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Review

Development and application of fluorescent protein-based indicators for live cell imaging

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ABSTRACT

Background: Developments in fluorescent molecular imaging techniques have enabled characterization of the localization and dynamics of particular molecules at the cellular and subcellular levels.
Highlight: Advances in imaging devices (fluorescence microscopy) and fluorescent indicators have contributed to the development of fluorescence imaging techniques. In particular, cloning of green fluorescent protein from the bioluminescent jellyfish *Aequorea victoria* and the subsequent development of other fluorescent proteins have revolutionized imaging technologies and accelerated their application in molecular and cell biological studies. There are two types of genetically encoded fluorescent indicators, namely, fluorescence resonance energy transfer-based ratiometric indicators and single fluorescent protein (FP)-based intensiometric indicators. The principal advantages of FP-based indicators are their superior sensitivities and specificities for molecular and physiological events, which are achieved by the incorporation of naturally evolved protein sensor domains. Consequently, numerous fluorescent indicators that enable monitoring of intracellular signaling, enzyme activities, apoptosis, cell cycle progression, and other cellular events have been developed. FP-based indicators can be expressed in specific cells in a temporally controlled manner; hence, they are favorable for long-term *in vitro* and *in vivo* experiments.
Conclusion: The advantages of FP-based indicators extend their application to understanding the functions of particular molecules and cellular events during physiological responses in live animals.

Contents

1. Introduction

Fluorescent molecular imaging can be used to visualize biological processes in live cells, tissues, and animals. This method enables characterization of the localization and dynamics of particular molecules at the cellular and subcellular levels, and has become an indispensable technique for understanding many biological processes [1–3]. The development of sensitive imaging

Abbreviations: CaM, calmodulin; cAMP, cyclic AMP; CFP, cyan fluorescent protein; Epac, exchange protein activated by cAMP; FP, fluorescent protein; FRET, fluorescence resonance energy transfer; GECI, genetically encoded Ca^{2+} indicator; GFP, green fluorescent protein; IP_3, inositol 1,4,5-trisphosphate; IP_3R, IP_3 receptor; M13, CaM-binding domain of myosin light chain kinase; PKA, protein kinase A; PLC, phospholipase C; YFP, yellow fluorescent protein

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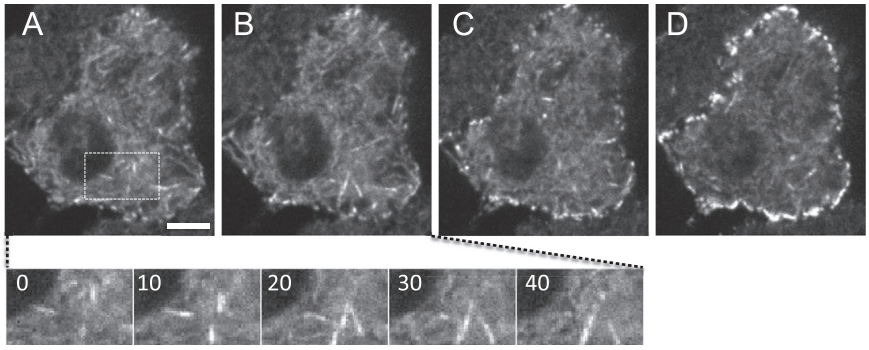

Fig. 1. Live cell imaging of YFP-STIM1 in HSY-EA1, a human parotid ductal cell line. HSY-EA1 cells expressing YFP-STIM1 were stimulated with 10 μM ATP in Ca^{2+}-free medium. The YFP signals were obtained at 5- s intervals via confocal laser scanning microscopy. The images shown are 160 s (A), 180 s (B), 340 s (C), and 440 s (D) after the stimulation. The smaller images show the area indicated in panel A at 10- s intervals. Scale bar: 10 μm.

