

## 論文の内容の要旨

### **Development of a Fluorescent Probe for Visualizing Telomeric Repeat-Containing RNA in Living Cells at the Single Molecule Level**

(生細胞内のテロメア RNA を一分子レベルで検出する蛍光プローブの開発)

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Mammalian cells have two types of RNA; messenger RNA (mRNA) and non-coding RNA (ncRNA). mRNA is considered as a genetic transporter which is transcribed from a gene in nucleus and is transported to cytoplasm for producing its protein. Recent RNA imaging studies revealed that the subcellular distribution of mRNA is asymmetry, resulting to local gene expression. However, there is no ideal method to study the dynamics of mRNA, especially at the single molecule level, in living cells. Much less is known about ncRNA. Genome-wide analysis of RNA found that more than half of the transcripts are ncRNA. Among ncRNAs, particular attention has focused on the transcripts referred to as long non-coding RNAs (lncRNAs), operationally defined as being longer than 200

nucleotides. Although, numerous studies showed that lncRNA has a critical role in gene expression, little is known about the mechanism of lncRNA.

In my PhD thesis, I develop a fluorescent probe for live cell imaging of endogenous RNA, applicable both mRNA and lncRNA, with single-molecule sensitivity. By using the probe, I investigated spatiotemporal information of  $\beta$ -actin mRNA and telomeric repeat-containing RNA (TERRA). The probe is designed to specifically target RNA. A domain of an RNA-binding protein, PUM-HD, was mutated to recognize the target sequence in RNA. Each split fragment of enhanced green fluorescent protein (EGFP) is conjugated to amino and carboxyl terminal of mutant PUM-HD. When the two probe molecules come close on the repeat region, EGFP is reconstituted between the adjacent probes.

I applied this probe to  $\beta$ -actin mRNA which produced cytoskeletal actin protein and a long non-coding RNA transcribed from a telomere, telomeric repeat-containing RNA (TERRA). Single-molecule imaging of  $\beta$ -actin mRNA revealed the localization of  $\beta$ -actin mRNA at the leading edge upon growth stimuli and the direct movement of  $\beta$ -actin mRNA along microtubules. Moreover, live-cell imaging of TERRA at the single-molecule level showed accumulation of TERRA around a telomere, a chromosome end which transcribes TERRA. By visualizing heterogenous nuclear ribonucleoprotein A1 (hnRNPA1), I found that TERRA localized in a telomere-neighboring region trapped diffusive hnRNPA1, thereby inhibiting hnRNPA1 localization to the telomere. Based on the single-particle analysis, I propose a mechanistic model how TERRA functions as a scaffold to hold hnRNPA1 around a telomere, inhibiting the localization of hnRNPA1 to the telomere.