Visualization of Single-Molecule Motion of a Non-Coding RNA in Living Cells.

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RNA is a recent target for bioimaging studies. Subcellular localization of particular RNAs has been investigated and the dynamics of them have been partially revealed. We recently developed protein-based RNA probes using an RNA-binding domain PUM-HD, and succeeded in visualization of single molecule motion of β-actin mRNA in living cells [1, 2]. In this study, we created a fluorescent probe for a non-coding RNA, telomeric-repeat containing RNA (TERRA) to monitor its single molecule dynamics in living cells.

The probe generated in the present study consists of a pair of split EGFP fragments and a PUM-HD mutant that was designed to binds specifically to the telomeric-repeat sequence. Upon multiple probe molecules bind to the repeat region, the EGFP fragments occur intermolecular reconstitution to recover its fluorescence (Fig. 1).

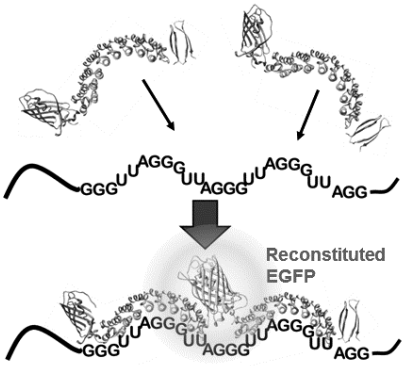

Figure. 1. Design and principle of TERRA probe developed in this study.

Using this probe, we performed simultaneous fluorescence observation of TERRA, telomere, and a telomere-related protein hnRNPA1 in living cells. A telomere probe (TRF1-iRFP) and TMR-labeled hnRNPA1-SNAPf were expressed in living cells in addition to the TERRA probe, and observed simultaneously with a TIRF microscope that equipped excitation lasers (488, 561, and 640 nm) and three cameras. In the observation, we found diffusing and static single molecule TERRA and hnRNPA1, and static telomeres in identical cells. By merging the images after distortion correction, we detected colocalization of TERRA and hnRNPA1 continuing 0.3 sec or more, which indicates complex formation of these molecules. Then we analyzed spatial distribution of the complex formation. The complexes were intensively formed in regions of 1 μm from individual telomeres. The complexes formed between static TERRA and diffusing hnRNPA1. Thus, we succeeded in live cell imaging and detail analysis of single molecule motion of a non-coding RNA TERRA and related proteins simultaneously. The principle of the probe and microscope observation will provide a general method for live cell single molecule RNA imaging studies [3].

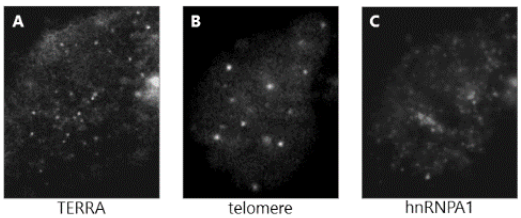

Fig. 2. Simultaneously observed fluorescence images of (A) TERRA, (B) telomer, and (C) hnRNPA1 obtained in an identical cell.

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