S59-4 Single-molecule imaging and quantification of mRNA at real time in a living cell

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In eukaryotic cells mRNA plays a key role in gene regulation. However, the function of mRNA is not fully understood because visualization of endogenous mRNAs in living cells has been difficult. Here we report techniques of quantifying specific mRNA and visualizing single mRNA molecules in living cells. First, endogenous c-fos mRNA in COS7 cells were labeled with a fluorescent antisense 2'*O*-methyl RNA oligonucleotide and analyzed by Fluorescence Correlation Spectroscopy. The association constant of the antisense probe and c-fos mRNA were determined. And concentration of c-fos mRNA was measured from the association constant and concentrations of free and bound probes. Next, single-molecule imaging of mRNA in the nucleus was performed as follows. Intronless and truncated ftz mRNA was synthesized and labeled with a Quantum Dot *in vitro* and it was microinjected into the nucleus of Cos7 cell. We succeeded in observing movement of individual mRNAs with temporal resolution of 30 ms and observation duration more than 60 s. Almost all of the mRNAs were moving, and the statistical analyses revealed anomalous diffusion between barriers with microscopic diffusion coefficient of 0.12 $\mu m^2/s$ and macroscopic diffusion coefficient of 0.025 $\mu m^2/s$. mRNAs were diffusing except chromatin labeled by histone2B-GFP. These results provide direct evidence of channeled diffusion of mRNA in interchromatin region.