The Green Fluorescent Protein (GFP)

Piyaraj P, MD
email : phunlerd.pi@bgh.co.th

Phunlerd Piyaraj, MD[1]

[1] Department of Parasitology, Phramongkutklao College of Medicine, Bangkok, Thailand.

Keywords:
green fluorescent protein (GFP), biomarkers, cellular expression, bioluminescence

Molecular biology is one of the most significant fields of biological science. The main aim of molecular biology is to explore and understand biological functions including all living beings. The knowledge gained could be extended to practical use in cell biology, pharmacy or medicine. Cellular biology is a key to visualise a blueprint of life; development of drugs, the use of biomarkers and the study of gene expression rely on molecular biology methodology.[1-4]

Back in the 20th century, limitations in experiments were common due to the complexity of cellular structures. Real-time monitoring of cellular processes was very difficult in the past with the tools available at the time.[5-9] The scientific community was waiting for a better and well-defined monitoring tool for further cellular exploration.[1]

A revolutionary technique was developed in the early 21st century which made a vast contribution to biological sciences.[2] The discovery of Green Fluorescent Protein (GFP) and its application enabled real-time monitoring of cellular functions in living organisms. This novel tool was a ground-breaking discovery. In the present day, many forms of cellular expression are described and clarified using GFP-based methodology.[1,3,4,10-16]

It had been observed for decades that some living organisms such as jelly fish or other sea creatures, lit up when photographed with a flash light. At first it was described in the 1950s that a green fluorescent substance within a jellyfish was responsible for a light emission effect. In the 1960s, after a dedicated hard working mission to extract more than 10,000 jellyfish to find a fluorescent substance, Osamu Shimomura finally isolated an associated protein from the crystal jelly fish Aequorea Victoria and visually described its bioluminescent effect.[5] Basically, GFP absorbs light between the blue to ultraviolet band and emits a bright green fluorescence after binding with Ca^{2+}. The term *'Green Fluorescent Protein'* or GFP was coined later in the 1970s by Morin and Hastings.[3] During the time from the 1970-1990s, GFP structures were broadly studied to gain a better understanding of its scientific merit.[1,3,6-13] Douglas Prasher realised its potential illumination effect and proposed that GFP could be inserted into cellular structures to observe their function. In the 1990s, he successfully cloned and mapped its genetic sequence. Unfortunately, his funding ran out and he was unable to carry out further experiments to merge the GFP gene into a living organism. The samples and data were sent to several institutions to carry on the study.[6,11]

In 1994, Martin Chalfie successfully incorporated the GFP gene, a native GFP extracted from Aequorea Victoria (also called wild-type GFP), into *E.coli* and *C.elegans* and induced bacteria to exhibit a green fluorescence after exposure to blue light.[12] Previous studies expanded the use of GFP by developing GFP derivatives

and produced various colors with quicker illumination. This development extended GFP applications to diverse laboratory techniques for studying biological science.[2,17,18]

A wild-type GFP (wtGFP) contains 238 amino acids. The GFP crystal structures were fully described in 1996. Its shape looks like a soda can, a typical barrel structure composed of one β-sheet with alpha helixes. It contains a chromophore within the centre of the barrel shape.[8] The chromophore is responsible for the bioluminescence.[5,10,11] Lukyanov described GFP's natural function as a light activated electron donor similar to the process that chlorophyll donates electrons in photosynthesis. The peak excitation spectrum is in a wavelength at 395nm and a smaller maximum at about 470nm. The fluorescent emission spectrum is at 505nm which is approximately a green colour band of visible light spectrum.[19]

GFP is non-toxic and provides low host specificity, which is why it can be used in various species of organisms from single cell organisms to multi-cellular living beings. When merging GFP into the organism's gene, it will mutually express a bioluminescent effect along with its normal cellular function without compromising the general physiology. Cellular activities can be observed in real-time with instant visualisation. The most common use of GFP is to monitor protein activities within a cell; the location, movement and protein cycle, or it is tagged to display gene expressions.[2,11,13,15] The flow of dynamicity can be imaged as a successful temporal study. GFP can be used as a biosensor to monitor intracellular parameters such as pH or metabolic activity. In medicine, GFP is widely used for functional tissue imaging for example when studying neuron activity. GFP is also valuable for studying cancer from its basic molecular properties to developing new treatments.[2]

