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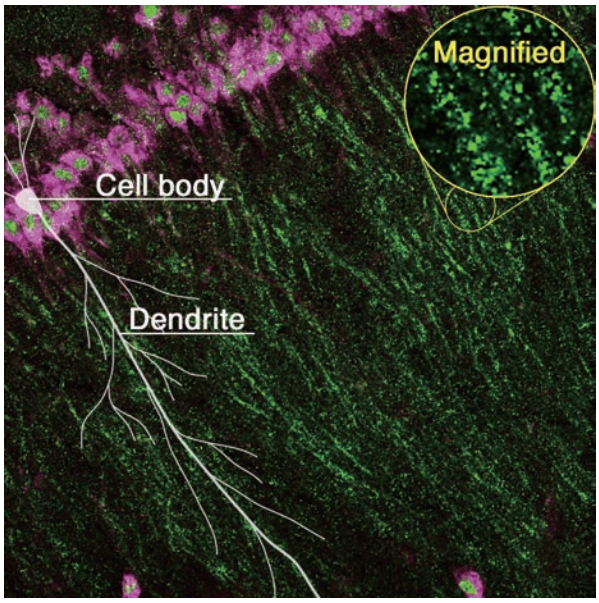

RNA granules (green) transported from the cell body (magenta) to dendrites in the hippocampus of the mouse brain.

The transportation 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 stably connect to each other, thereby forming long-term neural networks and memory. 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 membraneless organelles formed by liquid-liquid phase separation (LLPS) of RNA-binding proteins and mRNAs, which further recruit other factors such as ribosomes. They mediate the transport of mRNAs to the vicinity of synapses and synaptic stimulation-dependent local translation of the cargo mRNAs (Figure 1). We are currently using mice to research the mechanism of RNA granule assembly, RNA granule factors and their phase behavior regulating mRNA transport and local translation, their target mRNAs, and the roles of the locally synthesized proteins, so we can attain a better understanding of their relationship to the formation of synapses and neural networks, memory, learning, and behavior.

In addition to the physiological function, RNA granules are linked to neurodegenerative diseases such as amyotrophic lateral sclerosis (ALS) and frontotemporal lobar degeneration (FTLD). The basis of these diseases is thought to be the incorporation and aggregation of disease-causing proteins such as FUS and TDP-43 in RNA granules. Thus our research also aims to reveal the effects of such protein aggregation on mRNA transport and local translation.

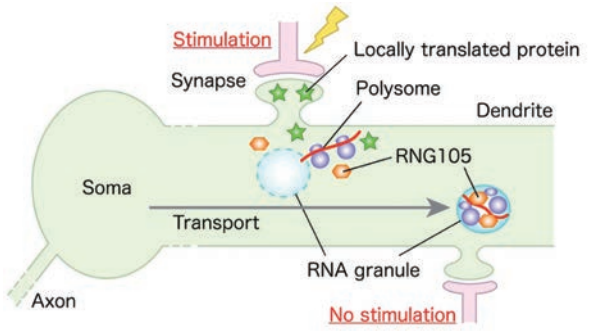

Figure 1. A model for local translation in neuronal dendrites. Specific mRNAs are recruited into RNA granules and transported to dendrites. Translation of the mRNAs is induced locally upon synaptic stimulation, which modifies local postsynapses to regulate synaptic connection and network formation.

I. Stress tolerance of conditioned fear memory conferred by alternatively spliced prion-like domain of Ilf3

LLPS is generally driven by protein intrinsically disordered regions (IDRs) that do not form three-dimensional structures. One class of IDR is the prion-like domain (PrLD), whose propensity to form aggregates has been well studied in regards to FUS and TDP-43 in neurodegenerative diseases. However, little is known about the physiological relevance of PrLDs in the brain.

We focused on an RNA-binding protein NFAR2, which has PrLD at the C-terminus. This PrLD is spliced out in the alternative splicing isoform NFAR1. Therefore, it is possible to investigate the role specific to PrLD by deleting PrLD of NFAR2, since NFAR1 without PrLD is intrinsically present and maintained even after the deletion of PrLD. NFAR1 and NFAR2 are synthesized from the *Ilf3* gene and both possess biochemical activities that they perform in common such as transcriptional regulation and stress-dependent translational inhibition. However, they differ in that NFAR2 alone can concentrate in the nucleoplasm and associate with cytoplasmic RNA granules via PrLD. To investigate the physiological relevance of PrLD, we generated NFAR2ΔPrLD mice, in which a stop codon was introduced into the NFAR2-specific exon.

Deletion of the PrLD lost the nucleoplasmic localization of NFAR2 and affected the genome-wide profiles of mRNA

expression and translation in the brain. In particular, PrLD deletion significantly altered chronic restraint stress (CRS)-induced changes in mRNA expression and translation in the amygdala, a brain region associated with emotional events such as anxiety and fear.

