Cruising inside Cells
バイオイメージングを目指した蛍光タンパク質のエンジニアリング

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The behavior of biochemical molecules moving around in cells makes me think of a school of whales wandering in the ocean, captured by the Argus system on the artificial satellite. When bringing a whale back into the sea --- with a transmitter on its dorsal fin, every staff member hopes that it will return safely to a school of its species. A transmitter is now minute in size, but it was not this way before. There used to be some concern that a whale fitted with a transmitter could be given the cold shoulder and thus ostracized by other whales for “wearing something annoying.” How is whale’s wandering related to the tide or a shoal of small fish? What kind of interaction is there among different species of whales? We human beings have attempted to fully understand this fellow creature in the sea both during and since the age of whale fishing.

In a live cell imaging experiment, a fluorescent probe replaces a transmitter. We label a fluorescent probe on a specific region of a biological molecule and bring it back into a cell. We can then visualize how the biological molecule behaves in response to external stimulation. Since fluorescence is a physical phenomenon, we can extract various kinds of information by making full use of its characteristics. For example, the excited energy of a fluorescent molecule donor (energy donor) transfers to an acceptor (energy acceptor) relative to the distance and orientation between the donor and acceptor. This phenomenon (FRET) can be used to identify interaction between biological molecules or structural change in biological molecules. Besides, we can apply all other characteristics of fluorescence, such as polarization, quenching, photobleaching, photoconversion, and photochromism, in experimentation.
For our laboratory that belongs to the advanced technology development core of the Brain Science Institute, we should, we believe, be as exclusive and flexible as possible, rather than focusing on some specific fields. We aim at pursuing the true nature of something flexible about cells with a breadth of vision. We attempt to grasp the meaning of a cell which automatically controls the ratio of output (reaction) to input (stimulation).

Cruising inside cells in a supermicro corps, gliding down in a microtubule like a roller coaster, pushing our ways through a jungle of chromatin while hoisting a flag of nuclear localization signal --- we are reminded to retain a playful and adventurous perspective at all times. What matters is mobilizing all capabilities of science and giving full play to our imagination. We believe that such serendipitous findings can arise out of such a sportive mind, a frame of mind that prevails when enjoying whale-watching.

We sought to develop a sensitive and quantitative technique capable of monitoring the entire flux of autophagy involving fusion of lysosomal membranes. We observed the accumulation inside lysosomal compartments of Keima, a coral-derived acid-stable fluorescent protein that emits different colored signals at acidic and neutral pHs. The cumulative fluorescent readout can be used to quantify autophagy at a single time point. Remarkably, the technique led us to characterize a novel autophagy pathway in Atg5-deficient cells, in which conventional LC3-based autophagosome probes are ineffective. Due to the large Stokes shift of Keima, this autophagy probe can be visualized in conjunction with other green-emitting fluorophores. We examined mitophagy as a selective autophagic process; time-lapse imaging of mitochondria-targeted Keima and GFP-Parkin allowed us to observe

simultaneously Parkin recruitment to and autophagic degradation of mitochondria after membrane depolarization.