nature chemical biology

Green fluorescent protein

The natural world serves as a constant source of inspiration for scientists and laymen alike, with few phenomena giving rise to more amazement than the generation of light, whether it be the spark of a firefly, the aurora borealis in the northern sky or the green glow of the *Aequorea victoria* jellyfish. Although other fluorescent and luminescent proteins or signals are known, *A. victoria*'s green fluorescent protein (GFP) and the other fluorescent protein (FP) family members are unique in that the emitted light is not derived from an external cofactor or as a byproduct of a chemical transformation, but rather is intrinsic to the protein sequence and requires no chaperones or mediators beside molecular oxygen. This independence makes the FPs invaluable for biological and chemical biological investigations as genetically encodable tags for localization experiments in live cells, as active indicators of cellular function, and as robust scaffolds for fluorescence resonance energy transfer (FRET) and other fluorescence measurements that monitor conformational and temporal dynamics.

GFP and other natural FPs have provided significant intellectual challenges and opportunities for chemical biology research. In particular, advances in understanding the mechanisms of chromophore formation and modulation have allowed the generation of a bevy of FPs with new spectral qualities tailored for almost any intended use. The versatility and ease of using and monitoring FPs, and particularly those that have been engineered for high stability or unique spectral properties, make this class of proteins one of the most important tools in the chemical biology toolbox. Indeed, the awarding of the 2008 Nobel Prize in chemistry to Shimomura, Chalfie and Tsien for the discovery and development of GFP reflects how pivotal these constructs and advances in this arena are to modern research. In this primer, we highlight just a few of these exciting scientific discoveries.

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KEY RESOURCES

Shimomura, O. *et al. J. Cell. Comp. Physiol.* **59**, 223–239 (1962).
Chalfie, M. *et al. Science* **263**, 802–805 (1994).
Heim, R. *et al. Nature* **373**, 663–664 (1995).
Tsien, R.Y. *Annu. Rev. Biochem.* **67**, 509–544 (1998).
Shaner, N.C. *et al. Nat. Methods* **2**, 905–909 (2005)
Zimmer, M. *Glowing Genes: A Revolution in Biotechnology* (Prometheus, Amherst, New York, USA, 2005).
Daunert, S. & Deo, S. *Photoproteins in Bioanalysis* (Wiley-VCH, Weinheim, Germany, 2006).
http://www.scholarpedia.org/article/Fluorescent_proteins
http://www.nature.com/nmeth/journal/v6/n1/index.html

Important events in the history of GFP

1962 Discovery of aequorin and GFP

Sierra Blakely

1979 Chromophore structure determined to be cyclic tripeptide

1992 Cloning and sequencing of GFP

1993 Chromophore structure fully elucidated

1994 GFP used as a reporter of gene expression in *E. coli* and *C. elegans*

Expression and purification of GFP

1995 GFP mutants enable use in mammalian cells

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1996 Crystal structure solved

1997 Development of GFP as FRET-based calcium sensor

1998 Chemical synthesis of GFP confirms reaction is autocatalytic

2000 Blinking observed by single-molecule experiments

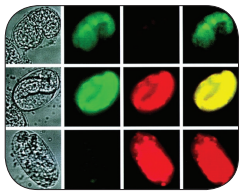
2001 GFP shows bone marrow cells can regenerate heart

2002 Photoactivatable GFP developed

2007 Molecular thermometers based on fluorescent protein blinking developed

Advances in GFP methodology

Some scientific questions center on tracking location not in space but in time. The development of fluorescent protein timers allows scientists to examine systems at distinct time points. Here FP maturation reports on the activity of a heat shock promoter under temperature stress.



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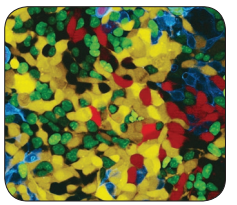
The continued development of GFP and other FP variants has resulted in a panel of genetically encoded fluorophores for complex biological applications. Their combination, such as shown here in HEK cells, can provide a straightforward means to map individual proteins or cells in a biological setting.



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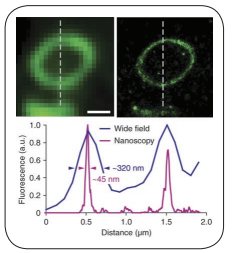
The development of photoswitchable fluorescent proteins that exchange between two distinct photostates has enabled scientists to achieve near molecular level resolution in fluorescence imaging, or 'nanoscopy'. Here two GFP-derived photoswitches improve resolution of *E. coli* membranes seven fold as compared to wide-field microscopy.



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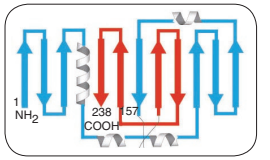
Detecting protein-protein interactions remains a significant challenge. In 2000, two reports applied split-intein technology to combine GFP pieces as a fluorescent readout of peptide or protein assembly.



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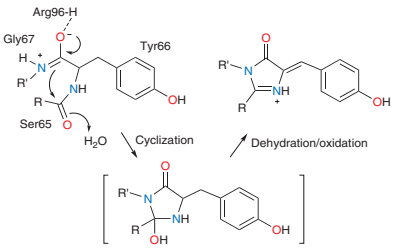
GFP folding and chromophore maturation

GFP is a single-domain protein of 238 amino acids. Protein folding triggers maturation of the chromophore, which is known to require three chemical steps—cyclization, oxidation and dehydration—although the order of the steps remains under investigation. The initial cyclization (reaction sequence, right), which occurs between Ser65 and Gly67, is promoted by backbone distortion and is mediated by the neighboring Arg96 as a proton donor. O_2-mediated oxidation of the Tyr66 side chain is the rate-limiting step of the reaction, occurring at a rate of 1.51×10^{-4} s^{-1} in a Ser65Thr mutant protein. The wild-type protein absorbs light at 395/475 nm and emits light at 508 nm; these and other properties can be tuned by exchanging residues in both the chromophore and the surrounding amino acids with non-natural or natural residues.



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