Given its huge impact on biological sciences, including subsequent benefits from GFP and the dedicated work of high academic merit, the GFP and its applications was awarded the Noble Prize in 2008 for chemistry. Osamu Shimomura, Marty Chalfie and Roger Tsien were acknowledged *"for the discovery and development of the green fluorescent protein, GFP."* Also, Douglas Prasher was highly praised as a co-contributor of GFP development.

References

1. Heim R, Cubitt AB, Tsien RY. Improved green fluorescence. *Nature* 1995;373:663-4.
2. Shaner NC, Patterson GH, Davidson MW. Advances in fluorescent protein technology. *J Cell Sci* 2007;120: 4247-60.
3. Morin JG, Hastings JW. Energy transfer in a bioluminescent system. *J Cell Physiol* 1971;77:313-8.
4. Merzlyak EM, Goedhart J, Shcherbo D, et al. Bright monomeric red fluorescent protein with an extended fluorescence lifetime. *Nat Methods* 2007;4:555-7.
5. Shimomura O, Johnson FH, Saiga Y. Extraction, purification and properties of aequorin, a bioluminescent protein from the luminous hydromedusan, Aequorea. *J Cell Comp Physiol* 1962;59:223-39.
6. Prasher DC, Eckenrode VK, Ward WW, et al. Primary structure of the Aequorea victoria green-fluorescent protein. *Gene* 1992;111:229-33.
7. Cody CW, Prasher DC, Westler WM, et al. Chemical structure of the hexapeptide chromophore of the Aequorea green-fluorescent protein. *Biochemistry* 1993;32:1212-8.
8. Ormö M, Cubitt AB, Kallio K, et al. Crystal structure of the Aequorea victoria green fluorescent protein. *Science* 1996;273:1392-5.
9. Brejc K, Sixma TK, Kitts PA, et al. Structural basis for dual excitation and photoisomerization of the Aequorea victoria green fluorescent protein. *Proc Natl Acad Sci U S A* 1997;94:2306-11.
10. Morise H, Shimomura O, Johnson FH, et al. Intermolecular energy transfer in the bioluminescent system of Aequorea. *Biochemistry* 1974;13:2656-62.
11. Prasher D, McCann RO, Cormier MJ. Cloning and expression of the cDNA coding for aequorin, a bioluminescent calcium-binding protein. *Biochem Biophys Res Commun* 1985;126:1259-68.
12. Chalfie M, Tu Y, Euskirchen G, et al. Green fluorescent protein as a marker for gene expression. *Science* 1994; 263:802-5.
13. Heim R, Prasher DC, Tsien RY. Wavelength mutations and posttranslational autoxidation of green fluorescent protein. *Proc Natl Acad Sci U S A* 1994;91:12501-4.
14. Campbell RE, Tour O, Palmer AE, et al. A monomeric red fluorescent protein. *Proc Natl Acad Sci U S A* 2002;99: 7877-82.
15. Patterson GH, Lippincott-Schwartz J. A photoactivatable GFP for selective photolabeling of proteins and cells. *Science* 2002;297:1873-7.
16. Chudakov DM, Belousov VV, Zaraisky AG, et al. Kindling fluorescent proteins for precise in vivo photolabeling. *Nat Biotechnol* 2003;21:191-4.
17. Mishin AS, Subach FV, Yampolsky IV, et al. The first mutant of the Aequorea victoria green fluorescent protein thatforms a red chromophore. *Biochemistry* 2008;47: 4666-73.
18. Rizzuto R, Brini M, De Giorgi F, et al. Double labelling of subcellular structures with organelle-targeted GFP mutants in vivo. *Curr Biol* 1996;6:183-8.
19. Lukyanov KA, Chudakov DM, Fradkov AF, et al. Discovery and properties of GFP-like proteins from nonbioluminescent anthozoa. *Methods Biochem Anal* 2006;47:121-38.