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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.

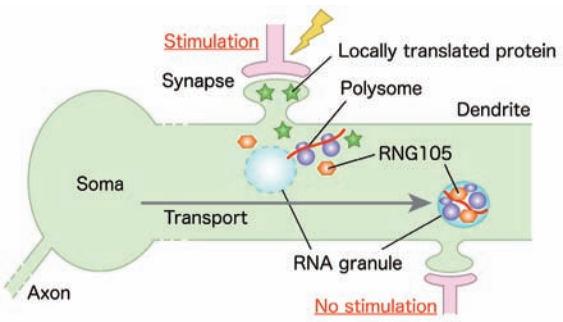

Figure 1. A model for local translation in neuronal dendrites. Specific mRNAs are recruited into RNA granules and transported to dendrites. Translation of the mRNAs is induced locally upon synaptic stimulation, which modifies local postsynapses to regulate synaptic connection and network formation.

I. Autism spectrum disorder (ASD)-like behaviors in RNG105 heterozygous mice

We previously identified RNA granule protein 105 (RNG105, also known as 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.

A recent study reported that a heterozygous mutation in the *Rng105/Caprin1* gene was found in an autism spectrum disorder (ASD) patient, but it remained unclear whether there is a causal relationship between RNG105 deficiency and ASD. We then subjected *Rng105*$^{+/-}$ mice to a comprehensive behavioral test battery, and revealed the influence of RNG105 deficiency on mouse behavior. *Rng105*$^{+/-}$ mice exhibited a reduced sociality in a home cage and a weak preference for novel mice. Consistently, the *Rng105*$^{+/-}$ mice also showed a weak preference for novel objects and novel place patterns. Furthermore, although the *Rng105*$^{+/-}$ mice exhibited normal memory acquisition in spatial reference tasks, they tended to have relative difficulty in reversal learning in the tasks. These findings suggest that the RNG105 heterozygous knockout leads to a reduction in sociality, response to novelty and flexibility in learning, which are implicated in ASD-like behavior.

II. Long-term memory deficits in RNG105 conditional knockout (cKO) mice

Although learning and memory, except for reversal learning, were normal in mice with a moderate deficiency of RNG105 (*Rng105*$^{+/-}$), learning and memory were remarkably impaired in mice with severe deficiency of RNG105: RNG105 conditional knockout (cKO) postnatally in the brain region caused reduced contextual and spatial long-term memories.

For example, RNG105 cKO mice showed severe deficits in the Morris water maze, a spatial long-term memory task in which a mouse is placed in a circular pool and learns the location of a hidden platform that allows the mouse to escape from water. Repeated trials enabled control mice to learn the platform location and escape on the platform faster than before the trials (Figure 2A). In contrast, the escape latency of RNG105 cKO mice did not shorten at all over the trials (Figure 2A). Following the last trial, a probe test was conducted: the platform was removed from the pool and the swimming path of the mice was visualized by tracking the mice. Control mice intensively searched around the target

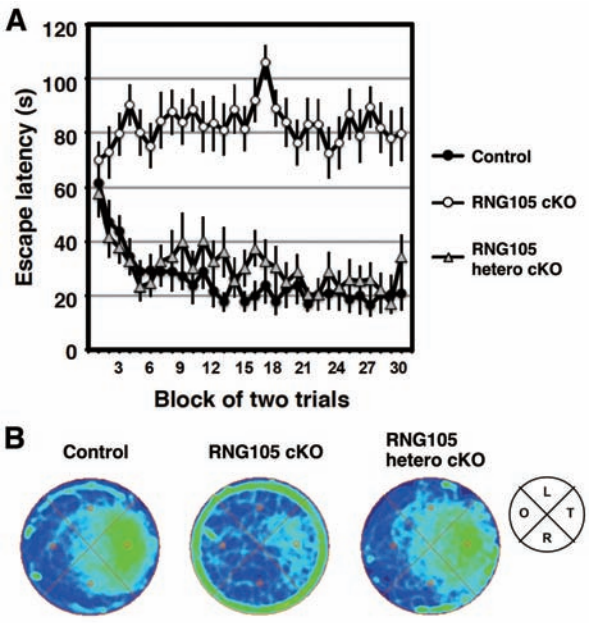

Figure 2. Spatial memory deficits of RNG105 cKO mice in Morris water maze. (A) Escape latency during the acquisition phase of the Morris water maze. (B) Spatial histograms of the mice's swimming paths during the probe trial of the Morris water maze. T indicates the target quadrant.

place where the removed platform had existed (Figure 2B). In contrast, RNG105 cKO mice showed a circular swimming path along the wall of the pool and reduced the search time around the target place (Figure 2B).

