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 exactly 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 currently using mice to research 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, so we can attain a better understanding their relationship to the formation of synapses and neural networks, memory, learning, and behavior.

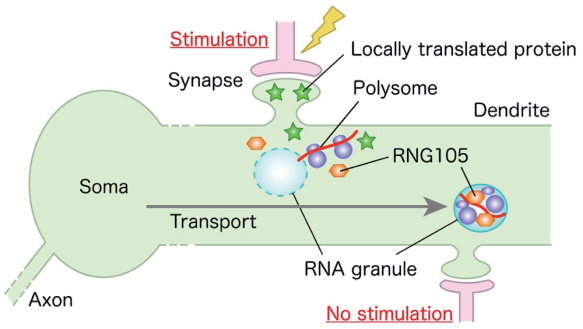

Figure 1. A model for local translation in neuronal dendrites. Specific mRNAs are recruited into RNA granules and transported to dendrites. Translation of the mRNAs is induced locally upon synaptic stimulation, which modifies local postsynapses to regulate synaptic connection and network formation.

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

RNA granules are membrane-less RNA–protein condensates formed by liquid-liquid phase separation (LLPS). They consist of not only dynamic liquid-phase shells but also stable solid-like cores, both of which are thought to function in numerous processes pertaining to 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 the case of these diseases, 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.

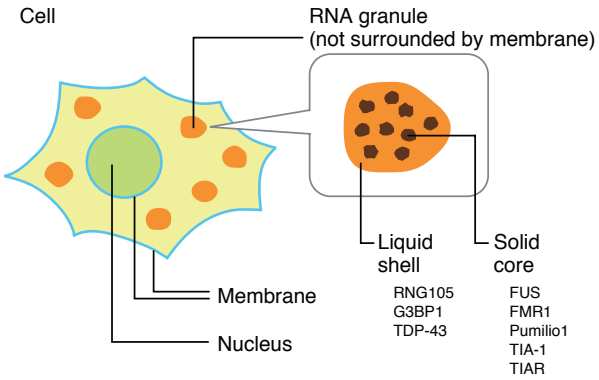

Figure 2. Membrane-less RNA granules contain liquid-like and solid-like substructures. Liquid-phase granules induced by RNG105, G3BP1, and TDP-43, and solid-like granules induced by FUS, FMR1, Pumilio1, TIA-1, and TIAR, combine into biphasic RNA granules.

We expressed 8 kinds of RNA granule scaffold proteins (scaffolds) as GFP and mRFP1-tagged proteins in cultured epithelial cells. Using these cells, we conducted fluorescence microscopy-based morphological and molecular-dynamics analyses (Shiina, J. Biol. Chem., 2019). These analyses demonstrate 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. However, it 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. Furthermore, they also raise questions as to whether the liquid and solid phase properties of RNA granules are also regulated by the scaffold proteins in neurons, and whether such phase properties impact mRNA transport and local translation in neuronal dendrites. We are testing these analyses using mice brains and primary cultured neurons.

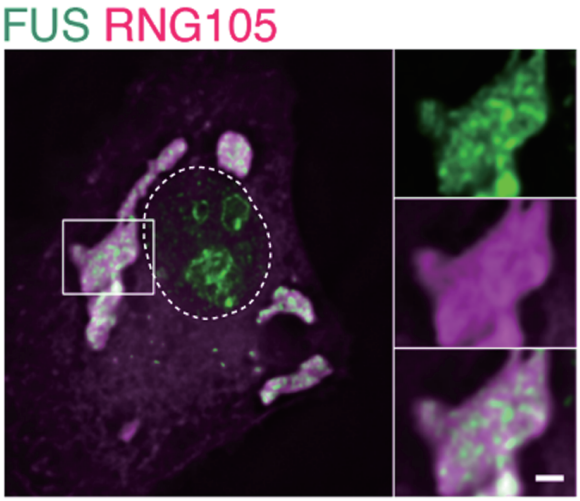

Figure 3. Liquid-type scaffolds (*e.g.* RNG105) and solid-type scaffolds (*e.g.* FUS) combine into the same granules and form individual liquid-like and solid-like substructures. The panels on the right are magnified images of the boxed area. Top: FUS, Middle: RNG105, Bottom: merged. The area surrounded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

II. RNG140 (Caprin2)-mediated translational regulation in eye lens differentiation

RNG105 and RNG140 are paralogous RNA-binding proteins that form distinct RNA granules. RNG105 is highly expressed in neurons and regulates mRNA transport and long-term memory formation (Nakayama *et al*., eLife, 2017), whereas RNG140 is highly expressed in the developing eye lens and plays a role in lens differentiation. Despite RNG140's function in translational regulation, the mechanism and its role within the eye has remained unclear.

We found that RNG140 binds to the translation initiation factor eIF3 using mass spectrometry of RNG140 immunoprecipitates from cultured CHO cells. Reporter translation assay revealed that RNG140 represses translation through mechanisms involving the suppression of eIF3-dependent translation initiation. Comprehensive ribosome profiling demonstrated that overexpression of RNG140 in CHO cells reduces translation of long mRNAs, including those associated with cell proliferation. In fact, RNG140 overexpression slowed the growth rate of CHO cells.

RNG140-mediated translational regulation also operates in the mouse eye, where RNG140 knockout increased the translation of long mRNAs. mRNAs involved in lens differentiation, such as crystallin mRNAs, are short, and were able to escape translational inhibition by RNG140 as well as be translated in differentiating lenses. These findings provide insight into the mechanistic basis of lens cell transition from proliferation to differentiation via RNG140-mediated translational regulation. Moreover, the preference for long mRNAs raised new questions about why and how RNA-binding protein complexes distinguish mRNA lengths in the coordination of proliferation and differentiation.

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

IDRs, which do not form three-dimensional structures, have been revealed to play key roles in LLPS. 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 an IDR and can associate with RNA granules. To investigate the physiological relevance of the IDR associated with NFAR2, we generated NFAR2ΔIDR mice, in which a stop codon was introduced in the exon encoding the IDR in the *Ilf3* gene.

Behavioral analysis demonstrated that NFAR2ΔIDR mice specifically displayed a decrease in fear-conditioned learning, but not in spacial 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 spacial 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.

Publication List:

〔Original paper〕

- Shiina, N. (2019). Liquid- and solid-like RNA granules form through specific scaffold proteins and combine into biphasic granules. J. Biol. Chem. *294*, 3532-3548. doi: 10.1074/jbc.RA118.005423

〔Review articles〕

- Ohashi, R., and Shiina, N. (2020). Cataloguing and selection of mRNAs localized to dendrites in neurons and regulated by RNA-binding proteins in RNA granules. Biomolecules *10*, 167. doi: 10.3390/biom10020167
- Roy, R., Shiina, N., and Wang, D.O. (2020). More dynamic, more quantitative, unexpectedly intricate: Advanced understanding on synaptic RNA localization in learning and memory. Neurobiol. Learn. Mem. *168*, 107149. doi: 10.1016/j.nlm.2019.107149