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 widely accepted 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 membrane-less macromolecular assemblies 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 their relation to the formation of synapses and neural networks, memory, learning, and behavior.

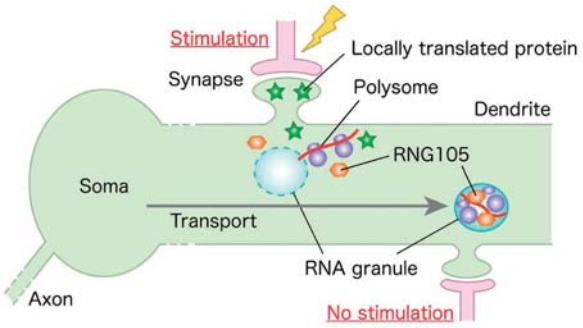

Figure 1. A model for local translation in neuronal dendrites. Specific mRNAs are recruited into RNA granules and transported to dendrites. Translation of the mRNAs is induced locally upon synaptic stimulation, which modifies local postsynapses to regulate synaptic connection and network formation.

I. RNG105 is essential for long-term memory formation

We previously identified RNA granule protein 105 (RNG105, also known as Caprin1), an RNA-binding protein, as a major component of RNA granules. RNG105 promotes the assembly of RNA granules and is responsible for the transport of its binding mRNAs in cultured cells.

To understand the role of RNG105 in learning and memory, we subjected RNG105 conditional knockout (cKO) mice, which lacked the *Rng105* gene in the brain region, to contextual fear conditioning tests. One of the tests was a passive avoidance test, in which an apparatus consisting of light and dark chambers was used (Figure 2). Mice received foot shock in the dark chamber. After that, if they remember the context, i.e., fear in the dark chamber, they would not enter the dark chamber and would stay in the light chamber. At 5 min after the foot shock, RNG105 cKO mice did not enter the dark chamber any more than control mice. However, at 1 day after the foot shock, they spent significantly longer time in the dark chamber than control mice. Furthermore, at 1 week after the foot shock, they spent as long a time as before the foot shock (Figure 2). These results indicated that not short-term memory, but long-term memory was markedly impaired by RNG105 cKO.

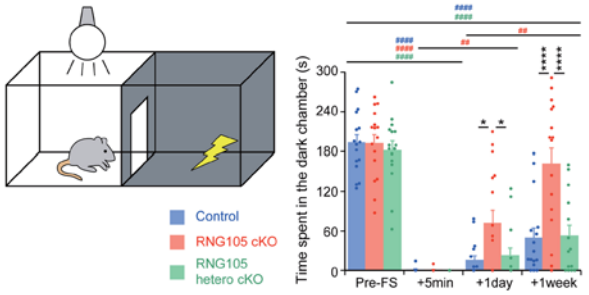

Figure 2. Contextual fear conditioning test. Time spent in the dark chamber before foot shock (Pre-FS) and at 5 min, 1 day and 1 week after the fear conditioning.

Another test was a contextual freezing test. In this test, mice received foot shock in a single chamber and their freezing responses were measured in the same chamber at 5 days after the foot shock. RNG105 cKO mice showed less freezing behavior than control mice, which indicated that RNG105 cKO mice did not well remember the place they learned to fear. These results supported the notion that RNG105 is essential for the formation of long-term memory.

II. RNG105 deficiency impairs AMPA receptor scaling on dendrites

To investigate the mechanism of how RNG105 cKO affects long-term memory, we measured electrophysiological responses (fEPSP) of neurons to synaptic stimulation in the hippocampus, the essential brain region for memory formation. fEPSP in RNG105 cKO mice was reduced by half compared to control mice both in the steady state and after synaptic long-term potentiation (LTP). This suggested that AMPA receptors (AMPARs), key factors for the synaptic transmission, were downregulated by RNG105 deficiency.

AMPAR downregulation by RNG105 deficiency was further suggested by RNA-seq analysis as follows. We comprehensively identified mRNAs whose dendritic localization was reduced in hippocampal neurons from RNG105 cKO mice. Subsequent gene ontology enrichment analysis revealed that a major category of the reduced mRNAs was "Regulation of Arf protein signal transduction", which included GTPase-activating proteins (GAPs) and guanine nucleotide exchange factors (GEFs) of small G protein Arf. Arf is known to regulate membrane trafficking, which includes the regulation of AMPAR surface expression on dendrites. These results suggested that cell surface expres-

sion of AMPARs on dendrites was affected by RNG105 deficiency.

We then quantified AMPARs expressed on the surface of dendrites in cultured neurons by immunostaining (Figure 3). Comparison between control and RNG105-deficient neurons revealed that the number of surface AMPARs on dendrites was significantly reduced by RNG105 deficiency. Furthermore, the scaling mechanism for AMPARs on the surface of dendrites was impaired in RNG105-deficient neurons: although normal neurons increase the surface number of AMPARs upon neuronal activity deprivation in order to maintain the basal activity, RNG105-deficient neurons did not increase the surface number of AMPARs even when their activity was blocked by tetrodotoxin (TTX) and APV (Figure 3). This impaired scaling of AMPARs is considered to underlie the reduced synaptic responses in the basal state, which may also attenuate synaptic responses in the LTP state.

Taken together, our study demonstrated that an element of RNA granules, RNG105, is essential for long-term memory formation. Our study further revealed RNG105-dependent dendritic localization of mRNAs as an underlying mechanism for AMPAR-dependent synaptic strength and long-term memory formation.

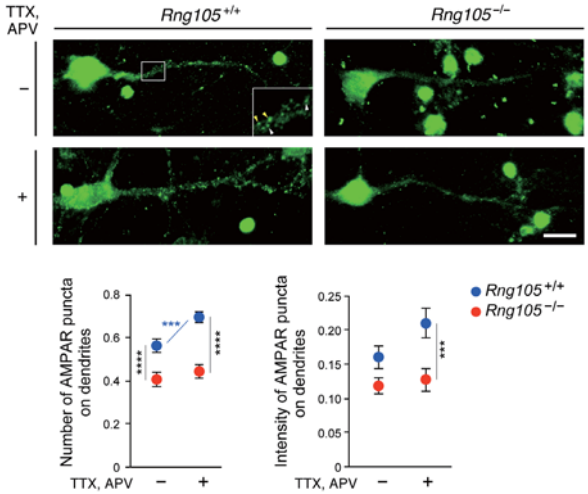

Figure 3. RNG105 deficiency impairs AMPAR scaling on dendrites. Primary cultured neurons from the cerebral cortex of control (*Rng105*$^{+/+}$) and RNG105 knockout (*Rng105*$^{-/-}$) littermates were immunostained for surface-expressed AMPAR subunit GluR1. Arrowheads in the inset indicate AMPAR puncta. The number of AMPAR puncta on dendrites is less in *Rng105*$^{-/-}$ neurons than in *Rng105*$^{+/+}$ neurons. Although the number of AMPAR puncta was increased by TTX and APV treatment in *Rng105*$^{+/+}$ neurons, it was not increased in *Rng105*$^{-/-}$ neurons. Bar, 10 μm.

Publication List:

〔Original paper〕

● Nakayama, K.*, Ohashi, R.*, Shinoda, Y., Yamazaki, M., Abe, M., Fujikawa, A., Shigenobu, S., Futatsugi, A., Noda, M., Mikoshiba, K., Furuichi, T., Sakimura, K., and Shiina, N. (2017). RNG105/caprin1, an RNA granule protein for dendritic mRNA localization, is essential for long-term memory formation. eLife *6*, e29677. (*: equal contribution)