SUMMARY OF Ph.D. DISSERTATION

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Title

Development of Multicolor Imaging Techniques Using Fluorescent Proteins

Abstract

Complex signal transduction networks regulate cellular functions. To investigate the spatiotemporal dynamics of various intracellular signals in living samples, fluorescence imaging is emerging as an important tool. Many genetically encoded sensors have been developed based on fluorescence resonance energy transfer (FRET) between fluorescent proteins. Moreover, combined use of the sensors within a single cell would be useful to analyze the dynamic relationship among the intracellular signals. However, the sensor has a broad spectral profile for two fluorescent proteins, thus, when the several sensors are present at the same location, imaging without the significant spectral overlaps is difficult. In this study, novel multicolor imaging techniques were developed to overcome this problem.

Chapter 1 describes the basis of current fluorescence imaging methods, focusing on the applications using fluorescent proteins. The limitations to overcome for an advance of the multicolor imaging techniques are clarified.

Chapter 2 describes a method to simultaneously image two intracellular signals using two FRET-based sensors in single living samples with a single excitation light. In recent reported methods for imaging of dual FRET pairs, to avoid the spectral overlaps, the two FRET donors are excited at two different wavelengths sequentially with a lag time. Therefore, these are inadequate to follow the signal changes in highly motile cells. To overcome this limitation, novel FRET sensors with variants of Sapphire and red fluorescent protein (RFP) for combined use with cyan and yellow fluorescent proteins (CFP/YFP) were constructed, and a method using four-color imaging and distinguishing fluorescent proteins by computational image processing was established. Using this method, for the first time, simultaneous imaging of intracellular cyclic AMP (cAMP) and cyclic GMP (cGMP) and that of intracellular cAMP and Ca^{2+} in highly motile cardiac myocytes were achieved.

Chapter 3 describes a development of a blue fluorescent cGMP biosensor, Cygnus, suitable for multiplexing with the other fluorescent sensors in a single living cell. In the previous chapter, a method for simultaneous imaging of cAMP and cGMP in a cell was established. For an advance toward the imaging of three cellular parameters in a cell, however, further multiplexing of FRET-based sensors would complicate the imaging experiments. To avoid the spectral overlaps of several fluorescent sensors, the FRET-based sensor was converted into a single wavelength indicator by using a dark FRET acceptor. Using this novel sensor, triple imaging of second messengers, cAMP, cGMP and Ca^{2+}, in a single cell was demonstrated. By confirmation of pH stability of the used FRET pair, it was shown that the Cygnus-type sensor has a potential as a complementary alternative to the current single fluorescent protein-based sensor, which is often pH sensitive. The biosensor suited to multicolor imaging would be a useful tool to investigate the complex interplay among multiple biological processes in a single cell.

Chapter 4 discusses interplays among the second messengers visualized in this study and further extended multicolor imaging, and gives the conclusions from this study.