II-H Development of Fluorescent and Bioluminescent Proteins for Imaging Intracellular Molecular Dynamics

A current focus of biological researches is to quantify and image cellular events in living cells and animals. To probe the fundamental cellular events in living cells, we are exploring a new way for developing fluorescent and bioluminescent reporter proteins based on protein splicing. With these reporter proteins, analytical methods to detect protein-protein interactions, intracellular localization of proteins and their dynamics, enzyme activities, gene expression, and production of small bio-molecules are being actively under development. We are also investigating analytical techniques such as complementary DNA library screenings and proteome analysis.

II-H-1 Quantitative Determination of Protein Nuclear Transport Induced by Phosphorylation or by Proteolysis

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Nucleocytoplasmic transport of proteins in eukaryotic cells is a fundamental process for gene expression. The transport is regulated by post-translational modifications of the proteins such as ligand-binding, phosphorylation, and proteolysis. For monitoring the nuclear transport of proteins induced by a ligand binding, we have recently developed a genetically-encoded bioluminescent indicator based on reconstitution of split fragments of *Renilla reniformis* (Rluc) by protein splicing of a DnaE intein.[1)] We herein describe that the technique is used for detecting phosphorylation- or proteolysis-induced nuclear transports of a target protein. Two model proteins, signal transducer and activator of transcription 3 (STAT3) and sterol-regulatory element binding proteins-2 (SREBP-2), were exemplified as phosphorylation- and proteolysis-induced nuclear transport, respectively. Each STAT3 or SREBP-2 is connected with C-terminal halves of Rluc and DnaE. If the protein translocates into the nucleus, the C-terminal Rluc meets the N-terminal Rluc, and full-length Rluc is reconstituted by protein splicing in the nucleus. The indicator with SREBP-2 enabled to quantify the intracellular concentrations of cholesterol. The indicator with STAT3 quantified the extent of the nuclear transport induced by representative cytokines. This simple assay based on protein nuclear transports allows the selection of suitable drugs among candidates, and has significant potential for risk assessments such as carcinogenic chemical screening *in vitro* and *in vivo*.

Reference

1) S. B. Kim, T. Ozawa, S. Watanabe and Y. Umezawa, *Proc. Natl. Acad. Sci. U.S.A.* **101**, 11542–11547 (2004).

II-H-2 A Stress Indicator for Noninvasively Imaging Endogenous Corticosterone in Living Mice

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The physical and emotional stress is one of the major controllers of physiological reactions and homeostasis in living animals. A stress hormone, corticosterone, is secreted from adrenal cortex into the blood vessel when animals sense the stress. The quantitative evaluation of corticosterone in living animals has been limited because of the unavailability of suitable methods *in vivo*. For a noninvasive molecular imaging of the stress, we developed a method for detecting physiological increases in the endogenous corticosterone caused by exo- and endogenous stress in living animals. We constructed a pair of genetically-encoded indicators composed of cDNAs of glucocorticoid receptor (GR), split *Renilla* luciferase (RLuc) and a *Synechocystis* sp. DnaE intein. The GR fused with C-terminal halves of RLuc and DnaE is localized in the cytosol, whereas a fusion protein of N-terminal halves of RLuc and DnaE is localized in the nucleus. If corticosterone induces GR translocation into the nucleus, the C-terminal RLuc meets the N-terminal one in the nucleus, and full-length RLuc is reconstituted by protein splicing with DnaE. Cell-based methods provided quantitative bioluminescence assay of the extent of GR translocation into the nucleus. We further demonstrated that the indicator enabled noninvasive imaging against two different types of imposed stress: a forced swimming and metabolic perturbation caused by 2-deoxy-D-glucose. This stress indicator should be valuable for screening pharmacological compounds and for tools to study the mechanism of physiological stress.