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 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.

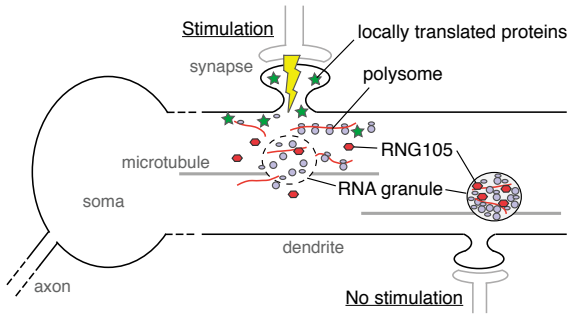

Figure 1. A model for local translation in neuronal dendrites. Specific mRNAs are recruited into RNA granules and transported to dendrites. Translation of the mRNAs is induced locally upon synaptic stimulation, which modifies local postsynapses to regulate synaptic connection and network formation.

I. Comprehensive behavioral analysis of RNG105 heterozygous mice: Implications in autism spectrum disorder (ASD)

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. 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).

A recent study aiming to detect genetic variants in ASD by whole-genome sequencing reported that a heterozygous *de novo* nonsense mutation in the *Rng105/Caprin1* gene was found in a patient with Asperger's syndrome, a form of ASD. The patient's intelligence quotient (IQ) was above average, but adaptive behavior and sociability were delayed. The report suggested that *Rng105/Caprin1* is a candidate risk gene for ASD, but it remained unclear as to whether there was a causal relation between RNG105 deficiency and ASD.

To investigate the influence of RNG105 deficiency on mouse behavior, we subjected RNG105 heterozygous ($Rng105^{+/-}$) mice to a comprehensive behavioral test battery. One of the marked changes in $Rng105^{+/-}$ mice was sociality. $Rng105^{+/-}$ mice showed reduced social interaction with familiar mice in a home cage test and reduced social interest/preference for novel mice over familiar mice in a three-chambered social approach test (Figure 2). The latter change can be characterized as indistinguishable responses of $Rng105^{+/-}$ mice to familiarity and novelty, as the $Rng105^{+/-}$ mice also showed reduced interest/preference for novel objects and places over familiar objects and places, respectively. In several maze tasks, $Rng105^{+/-}$ mice showed normal acquisition of memory, and in some of the tasks, they showed higher performance compared to wild-type mice. However, they showed relative difficulty in reversal learning,

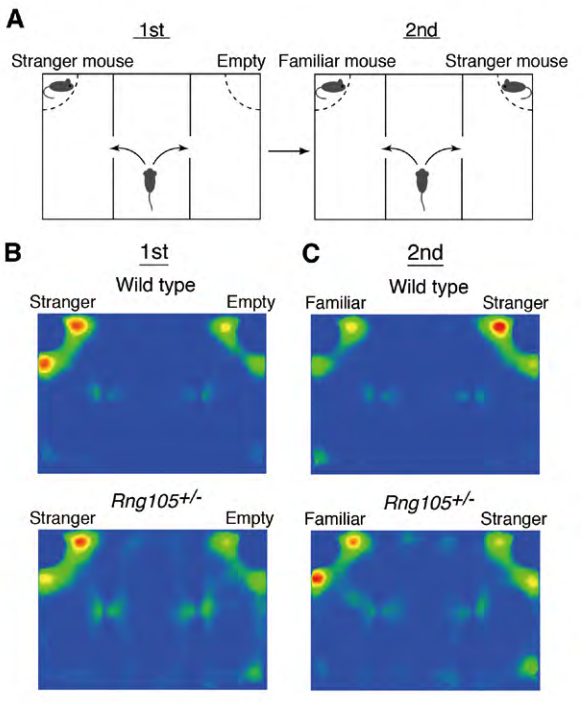

Figure 2. Three-chambered social approach test. (A) Schematic diagram of the test. In the first session, one of the cages (dotted lines) contains a stranger mouse. A test mouse is allowed to freely explore the three chambers. In the second session, a familiar mouse and a stranger mouse are put in each cage. (B and C) Heat maps showing the average traces of wild-type mice (top panels) and $Rng105^{+/-}$ mice (bottom panels) in the first session (B) and the second session (C). Social interaction with a stranger mouse is normal, but there is a lack of interest/preference for a stranger mouse over a familiar mouse in $Rng105^{+/-}$ mice.

in which the target was changed from the initial position. These results suggest that social interaction, responses to novel situations, and flexibility to changes were reduced in *Rng105*$^{+/-}$ mice.

