【Grant-in-Aid for Scientific Research (S)】

Broad Section G

Title of Project : The pursuit of both brightness and photostability in fluorescent protein technology

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【Purpose and Background of the Research】

Over the last few decades, thanks to improvements in gene transfer and protein targeting techniques, scientists have succeeded in labeling subcellular components such as the endoplasmic reticulum (ER) and mitochondria with fluorescent proteins (FPs), enabling for efficient observation of these organelles. However, each cell may have a capacity for FP expression, and exceeding this capacity may result in mis-localization of the FP or perturbation of cellular functions. While this problem has been solved to some extent by the development of folding mutants of FP, cells with moderate expression levels should be more acceptable. For reliable fluorescence readouts, however, the visualization of low–copy number targets requires a strong excitation light, which often causes a substantial level of photobleaching of FPs. In addition, recent fast observations of subcellular components revealed rapid motion of their fine structures. However, the continuous acquisition of images inevitably leads to more noticeable FP photobleaching than conventional time-lapse image acquisition. Highly photostable FPs are required to ameliorate these undesirable situations.

【Research Methods】

The development of photostable FPs has nearly always been accompanied by a decrease in brightness. In most cases, molecular oxygen (O_2) is a double-edged sword. Maturation of FP chromophores requires oxidation reaction(s) that involve O_2, and therefore high accessibility to O_2 contributes to the increase in practical brightness. On the other hand, decomposition of FP chromophores is caused by the O_2 attack while FP chromophores remain in the singlet or triplet excited state. In this regard, enhanced O_2 accessibility should decrease photostability. Accordingly, there exists a trade-off between brightness and photostability in FP technology.

A solution to this problem was uncovered through our molecular cloning and mutagenesis studies on a wild-type FP. By yet unknown mechanisms, an engineered FP is fairly bright and extremely photostable. The FP is over one order of magnitude more photostable than any of the currently available FPs, contributing to the improvement of spatiotemporal resolution and the dramatic extension of the observation period. To fully benefit from the rich photon budget, we are going to try some unusual illumination modalities for sustainable live imaging in consideration of phototoxicity. For example, we will analyze the dynamic architecture of the ER by cell-wide and spatiotemporally well-resolved imaging (three-dimensional structured illumination microscopy (3D-SIM)) that spans a long period with no intervals

Of particular interest is how the new FP interacts with O_2 to synthesize and degrade its own chromophore. Atomic-level understanding of the mechanism underlying its unique photostability will require determination of the crystal structure. Also, single-molecule imaging of the FP will help us to elucidate photophysical events including blinking, an indicator of intersystem crossing to the triplet state, in addition to photobleaching.

【Expected Research Achievements and Scientific Significance】

The transfection with FP constructs usually results in marked heterogeneity in fluorescence brightness among transfected cells. To observe microscopic objects over time, furthermore, conventional methods usually adopt the time lapse mode with prescribed intervals. However, there is concern that considerably bright cells may exhibit artifactual signals and that temporal down-sampling may miss transient but meaningful signals. By using the new photostable FP it will be possible to select moderately bright cells intentionally for observation with continuous illumination. And researchers will tune experimental conditions to create situations in which imaging performance is not limited by the photobleaching of fluorescent dyes.

【Publications Relevant to the Project】

- Nixon-Abell, J. et al. Increased spatiotemporal resolution reveals highly dynamic dense tubular matrices in the peripheral ER. Science 354, aaf3928 (2016).
- Shaner, N.C. et al. Improving the photostability of bright monomeric orange and red fluorescent proteins. Nat. Methods 5, 545–551 (2008).

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