Development of a fluorescent protein with deep blue color

Takeharu Nagai, Wataru Tomosugi, Tomoki Matsuda, Ippei Kotera and Kenta Saito
Lab. for Nanosystems Physiology, RIES, Hokkaido University

Application of green fluorescent protein (GFP) from the jellyfish *Aequorea victoria* and its color variants, as well as GFP-like proteins from other organisms, has revolutionized our ability to analyze a wide range of biological issues such as gene expression, protein localization, and cell motility in living specimen. So far, GFPs with altered fluorescence spectra have been created. For example, blue and cyan variants were made by substitution of Tyr at 66 (Y66) in the *Aequorea* GFP chromophore for His and Trp, respectively. To further increase the colour repertory of fluorescent proteins, we tried to substitute Y66 for phenylalanine (Y66F). However, the GFP variant with Y66F mutation (GFP-Y66F) was dimly fluorescent, making it unsuitable for cellular imaging. Then, we sought to generate enhanced GFP-Y66F by combination of site-directed and semi-random mutagenesis. The mutated gene was bacterially expressed and subjected to screening in terms of the brightness of bacterial colony when illuminated by ultra-violet light. Of several hundreds colonies tested, we found a bright fluorescent colony with deep blue color. Spectroscopical analysis of the purified protein revealed that absorption and emission peak was 360 nm and 420 nm, respectively. Both peaks are the shortest among the fluorescent proteins reported so far. Due to its deep blue color, this protein was named as ultra-marine fluorescent protein, UMFP. Most interesting feature of UMFP is its high fluorescence stability in various range of pH, enabling quantitative fluorescence imaging in acidic environment such as in lysozome and Golgi body. In addition to this feature, UMFP can be used as an ideal FRET donor in pair of CFP acceptor since the emission spectrum of UMFP is completely overlapping with the absorption spectrum of CFP. Accordingly, UMFP-CFP fusion protein showed efficient FRET confirmed by 65% quenching of UMFP signal. The application of UMFP for cell biological analysis will be discussed.