LABORATORY OF NEURONAL CELL BIOLOGY

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The transport of specific mRNAs and local control of translation in neuronal dendrites are part of 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 understanding 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, which mediate the transport and local translation of their mRNA cargoes in response to synaptic stimulation (Figure 1). We are researching the mechanism of RNA granule assembly, 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 relationship to the formation of synapses and neural networks, memory, learning, and behavior.

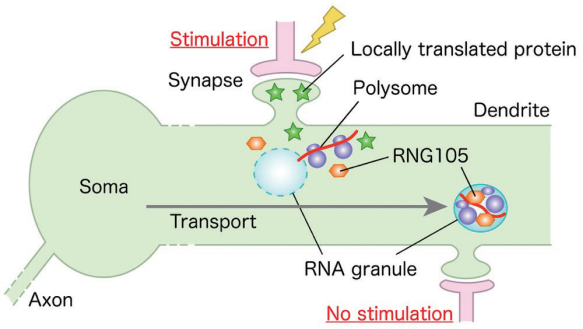

Figure 1. A model for local translation in neuronal dendrites. Specific mRNAs are recruited into RNA granules and transported to dendrites. Translation of the mRNAs is induced locally upon synaptic stimulation, which modifies local postsynapses to regulate synaptic connection and network formation.

I. Liquid and solid-phase RNA granules form through specific proteins and combine into biphasic granules

RNA granules consist of membrane-less RNA–protein assemblies and contain dynamic liquid-like shells and stable solid-like cores, which are thought to function in numerous processes in mRNA sorting and translational regulation (Figure 2). Abnormalities in RNA granule dynamics are associated with neurodegenerative diseases, such as amyotrophic lateral sclerosis (ALS) and frontotemporal lobar degeneration (FTLD), in which solidification and aggregation of RNA granule components are facilitated in neurons. However, how these distinct liquid-like and solid-like substructures are formed, whether they are assembled by different scaffolds, and whether different RNA granule scaffolds induce these different substructures remains unknown.

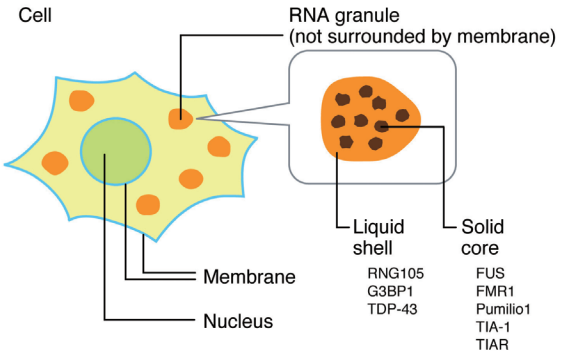

Figure 2. Membrane-less RNA granules contain liquid-like and solid-like substructures. Liquid-phase granules induced by RNG105, G3BP1, and TDP-43, and solid-like granules induced by FUS, FMR1, Pumilio1, TIA-1, and TIAR, combine into biphasic RNA granules.

We expressed 8 kinds of RNA granule scaffold proteins (scaffolds) as GFP and RFP-tagged proteins in cultured cells, and conducted fluorescence microscopy-based morphological and molecular-dynamics analyses (Shiina, N., J. Biol. Chem. doi: 10.1074/jbc.RA118.005423). These analyses demonstrated that the scaffolds can be largely classified into two groups, liquid and solid types, which induce the formation of liquid-like and solid-like granules, respectively, when expressed separately in cultured cells. Liquid-like granules were induced by RNG105 (also known as Caprin1), G3BP1, and TDP-43, whereas solid-like granules were induced by FUS, FMR1, Pumilio1, TIA-1, and TIAR (Figure 2). Furthermore, we found that when co-expressed, the liquid-type and solid-type scaffolds combine and form individual liquid- and solid-like substructures in the same granules (Figure 3). The combination of the different types of scaffolds reduced the immobile fractions of the solid-type scaffolds and their dose-dependent ability to inhibit translation in granules, but had little effect on the dynamics of the liquid-type scaffolds or their dose-dependent ability to increase translation in granules. These results suggest that liquid- and solid-type scaffolds form different substructures in RNA granules and the relative effect of each type on their scaffold counterpart varied. These findings provide a detailed insight into the assembly mechanism and distinct dynamics and functions of core and shell substructures in RNA granules.

