A TECHNOLOGY FOR RNA LABELING IN LIVING CELLS TO MONITOR SINGLE-MOLECULE DYNAMICS UNDER FLUORESCENCE MICROSCOPY

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We introduce here a novel mPUM technology, by which a variety of RNAs are labeled and visualized in living cells in single molecule sensitivity through TIRF and oblique illumination-fluorescence microscopy. Localization and dynamics of RNAs in living cells often concerns regulation of intracellular events. Specific localization of mRNAs implicated in cell expansion, taxis, and differentiation. Gene regulation and repairing is supported by non-coding RNAs (ncRNAs). Despite the importance of RNAs, details of their localization and dynamics in living cells have not fully analyzed due to the lack of RNA probes for fluorescence live-cell imaging. The mPUM technology is a possible candidate to provide a solution for the problem. An mPUM, which is a mutant of RNA binding protein PUM-HD, binds to a specific 8-base RNA sequence. An mPUM that binds to a particular RNA sequence can be designed in custom-made manner. We prepared several mPUM-based RNA probes that bind to target RNAs and possess a fluorescent protein such as GFP or its split fragments. The GFP fragments reconstitute and recover fluorescence upon binding of the PUM-HD mutants to the target RNA. This probe was expressed in living cells and subjected to oblique fluorescence microscope observation [1].

The first target was β-actin mRNA, whose localization and dynamics has been well studies and therefore is an ideal target to assess the ability of the mPUM technology [2, 3]. In the observation of β-actin mRNA in living cells, fluorescent spots that are representing single probe molecules attaching to β-actin mRNAs were localized on and moved along microtubules. These results indicate that the present probe has an ability to visualize trafficking of single β-actin mRNA molecules in living cells. The next target was a telomere repeat-containing RNA, TERRA, which is implicated in regulation of telomere length [4]. In the observation of TERRA, single TERRA molecules showed transient confinement around the telomere DNA and colocalization with telomere related proteins. Based on these findings, a possible mechanism of TERRA function was proposed [4].

Thus, the mPUM technology has ability to monitor diffusion motions of various RNAs in living cells under a TIRF and oblique fluorescence microscopes. Combination of the mPUM technology and advanced microscope techniques including robust single-molecule tracking and super-resolution microscopy will provide important clues to understand functions and mechanisms of RNAs in living cells.

[1] H. Yoshimura, "Live Cell Imaging of Endogenous RNAs Using Pumilio Homology Domain Mutants: Principles and Applications." *Biochemistry*, In press
[2] H. Yoshimura, A. Inaguma, T. Yamada and T. Ozawa, "Fluorescent probes for imaging endogenous β-actin mRNA in living cells using fluorescent protein-tagged pumilio." *ACS Chem. Biol.,* **7,** 999-1005 (2012).
[3] T. Yamada, H. Yoshimura, A. Inaguma and T. ozawa, "Visualization of nonengineered single mRNAs in living cells using genetically encoded fluorescent probes." *Anal. Chem.*, **83,** 5708-5714 (2011).
[4] T. Yamada, H. Yoshimura, R. Shimada, M. Hattori, M. Eguchi, T. K. Fujiwara, A. Kusumi, T. Ozawa, "Spatiotemporal analysis with a genetically encoded fluorescent RNA probe reveals TERRA function around telomeres." *Sci. Rep.*, **6,** 38910 (2016).