Genetically-encodable and customizable RNA-binding proteins for live-cell imaging and manipulation of authentic RNAs

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RNAs do not only serve as the blueprint for the protein assembly, but also play wide variety of essential functions in cells. Furthermore, expression levels of mRNAs can be indicators of cell differentiation and signal transduction. Thus, visualization and manipulation of RNAs in living cells would be beneficial for both basic and applied sciences. To this end, aptamer sequences such as MS2 stem loop or Broccoli, or synthetized fluorescent oligos such as molecular beacons were developed. However, modification of target RNA sequences and the lack of the method for efficient probe introduction have sometimes been critical problems. That's why a fully genetically-encodable probe to visualize authentic, unmodified RNAs has been expected to be developed.

Here, we report the development of customizable RNA-binding protein (dRBP), which is genetically-encodable and programmable to bind to the RNA of interest. We first established an ELISA-like in vitro assay system using our bright bioluminescent protein, Nano-lantern [1], and it was shown that the dRBPs have high affinity (1-10 nM) specifically to the target RNAs. To examine the avidity of our dRBPs to bind to target RNAs in living cells, we designed GFP-fused dRBPs for authentic, unmodified RNAs such as beta-actin mRNA. Immunoprecipitation of the probe followed by quantitative PCR analysis demonstrated that the target, authentic beta-actin mRNA was specifically recognized in vivo. We also showed that GFP-fusion of dRBPs was applicable to the visualization of the dynamics of the authentic RNAs including beta-actin mRNA or long non-coding RNA Neat1_2 in living cells. Furthermore, manipulation of the localization of the beta-actin mRNA using the dRBP fused to constitutively active kinesin resulted in the neurite-like elongation of cellular processes. These data collectively suggest that our new probe for RNA would serve as a powerful tool for the imaging and manipulation of unmodified, authentic RNAs in living cells or organisms.

[1] A. Takai et al., "Expanded palette of Nano-lanterns for real-time multicolor luminescence imaging", *Proc. Natl. Acad. Sci. U. S. A.*, **112**(14), 4352-4356, 2015