

S42-3 **Imaging and quantification of endogenous mRNA in single living cell**

○Kohki OKABE¹, Yoshie HARADA², Takashi FUNATSU¹

¹Univ. of Tokyo, Grad. Sch. of Pharm. Sci., ²Kyoto Univ., iCeMS

In eukaryotic cells mRNA plays a key role in gene regulations. Direct observation of endogenous mRNA in living cells is of great importance because it will help to establish a therapy using small interfering RNAs as well as the understanding of gene regulation. Here, we developed a novel method for imaging and quantification of specific endogenous mRNA in living cells using antisense probe bearing artificial nucleic acid backbone. To detect the target mRNA selectively, we utilized two antisense probes having different fluorophores. These probes are complementary to an adjacent sequence of target mRNA and therefore FRET occurs upon hybridization. By observing the FRET fluorescence, real time imaging of a specific endogenous mRNA was performed. Furthermore, endogenous mRNA in living cells was quantified with fluorescence correlation spectroscopy (FCS). Quantitative analysis of antisense probe in living cell by FCS showed two fractions having different diffusion times, suggesting that probes hybridized with mRNA showed slower diffusion times than those of unbound probes. Using a model for hybridization of antisense probe and kinetic parameters such as K_d and the concentration ratio of bound/free probe, the concentration of specific endogenous mRNA in each single cell was determined. This novel method to image and quantify endogenous mRNAs in single living cells will help us to elucidate the characteristics of mRNA such as distributions and functions.