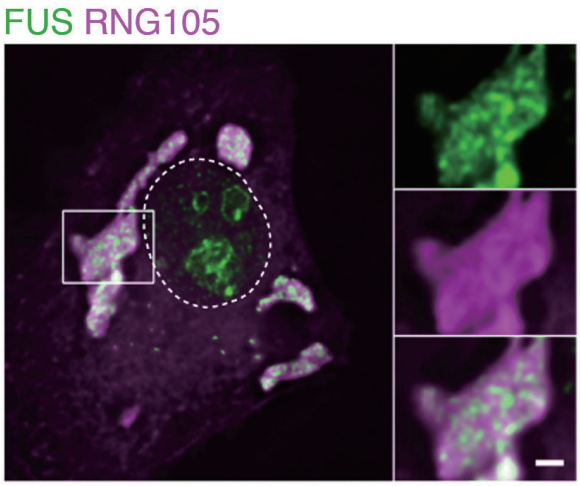

Figure 3. Liquid-type scaffolds (*e.g.* RNG105) and solid-type scaffolds (*e.g.* FUS) combine into the same granules and form individual liquid-like and solid-like substructures,. The panels on the right are magnified images of the boxed area. Top: FUS Middle: RNG105 Bottom: merged. The area surrounded by the dotted line contains the nucleus. Scale bar: 2 μm.

II. Dendritic localization of mRNAs for Arf GEFs and GAPs that are involved in spine formation and maturation in dendrites

Local protein synthesis plays an important role in synaptic plasticity and memory formation. To achieve local translation in dendrites, specific mRNAs are required to be localized to dendrites. RNG105 is a major RNA-binding protein localized to RNA granules, and its deficiency in mice leads to the impairment of higher-order brain functions such as long-term memory and sociability. We previously found that many dendritic mRNAs are reduced in the dendritic layer of the hippocampus in RNG105-deficient mice, particularly mRNAs for ADP-ribosylation factor (Arf) regulators, Arf GEFs and Arf GAPs.

First, we aimed to visualize and quantify the dendritic localization of 8 kinds of mRNAs for the Arf regulators using the MS2 system in mouse cerebrum primary cultured neurons. We found that Arf GEF mRNAs and GAP mRNAs were localized to dendrites in a different way; most of the Arf GEF mRNAs were localized to dendrites independently on KCl stimulation (neuronal depolarization). Despite this, most of the Arf GAP mRNAs were localized to dendrites in a KCl stimulation-dependent manner. Next, we analyzed the knockdown effects of the Arf regulators on the formation of dendritic postsynapses (spines) in primary cultured neurons. Knockdown experiments with shRNA demonstrated that the Arf GEFs and GAPs were classified into two groups: one group consisted of positive regulators of spine formation and maturation, and the other consisted of negative regulators of immature spine formation. These results suggested that Arf GEFs and GAPs both play important roles in tuning spine formation and maturation through their mRNAs being localized to dendrites at a specific timing.

III. Comprehensive behavioral analysis of mice that lack the intrinsically disordered region (IDR) of NFAR2, a stress responsive translation regulatory factor

RNA-binding proteins possessing IDRs, which do not form three-dimensional structures, have been revealed to play an important role in RNA-protein complex assembly and translation regulation. We focused on an RNA-binding protein, NFAR2, that possesses an IDR. NFAR1 and NFAR2 are splicing variants transcribed from a single *ILF3* gene, and inhibit translation of their binding mRNAs in a stress-dependent (*i.e.* oxidative stress) manner. However, they differ in that only NFAR2, but not NFAR1, has the IDR and can associate with RNA granules. To investigate the physiological relevance of the IDR of NFAR2, we generated NFAR2ΔIDR mice, in which a stop codon was introduced in the exon encoding the IDR in the *ILF3* gene.

Comprehensive behavioral analysis demonstrated that NFAR2ΔIDR mice displayed phenotypes that displayed weight loss, body temperature elevation, hyperactivity, decreased anxiety-like behavior, and decreased startle response. Regarding learning and memory, NFAR2ΔIDR mice displayed a decrease specifically in fear-conditioned learning, but not in spatial learning. Furthermore, chronic stress, which is known to induce oxidative stress in the brain, exacerbated the fear-conditioned learning of NFAR2ΔIDR mice without affecting their spatial learning. These results suggested that the IDR of NFAR2 is responsible for specific higher-order brain functions such as fear-conditioned learning under stress conditions.