances the assembly of RNG105-containing RNA granules.

Another domain of NFAR2, the DZF domain, was not necessary for the interaction with the RNG105 mRNP complexes, but was 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).

Analysis of translation activity at the single cell level by ribopuromycilation assay revealed that NFAR2 repressed translation, but the NFAR2-dependent repression was de-repressed by NF45.

Our results suggest a model in which 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 that is linked with translation repression. The connector function may be enhanced by phosphorylation by PKR, whereas it is blocked by NF45 binding, which disassembles RNA granules and de-represses translation. 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 has been recently suggested to be associated with neurodegenerative diseases such as amyotrophic lateral sclerosis (ALS) and frontotemporal lobar degeneration (FTLD).

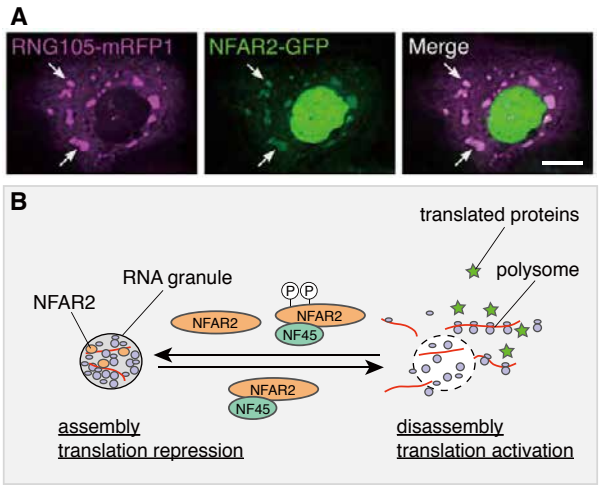

Figure 3. Effects of NFAR2 and NF45 on the assembly of RNA granules and translation activity. (A) A6 cells were co-transfected with RNG105-monomeric red fluorescent protein 1 (mRFP1) and NFAR2-GFP. NFAR2 was predominantly localized to the nucleus, and also co-localized to and enlarged RNG105-containg RNA granules (arrows). Scale bar, 10 μm. (B) A model of RNA granule assembly and translation activity modulated by NFAR2 and NF45. NFAR2 enhances RNA granule assembly and represses translation. NF45 binds to NFAR2, disassembles RNA granules and de-represses translation. Phosphorylation of NFAR2 by PKR reduces the effect of NF45.

Publication List

〔Original paper〕

● Shiina, N., and Nakayama, Kei. (2014). RNA granule assembly and disassembly modulated by nuclear factor associated with double-stranded RNA 2 and nuclear factor 45. J. Biol. Chem. *289*, 21163-21180.