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. We are researching 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.

I. Roles of mRNA transport and local translation in the formation of neuronal networks

Specific mRNAs are recruited into "RNA granules" in neuronal dendrites. RNA granules are macromolecular complexes composed mainly of mRNAs and ribosomes, and mediate the transport and local translation of their mRNA cargoes in response to synaptic stimulation (Figure 1).

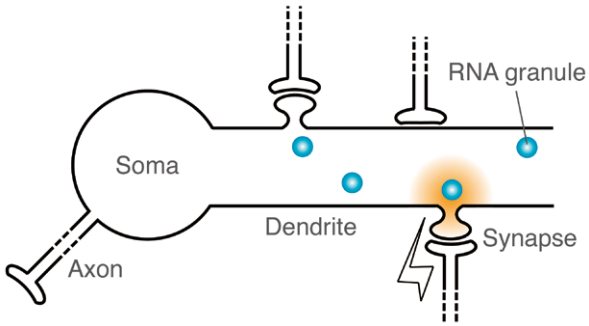

Figure 1. A model for local translation in neuronal dendrites. Specific mRNAs are recruited into RNA granules and transported to dendrites. Translation of the mRNAs is induced locally upon synaptic stimulation, which modifies local postsynapses to regulate synaptic connection and network formation.

We have identified RNA granule protein 105 (RNG105), an RNA-binding protein, as a component of RNA granules. RNG105 dissociates from RNA granules after synaptic stimulation, which is accompanied by the induction of mRNA translation near the granules, suggesting RNG105's involvement in the delivery of its cargo mRNAs to the site for local translation and/or the control of local translation.

To understand the function of RNG105, we have generated RNG105 knockout mice. Furthermore, we have identified about 60 RNG105 cargo mRNAs in neurons. The cargo mRNAs, e.g., those encoding Na^+/K^+ ATPase (NKA) subunit isoforms, are transported to dendrites together with RNG105, but their dendritic transport is markedly reduced in neurons from RNG105 knockout mice. These results indicate the role of RNG105 in the dendritic transport of mRNAs. The RNG105 knockout neurons exhibit reduced dendritic synapse formation and reduced dendritic arborization, which results in poor development of neuronal networks (Figure 2). The perturbed formation of dendritic synapses and networks is mimicked by inhibition and knockdown of NKA subunit isoforms. Taken together, these results suggest that 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.

We are currently investigating the roles of proteins encoded by RNG105 cargo mRNAs in the formation of synapses and neuronal networks. We are also investigating *in vivo* formation and function of neuronal networks in fetuses of RNG105 knockout mice because RNG105 knockout neonates die soon after birth due to respiratory failure, which is associated with defects in fetal brainstem development. Furthermore, we are generating conditional RNG105 knockout mice to investigate the role of RNG105 in higher brain functions, e.g., memory and learning, in adult mice.

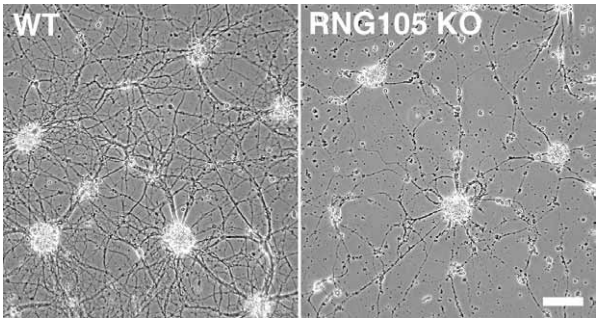

Figure 2. Phase contrast images of primary cultured neurons from wild-type (WT) and RNG105 knockout (KO) fetal brains. RNG105 knockout neurons formed poor networks compared to wild-type neurons. Scale bar, 100 μm.

II. Divergence of mRNA transporting machinery

We have also identified an RNG105 paralog, RNG140. RNG105 and RNG140 are conserved only in vertebrates and an RNG105/RNG140 homolog is found in higher invertebrates, i.e., urochordates and insects. The genes are highly expressed in the central nervous system, suggesting a link between the function of the genes and neuronal functions. RNG140 has RNA-binding domains highly conserved with RNG105 and directly binds to RNA. RNG140 as well as RNG105 induces the formation of RNA granules where mRNAs are recruited. However, RNG140-induced RNA granules do not contain RNG105, and vice versa, indicating that RNG105 and RNG140 induce distinct RNA granules (Figure 3). RNG105-induced RNA granules are similar to stress-induced stress granules in terms of

molecular components and stress inducibility, but RNG140-induced RNA granules are not similar to any other previously known RNA granules. The timing of expression of the two genes during development is also different: RNG105 is highly expressed in embryos, but RNG140 is highly expressed in adults.

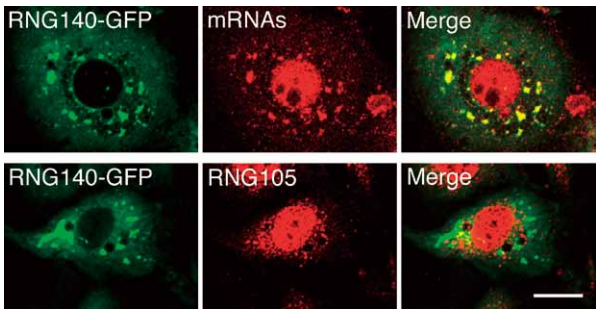

Figure 3. Staining of cultured fibroblasts expressing RNG140-green fluorescent protein (GFP). RNG140-GFP induced the formation of cytoplasmic RNA granules. The cells were stained for mRNAs with poly(dT) probes (top panels) and for RNG105 with an anti-RNG105 antibody (bottom panels). RNG140-GFP-induced granules contained mRNAs but not RNG105. Scale bar, 10 μm.

In spite of localizing to distinct RNA granules, RNG105 and RNG140 gene knockdown show similar effects on neurons: suppression of either gene reduces dendritic arborization and dendritic synapse formation. However, the knockdown effects of RNG140 are not rescued by RNG105, and vice versa, suggesting that RNG105 and RNG140 play similar roles in the development of dendrites and dendritic synapses through different pathways. Thus, RNG105 and RNG140 are localized to different kinds of RNA granules and play roles in the development of dendritic structure at distinct developmental stages.

We are currently identifying components included in the RNG140 RNA granules and investigating if the RNG140 RNA granules transport the same cargo mRNAs as RNG105 RNA granules. We will further generate knockout mice for RNG140 and RNG105- and RNG140-associated mRNAs and proteins to analyze their roles in higher order brain functions.

Publication List

〔Original papers〕

- Shiina, N., and Tokunaga, M. (2010). RNA granule protein 140 (RNG140), a paralog of RNG105 localized to distinct RNA granules in neuronal dendrites in the adult vertebrate brain. J. Biol. Chem. *285*, 24260-24269.
- Shiina, N., Yamaguchi, K., and Tokunaga, M. (2010). RNG105 deficiency impairs the dendritic localization of mRNAs for Na^+/K^+ ATPase subunit isoforms and leads to the degeneration of neuronal networks. J. Neurosci. *30*, 12816-12830.