Fluorescent Proteins

Filters, Mirrors and Wavelengths

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Fluorescent proteins have become a mainstay of today's biomolecular research. Their small size, ease of use, wavelength variability and no substrate requirement make these genetic elements useful tools to answer countless numbers of experimental questions. Here we describe several of the commonly used technologies associated with fluorescent proteins. In addition an extensive list of fluorescent proteins, associated excitation and emission wavelengths and suggested filters and mirror combinations is provided.

Introduction

In the past 15 years, green fluorescent protein (GFP) has changed from a virtually unknown protein to a common molecular detection and imaging tool used in multiple scientific fields such as biology, chemistry, genetics, and medicine (Figure 1). The ability to auto-catalyze along with the relatively easy genetic encodability of GFP makes it ideal for minimizing the invasiveness of many procedures used to study biological processes. GFPs and GFP-like proteins (i.e., chromoproteins and other fluorescent proteins) are extremely useful due to their stability and also because their chromophore, (i.e. the protein region responsible for the color of GFP) is formed in an autocatalytic cycling of the 65SYG67 sequence (Figure 2). Because GFP doesn't require a cofactor it can fluoresce under multiple conditions [1].

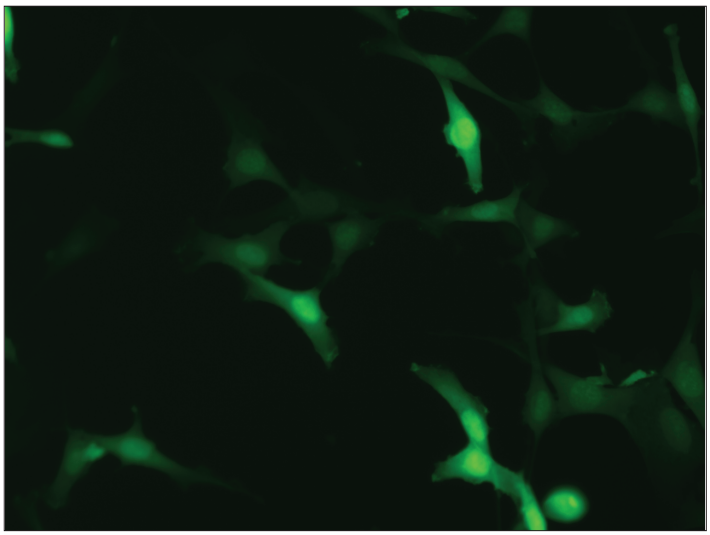

Figure 1. Green fluorescence in NIH3T3 cells expressing GFP.

Originally discovered in the jellyfish *Aequorea victoria*, GFP is a naturally fluorescent monomeric protein that is composed of 238 amino acids [2]. It is activated in *A. Victoria* by the naturally occurring bioluminescent protein aequorin, which releases blue light after binding with calcium. Absorption of the blue light emission by GFP excites the protein fluorescence and it then emits green light.

In addition, when GFP is transfected into cells as a fusion protein, it does not alter the function or location of the protein chimera. This makes GFP versatile because it can be used to localize proteins as they move around the cell, as well as monitor protein translation [3].

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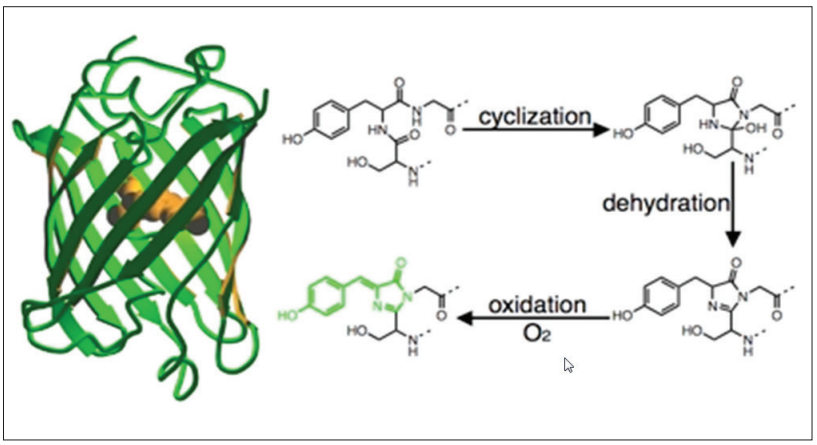

Figure 2. **Structure of Green Fluorescent Protein.** Three amino acid residues, Ser65, Tyr66 and Gly67 of the wild type A. victoria GFP spontaneously undergo sequential post-translational reactions in order to form the chromophore in the core of the barrel protein [4].

