Toward deciphering biological phenomena by genetically-encoded molecular spies

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Our primary goal is to better understand how biological molecules function in space and time. To this end, we are developing several techniques to visualize physiological events at molecular level in living cells and whole body. One approach is the use of the green fluorescent protein and its derivatives (FPs) which are spontaneously fluorescent. To expand color palette of FPs, we recently invented a pH-insensitive ultramarine fluorescent protein, Sirius, with enhanced photostability and an emission peak at 424 nm, the shortest wavelength among fluorescent proteins reported to date. The pH-insensitivity of Sirius makes possible prolonged visualization of biological events in an acidic environment. Combination of FPs with fluorescence resonance energy transfer (FRET) technique allows us to develop functional indicator, thereby we can visualize localized molecular events in their natural environment in vivo. For example, we have developed an ultra-sensitive Ca^{2+} indicator by introducing some modification into Ca^{2+} sensing domain of YC3.60. Its small K_d value (20 nM) allows us to detect Ca^{2+} dynamics even at 10-150 nM ranges without affecting cellular viability. Large dynamic range (1400 %) also enables us to detect the signaling pattern in 100,000 cellular networks at single cell resolution, being the largest scales to be achieved so far. Furthermore, we applied the FRET technique to make a photoconvertible fluorescent protein, Phamret, which can be highlighted by UV stimulation inducing a change in fluorescence emission from cyan to green color. Phamret can be monitored by single-excitation-dual-emission mode allowing mobility analyses over a broad range of kinetics. In this seminar, I will introduce not only several kinds of FP-based indicators mentioned above but also the use of autoluminescent indicators in conjunction with optogenetic technology.

Selected references

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