III. Reduced structural synaptic plasticity in RNG105 cKO mice

Memory formation is generally correlated with structural plasticity of postsynapses (spines) on dendrites, i.e., stimulation-induced long-lasting increase in the size of spines. Therefore, we measured the stimulation-dependent changes in spine size in RNG105 cKO cultured neurons. In response to synaptic stimulation by glutamate uncaging, spines near the uncaging locus increased in volume ~2.5-fold and sustained the increased state at least for 60 min in control neurons (Figures 3A and 3B). In contrast, in RNG105 cKO neurons, spine volume during the sustained phase was significantly reduced (Figure 3B). These results suggested that structural plasticity of spines was reduced by RNG105 deficiency.

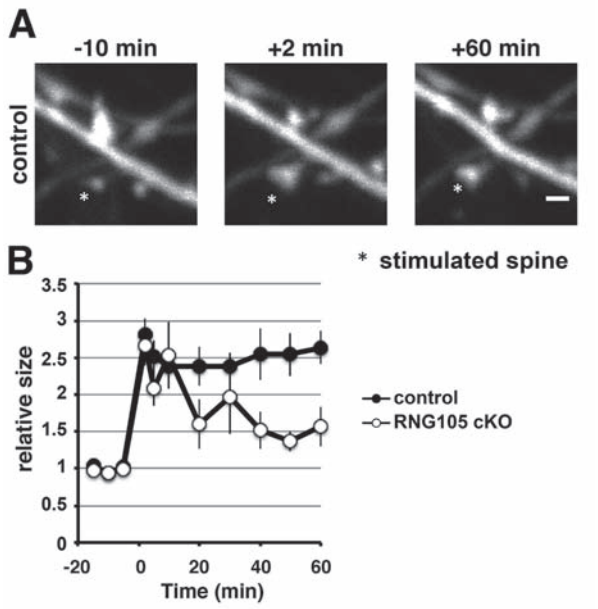

Figure 3. Reduced structural plasticity of spines in RNG105 cKO neurons. (A) Representative spine images before and after synaptic stimulation by glutamate uncaging. Scale bar, 1 μm. (B) Time-lapse graph of the effect of synaptic stimulation on spine size.

IV. Reduced localization of mRNAs in dendrites of RNG105 cKO neurons

To investigate the mechanism of how RNG105 cKO affects synaptic plasticity and long-term memory, we comprehensively identified mRNAs whose dendritic localization was reduced in RNG105 cKO neurons. The soma and dendrites of neurons were isolated by microdissection of the hippocampal stratum pyramidale (SP) layer and stratum radiatum (SR) layer, respectively, and then subjected to RNA-seq analysis using next-generation sequencing. By comparing mRNAs from the soma and dendrites, we identified 1,122 dendritically enriched mRNAs and 2,106 somatically enriched mRNAs. Enrichment of most of the dendritic mRNAs in dendrites was significantly reduced in RNG105 cKO neurons, which suggested that RNG105 is responsible for the dendritic localization of many different mRNAs.

Gene ontology enrichment analysis of the identified mRNAs revealed that categories in which significantly large number of mRNAs were enriched were quite different between somatic and dendritic mRNAs. Major categories for dendritic mRNAs were “Regulation of Arf protein signal transduction” which included GTPase-activating proteins (GAPs) and guanine nucleotide exchange factors (GEFs) of small G protein Arf and “Structural constituent of ribosomes” which included ribosomal subunit proteins. Arf is known to regulate membrane trafficking between the cell surface and endosomes through controlling endocytosis and exocytosis, which includes glutamate receptor (AMPA receptor) surface expression in neurons. Arf is also known as an important regulator of actin cytoskeleton dynamics and dendritic spine formation via Rac1 activation. “Regulation of small GTPase-mediated signal transduction” was also a category in which dendritic mRNAs were enriched, which included regulators of small G proteins such as Ras, Rho and Rac, known to be involved in synaptic and actin regulation. Dendritic mRNAs and gene ontology categories identified in this study provide insight into underlying mechanisms for dendritic mRNA localization-dependent long-term synaptic plasticity and memory, which will be addressed in the future.

Publication List:

〔Original paper〕

- Ohashi, R., Takao, K., Miyakawa, T., and Shiina, N. (2016). Comprehensive behavioral analysis of RNG105 (Caprin1) heterozygous mice: Reduced social interaction and attenuated response to novelty. Sci. Rep. *6*, 20775.