Mutations and variants

Characteristics of GFP such as the fluorescence color and intensity can be altered by changing the amino acid residues around the chromophore [1]. These mutations of GFP provide a number of color variants such as blue and yellow. These variations of GFP can be used to construct fluorescent chimeric proteins to be expressed in living cells, tissues, and entire organisms, after transfection with engineered vectors. Other colors such as red fluorescent proteins have been isolated from other species including coral reef organisms, and are used in assays requiring a fluorescent protein with different characteristics than GFP [6]. RFP emits in a longer wavelength which makes it compatible with existing confocal and wide field microscopes. RFP also has an increased capacity to image entire organisms, which are more transparent to red light. Table 1 provides a comprehensive listing of Fluorescent proteins available, their spectral characteristics and suitable optical filter sets.

Many mutations to GFP were created to optimize GFP efficiency or increase expression. For example, a laboratory mutation called GFPuv has been optimized to fluoresce when excited with ultra violet (UV) light. The mutation causes a brighter fluorescence and more desirable expression properties. It contains three amino acid substitutions (Phe to Ser at #99, Met to Thr at #153, and Val to Ala at #163) that alter protein folding and ultimately the formation of the chromophore [6]. When GFPuv is expressed in E.coli, it is more soluble than the wild type GFP (wtGFP). GFPuv was used with Bacillus subtilis to accurately analyze the spore surface's display system, which required a fluorescent protein with superior expression than wtGFP. When GFPuv was expressed in B. subtilis, the presence of a CotG-GFPuv protein on the spore was confirmed [9]. When the CotG anchoring motif was removed, no fluorescence was recorded confirming the role of CotG as an essential anchoring motif in the spore surface's display system. It was reported that the spore displaying GFPuv could be used for other signaling applications that use extracellular or intracellular stimuli [7].

Other alterations have been made to improve protein folding and chromophore formation. Besides amino acid substitutions such as the GFPuv mutation, many other silent mutations have been made to the wild type sequence. Codon changes in the DNA sequence for example, can reflect the intended host's codon bias. A mutation that was created by destroying a cryptic intron within wtGFP named mGFP4 has been extremely useful in higher plants because of its ability to give off a brighter green fluorescence that stands apart from the regular green coloring of plants when expressed [8]. The ability of GFP to be introduced into the genome of other organisms has opened many new doors in cellular, molecular and developmental biology. Successful GFP expression has taken place in many different cells including bacteria [7], yeast [9], plants [10], and mammals [11]. Whole organisms have been genetically altered to glow completely green [12]. Many of these fluorescent proteins can function as a protein tag, a bioluminescent reporter that can easily be expressed without interfering with the tagged protein's regular function and movement.

Other mutations of GFP can provide blue emission properties (BFP), but these mutants typically have low fluorescence quantum yield and rapid photobleaching making their efficacy limited. Using mutational analysis targeting residues adjacent to the BFP chromophore a variant with enhanced quantum yield, 0.55 was found (cf. 0.34 in original BFP). This BFP mutant, called Azurite also has reduced pH sensitivity and is more resistant to photobleaching [16]. It is well expressed in bacterial and mammalian cells and its discovery expands the palette of fluorescent proteins that can be used for detection and imaging.

When some proteins are tagged with fluorescent proteins, interactions between the tagged protein and fluorescent protein can upset targeting and/or yield undesirable results. Many wild type yellow to red fluorescent proteins are active as tetramers and are toxic or disruptive to the native protein [10]. The fluorescent protein mRFPI, "DsRed" from the Discosoma species. (mushroom coral) is a true monomer fluorescent protein that was developed by directed evolution to increase the speed of maturation and to break each subunit interface while restoring fluorescence. Since its initial development, several alterations to mRFPI have led to further improvements. These improvements include more rapid maturation, greater toleration of N-terminal fusions, higher fluorescence quantum yields, and greater photostability enabling them to be used in a wider array of assays [10].