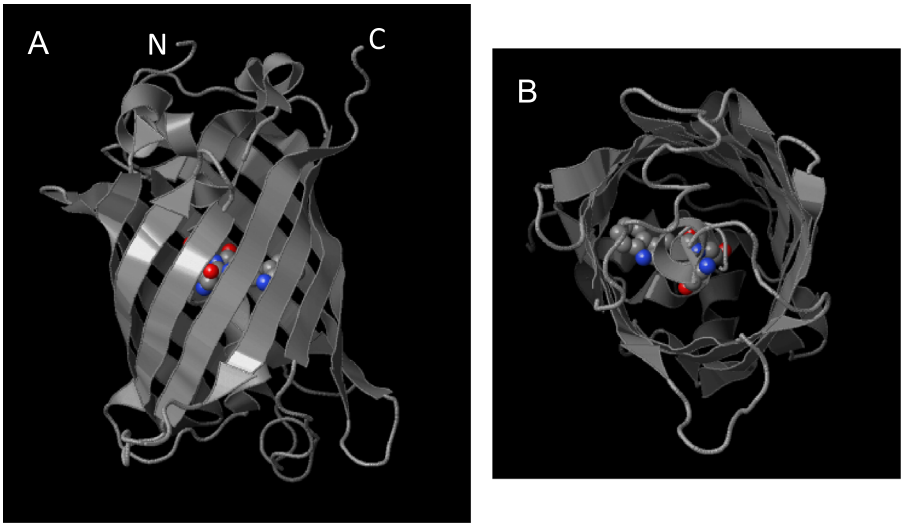

Fig. 2. The structure of GFP. The overall structure of the β-barrel of GFP is shown from the side (A) and bottom (B). The chromophore is shown in a space-filling representation. Data from Protein Data Bank Japan (PDBj).

devices such as confocal laser scanning and two-photon fluorescence excitation microscopes has contributed to recent advances in fluorescence imaging. In addition, the generation of optimized image detectors such as cooled charge-coupled cameras, electron multiplying charge-coupled cameras, and complementary metal oxide semiconductors has aided the evolution of conventional fluorescence microscopy to total internal reflection microscopy, which can be used for single molecule imaging. More recently, photoactivated localization microscopy techniques have been developed for super-resolution imaging [3].

Improvements in fluorescent indicators have also been crucial to the development of effective fluorescence imaging techniques. In the 1990s, fluorescent indicators were developed mainly using organic chemistry; thus, the targets of imaging studies were limited by the scope of the available fluorescent indicators such as Fura-2, which detects Ca^{2+} [4]. However, the discovery and subsequent cloning of green fluorescent protein (GFP) from the bioluminescent jellyfish *Aequorea victoria* revolutionized the visualization of biological systems [1,2,5]. One of the most important and attractive features of GFP as a biological marker is that it generates fluorescence in the absence of additional cofactors in a variety of species, including vertebrates, invertebrates, plants, fungi, and bacteria [3,6]. The simplest application of GFP is as a passive marker fused to a target protein of interest, which enables visualization of its spatiotemporal distribution in live cells (Fig. 1). In addition, molecular biology-based engineering of fluorescent proteins (FPs), such as circular permutations and fluorescence resonance energy transfer (FRET) between two differently colored FPs, has allowed researchers to design and create a variety of fluorescent indicators capable of detecting ions, intracellular messengers, phosphorylation, cell cycle stages, cell death, and other molecules or events [1–3,7–9]. This review focuses on FP-based fluorescent indicators that detect concentrations of intracellular messenger molecules.

2. Fluorescent proteins

GFP from the jellyfish *A. victoria* comprises 238 amino acids and has a cylindrical structure with a diameter of 30 Å and a length of 40 Å. The fluorophore of GFP consists of residues Ser65, dehydroTyr66, and Gly67, which form a tripeptide located in the geometric center of the cylinder (Fig. 2). This folding motif, which contains an alpha helix inside a beta sheet structure, was named the beta-can [3,10].

Considerable efforts have been made to develop GFP variants with altered excitation and emission wavelengths, enhanced brightness, and improved pH resistance [11,12]. The growing popularity of GFP and its variants prompted researchers to search for new alternatives and resulted in the discovery and cloning of a number of distinct FPs from marine organisms [3]. FPs can be applied to a wide variety of studies related to various aspects of biological systems, and the range of these applications is expanding continuously. Placing FPs under the control of a promoter of interest allows the characterization of promoter activities in specific gen-