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Perspective

Fluorescent Protein has been Engineered to Produce a Plethora of Diversely Colored Mutants

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INTRODUCTION: The unique property of being self-sufficient to form a visible wavelength chromophore from a sequence of three amino acids within their own polypeptide sequence distinguishes fluorescent proteins from other structurally homologous proteins. Using fluorescence microscopy, biologists commonly introduce a gene that encodes an engineered fluorescent protein into living cells and then examine the location and dynamics of the gene product. The biological research community was quick to recognize the unique value of a genetically encoded fluorophore as a marker of gene expression and protein localization, armed with the arsenal of potent molecular biology techniques developed over the previous two decades. In hindsight, the first demonstrations of recombinant expression in organisms other than jellyfish and the cloning of the gene represent a clearly discernible turning point in the history of fluorescent protein research. By allowing researchers to use molecular cloning techniques to fuse the fluorophore moiety to a wide variety of protein and enzyme targets in order to monitor cellular processes in living systems using optical microscopy and related methods, the early 1960s discovery of green fluorescent protein ultimately heralded a new era in cell biology. The green fluorescent protein and its color-shifted genetic derivatives have been of great use in many thousands of live-cell imaging experiments thanks to recent technological advancements in wide field fluorescence and confocal microscopy, such as ultrafast low light level digital cameras and multitracking laser control systems. Although the gene for green fluorescent protein was first cloned in 1992, it wasn't until several years later that fusion products were used to track gene expression in bacteria and nematodes that the gene's significant potential as a molecular probe was realized.

DESCRIPTION: Green fluorescent protein has been engineered to produce a plethora of diversely colored mutants, fusion proteins, and biosensors that are collectively referred to as fluorescent proteins since these initial studies. The fluorescence can be destroyed by denaturation as well as mutations in residues that surround the fluorophore. However, GFP's beta barrel structure's amino acids are extremely stable due to its stable structure. This protects GFP from temperature, pH, and chemicals like urea changes, resulting in a high fluorescence quantum yield of 80%. Yellow fluorescent protein, or YFP, is a GFP mutation. In this instance, a mutation was introduced after it was discovered that GFP contained a threonine residue close to the chromophore. By changing a tyrosine residue to a histidine at position 66, blue fluorescent protein was made. This fluorescent protein has a maximum emission wavelength of 450 nm. A fluorescent protein with an emission maximum at 500 nm is produced when tryptamine replaces tyrosine. Cyan fluorescent protein, or CFP, is the latter. However, a number of issues have hindered the use of RFP. Because RFP is an obligate tetramer, it aggregates in large quantities within cells. Because of this, RFP's application for reporting a protein's location is severely limited.

CONCLUSION: Unique properties, such as red-shifted emission above 600 nm or photo conversions from a green-emitting state to a red-emitting state, can be displayed by fluorescent proteins with other chromophores, such as UnaG with bilirubin. They can have enough distance between their excitation and emission wavelengths to convert red to green light.

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