Processes that are involved in the fluorescence of GFP have been investigated. Excited state proton transfer (ESPT), for example, is the process that causes and controls fluorescence of GFP and is the main focus of a recent study involving the discovery of an alternate proton acceptor for ESPT. A proton moves from the chomophore to an ionized side chain (E222), leading to the formation of an anionic chromophore. If this side chain is re-oriented or swapped out for another chain, ESPT is disabled causing a considerable loss of green emission when the chomophore is excited [14]. However, when a second mutation is introduced (this particular study used a GFP protein variant called H148D), green emissions were restored. This and other similar results with the E222/H148D double mutation support the theory that the D148 side chain is the proton acceptor in ESPT [14]. Further exploration of fluorescent emissions using time-resolved fluorescent and vibrational spectroscopy demonstrate a low barrier hydrogen bond between the phenyl hydroxyl of the chromophore and the D148 side chain. Besides furthering our understanding of proton transfer between proteins, this indicates that the hydrogen bond network in wtGFP can be replaced by a single residue [14].

Gene Expression

The assessment of gene expression has become one of the most utilized tools in molecular and cellular biology today. Both the permanent and transient expression of transfected cloned DNA sequences, aids in the determination of the transcriptional activity of promoters. Unfortunately, in most instances the natural product of the promoter cannot be assayed in a quantitative manner. In the past, this problem was solved by joining the promoter with a reporter gene which coded for a protein with unique enzymatic activity, such as β-galactosidase that could be assayed easily. The level of gene activity would then be monitored as a function of that enzymatic activity. These assays, while easy to perform and generally quite quantitative, suffer from their inability to be measured in real time and generally it was necessary to make cell lysates and perform reactions on these lysates later. The biggest issue with these experiments was the stability of the enzyme both while in storage and during the actual assay. Recently this problem has been eliminated with the use of inherently fluorescent proteins, such as GFP, which has been a way to evaluate gene expression and transfection efficiency.

Clearly, there are many uses for monomeric fluorescent proteins, many of which have been developed in recent years. Microscopy of fluorescent protein-based fluorescence resonance energy transfer (FRET) pairs have been used as reporters but hardware limitations often present difficulties that complicate cell screenings and other processes in cellular biology. Scientists have tried to remedy this by screening monomeric fluorescent protein pairs to find the best combination that would provide the desirable high dynamic range FRET changes, high pH, high photo-stability, fast maturation and bright fluorescence along with a reliable detection in any imaging system. Perhaps the most widely used biosensor design to screen new or improved FRET pairs involves a protease cleavage assay (Figure 3). The simple motif consists of two fluorescent proteins linked together by a short peptide that contains a consensus protease cleavage site. In general, the sensor exhibits very strong resonance energy transfer that is completely abolished upon cleavage of the linker sequence. Using this information, it is possible to monitor interactions of proteins in living cells and generate FRET based sensors. For example, Casper3-GR (Everogen), a commercially available derivative of CasperR3 [15], provides a practical detection for apoptosis and similar constructs could be very useful for cell biology studies and high-throughput screening assays in the future.

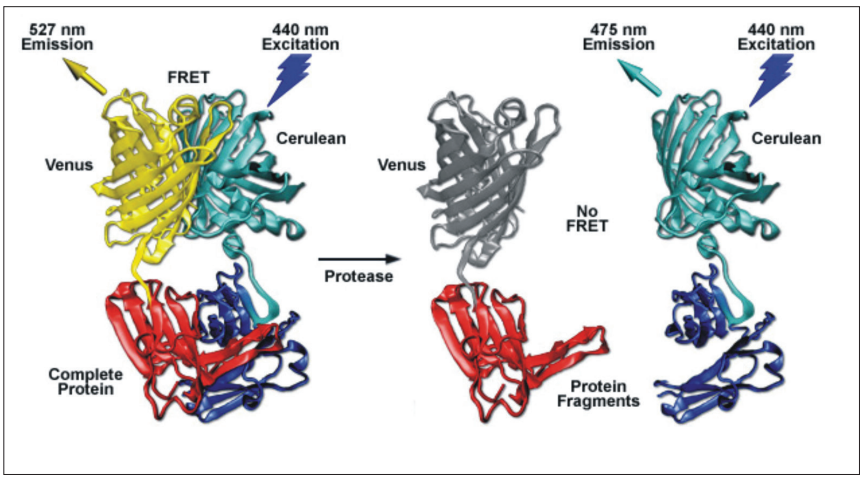

Figure 3. **Fluorescent Protein FRET Biosensor for Protease Activity.** Two fluorescent proteins (Cerulean and Venus) are linked by a short peptide containing a consensus protease cleavage site.