Consistent with its effect on CRS-induced changes in mRNA expression and translation, PrLD deletion affected tolerance of amygdala-associated learning and memory to CRS: conditioned fear memory in wild-type mice was unaffected by CRS, but it was exacerbated in NFAR2ΔPrLD mice due to this factor. These results suggested that PrLD of NFAR2 conferred tolerance of fear-associated memory formation to stressful environments.

II. Incorporation of FUS and TDP-43 into RNA granules releases RNG105 (Caprin1) from the granules

FUS and TDP-43 are predominantly localized in the nucleus when the cell is healthy, but translocate to the cytoplasm and incorporate into RNA granules when stricken by neurodegenerative diseases. This incorporation is thought to affect the fluidity of the components of RNA granules formed by LLPS, but little is known about their effects. We overexpressed either disease-related FUS mutants or disease related TDP-43 mutants in primary cultured neurons from the mouse cerebral cortex and analyzed their effects on the fluidity of RNA granule components using fluorescence recovery after photobleaching (FRAP) and cell permeabilization assays (Shiina, *J. Biol. Chem.*, 2019).

FUS and TDP-43 did not affect the fluidity of FMRP or PUM2 in dendritic RNA granules, but did affect the fluidity of RNG105 (Caprin1) (Figure 2). The fluidity of RNG105 was significantly increased, resulting in the release of RNG105 from RNA granules into the cytoplasm of dendrites. Since RNG105 is required for mRNA transport to dendrites and formation of long-term memory (Nakayama *et al.*, *eLife*, 2017), loss of RNG105 in dendritic RNA granules may affect these functions. We are currently investigating the molecular mechanisms underlying the changes in fluidity specific to RNG105 and the effects of FUS and TDP-43 incorporation into RNA granules on mRNA recruitment into RNA granules, local translation in the vicinity of RNA granules, and synapse formation in dendrites.

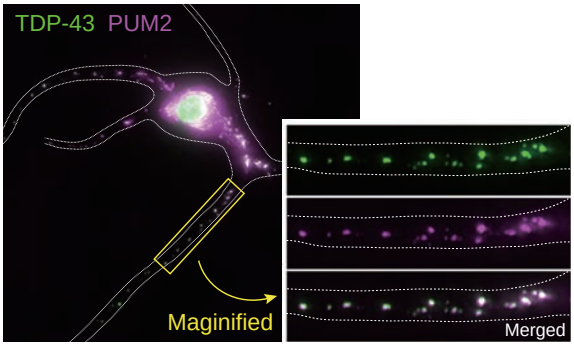

Figure 2. Incorporation of neurodegenerative disease-related proteins into RNA granules. Primary cultured neurons from mouse cerebral cortex were transfected with disease-related proteins (TDP-43-GFP in this case) together with RNA granule components (PUM2-mRFP1 in this case). The fluidity of these proteins in RNA granules was measured by FRAP and cell permeabilization assays.

III. 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, 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 underlying mechanism and its role within the eye has remained unclear.

We found that RNG140 binds to the translation initiation factor eIF3 through the application of 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 and be translated in differentiating lenses (Figure 3). 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.

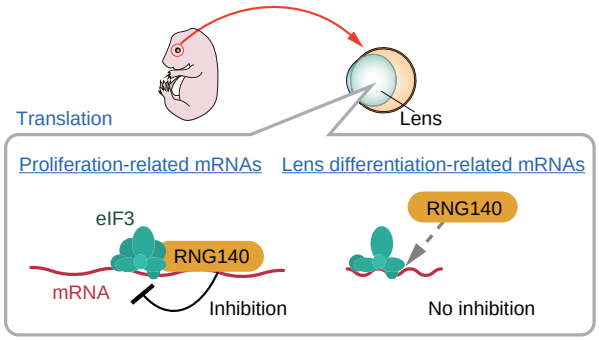

Figure 3. mRNA length-selective inhibition of translation by RNG140 in eye lens differentiation.

Publication List:

〔Original papers〕

- Nakayama, T., Okimura, K., Shen, J., Guh, Y.-J., Tamai, T.K., Shimada, A., Minou, S., Okushi, Y., Shimmura, T., Furukawa, Y., *et al.* (2020). Seasonal changes in NRF2 antioxidant pathway regulates winter depression-like behavior. Proc. Natl. Acad. Sci. U.S.A. *117*, 9594–9603. DOI: 10.1073/pnas.2000278117
- Nakazawa, K., Shichino, Y., Iwasaki, S., and Shiina, N. (2020).

Implications of RNG140 (caprin2)-mediated translational regulation in eye lens differentiation. J. Biol. Chem. *295*, 15029–15044. DOI: 10.1074/jbc.RA120.012715

〔**Review article**〕

- 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*. DOI: 10.3390/biom10020167