Engineering fluorescent proteins to visualize biological functions

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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 experimental ability to analyze a wide range of biological issues such as gene expression, protein localization, and cell motility in living specimen. The usefulness of GFPs derives from the finding that the fluorescent property of GFPs requires no other cofactor: The fluorophore, is formed spontaneously by autocatalytic reactions of the tripeptide, Ser65-Tyr66-Gly67. This property allows us to produce fluorescence in any regions of interest in living cells or organisms just by introducing the genes using appropriate techniques such as lipofection and virus-mediated gene transfer.

Advancement in the fluorescence imaging methods and microscopy systems is also enabling higher resolution imaging of fluorescence signals from GFPs fused to proteins of interest, providing novel and unsuspected insights into the dynamic movement of biomolecules and their active interactions with the cellular milieu. Among these methods, fluorescence resonance energy transfer (FRET) between two GFP-like fluophores has been used to develop genetically encoded fluorescent indicators to quantify critical cellular events including ion concentration changing, voltage changing, and enzyme activation. For example, “cameleon” was the first developed FRET-based indicator that is composed of calmodulin, a Ca^{2+}-binding protein, and its target peptide M13 derived from myosin light chain kinase, sandwiched by cyan- (CFP) and yellow-fluorescent protein (YFP). When Ca^{2+} binds to calmodulin, the Ca^{2+}-calmodulin tightly complexes with M13, thereby triggering an efficient FRET from CFP to YFP. By putting cameleon in living samples as a molecular spy, we can measure the precise dynamics of intracellular Ca^{2+} (e.g. amplitude and frequencies). This enables us to better understand how information from events such as large Ca^{2+} spike and persistent Ca^{2+} oscillation can be decoded to information to execute various biological responses. Like cameleon, many genetically-encoded functional indicators for voltage changing and enzyme activation have been developed, opening a new avenue to decipher complex biological phenomena including pathogenesis and brain function.

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