Furthermore, RNG105-deficient neurons showed a reduction in AMPA glutamate receptor (AMPAR) cell surface distribution in dendrites (Figure 3), which has been reported in other ASD-like mutant mice and is thought to be related with the neuropathology of ASD. The behavioral test battery, together with the analysis of AMPAR distribution, suggest that an RNG105 deficiency leads to ASD-like behavior. In particular, *Rng105*$^{+/-}$ mice performed well in specific tasks, suggesting that the phenotype of *Rng105*$^{+/-}$ mice was related to Asperger's syndrome-like behavior.

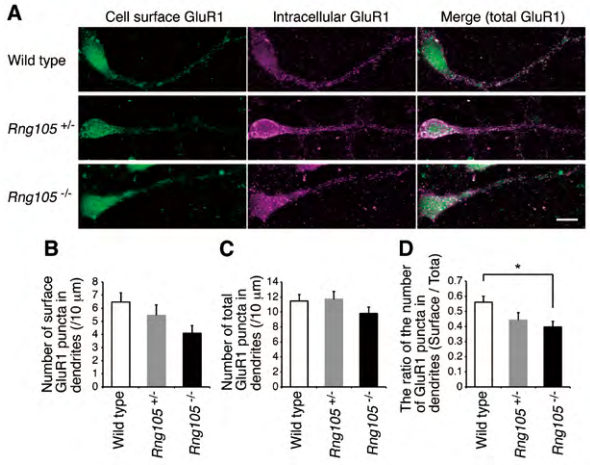

Figure 3. The cell surface distribution of AMPAR subunit GluR1 is reduced in dendrites of RNG105-deficient neurons. (A) Immunostaining for GluR1 in cultured neurons (9 DIV) from cerebral cortexes of E17.5 wild-type, *Rng105*$^{+/-}$ and *Rng105*$^{-/-}$ mice. Scale bar, 10 μm. (B and C) The number of surface (B) and total (C) GluR1 puncta in dendrites. (D) The ratio of surface/total number of GluR1 puncta in dendrites. *p<0.05, one-way ANOVA followed by Turkey-Kramer test.

II. Learning and memory deficits in RNG105 conditional knockout (cKO) mice

Although learning and memory were normal in mice with a moderate deficiency of RNG105 (*Rng105*$^{+/-}$), learning and memory were markedly impaired in mice with severe deficiencies of RNG105: RNG105 conditional knockout (cKO) in the brain after birth led to reduced contextual and spatial memories.

Because memory formation is highly correlated with increases in the size of spine heads (postsynapses) on neurons, we measured the size of spines in the hippocampus of RNG105 cKO mice. The size of the spine heads was significantly smaller in RNG105 cKO mice than in wild-type mice, which may underlie the impaired memory formation in RNG105 cKO mice.

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). To investigate the role of RNG140 in higher brain functions in adult mice, we have started to generate RNG140 knockout mice with the CRISPR/Cas9 system.

III. RNA granule dynamics regulated by PRMT1

Regulation of RNA granule assembly and disassembly is emerging as a key mechanism of translational control, defects of which are linked with neurodegenerative diseases such as amyotrophic lateral sclerosis (ALS) and frontotemporal lobar degeneration (FTLD). We conducted proteomic analyses to identify proteins associated with RNG105, and identified PRMT1 as a factor which disassembles RNG105-containing RNA granules.

PRMT1 is a major arginine methyltransferase that methylates arginine residues in the arginine and glycine-rich (RG-rich) domain. We found that the RG-rich domain of RNG105 was bound and methylated by PRMT1. A methyltransferase activity-deficient mutant PRMT1 also bound to RNG105, but had reduced ability to methylate RNG105 and disassemble RNA granules. These results suggested that not only the association with RNG105, but also the methylation activity of PRMT1 is required to disassemble RNA granules. Because various RNA granule components, including an ALS and FTLD risk factor FUS, have the RG-rich domain, it will be a future challenge to investigate whether methylation of these RNA granule factors is implicated in the regulation of RNA granule dynamics and neurodegenerative disease.

Publication List:

〔Original paper〕

- Tsuboi, D., Kuroda, K., Tanaka, M., Namba, T., Iizuka, Y., Taya, S., Shinoda, T., Hikita, T., Muraoka, S., Iizuka, M., Nimura, A., Mizoguchi, A., Shiina, N., Sokabe, M., Okano, H., Mikoshiba, K., and Kaibuchi, K. (2015). Disrupted-in-schizophrenia 1 regulates transport of ITPR1 mRNA for synaptic plasticity. Nat. Neurosci. *18*, 698-707.