GFP is used frequently in experiments requiring gene expression because it is easily fused with the target gene and usually doesn't interfere with normal gene function. It is known that transposable elements containing GFP can be used to detect gene expression, inactivate gene function and also to induce misplaced or over-expression of genes. These characteristics have been used in conjunction with a technique known as a promoter trap. For the promoter trap, a promoterless cDNA (GAL4 cDNA) was used to express GFP when the construct was inserted in a transcriptional unit [16]. Once inserted, GAL4 activates a GFP-encoding gene that was also contained in the transposon (Figure 4). Thus identifying gene promoters specific to tissues by a gain of function. Because this construct would be expected to prematurely terminate the endogenous gene, a series of FLP recombinase sites were inserted into the vector. This transposon containing the GFP-encoding gene and the GAL4 cDNA can be inserted and removed by FLP recombinase, which allows it to stimulate conditional misexpression of the tagged gene. This promoter trap is used because it allows for the study of gain- and loss-of function within a particular insertion. For example, the promoter trap technique has been used to identify a group of cells that innervate a part of the mushroom body, a pair of structures in the brain of insects that are involved in learning and memory particularly with smell [16].

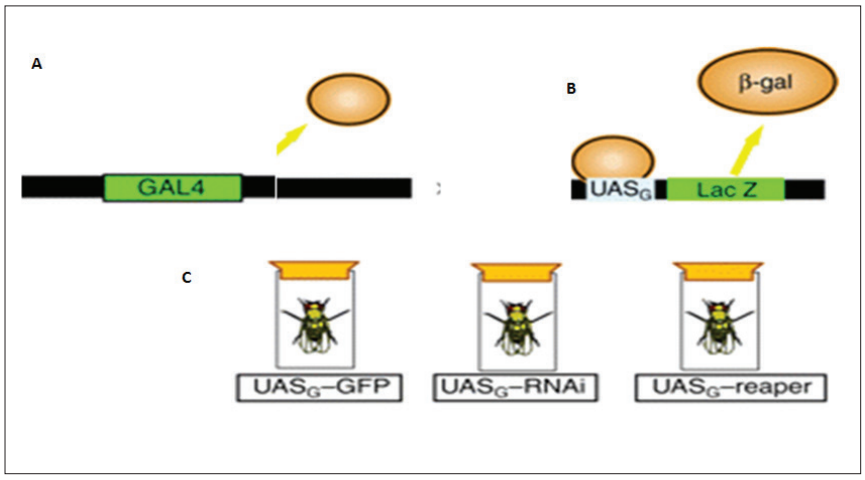

Figure 4. (a) GAL4 is a yeast transcription factor. (b) It binds a specific yeast promoter (UAS_G). Any gene controlled by UAS_G will be switched on in a cell expressing GAL4. (c) Crossing a GAL4 line with *Drosophila* containing the appropriate UAS_G construct, any gene can be expressed cell-specifically [16].

Non-Imaging Assays

Many proximity assays, such as LanthaScreen®, use fluorescence resonance energy transfer (FRET) to improve signal to background ratios. This is a time-resolved FRET (TR-FRET) that is often used to quantify a multitude targets such as protein kinases, nuclear hormone receptors or proteases. TR-FRET assays use a long-lifetime lanthanide chelate as the donor fluorescent species and a GFP moiety as the acceptor. A visual example of TR-FRET is shown in Figure 5. The time-resolved component is important because lanthanide chelates have a unique excited state lifetime which can last longer than a millisecond, significantly longer than the average excited state lifetime of most fluorescent compounds, normally measured in nanoseconds. This measurement is equivalent to the average time that the targeted molecule will spend in the excited state after being excited with photons [17]. The long half life of the donor molecule allows for a delay of 50-300 microseconds after the cessation of excitatory light and the measurement of FRET signal. This delay provides the means to overcome common background interferences which have much shorter fluorescent half lives [17].

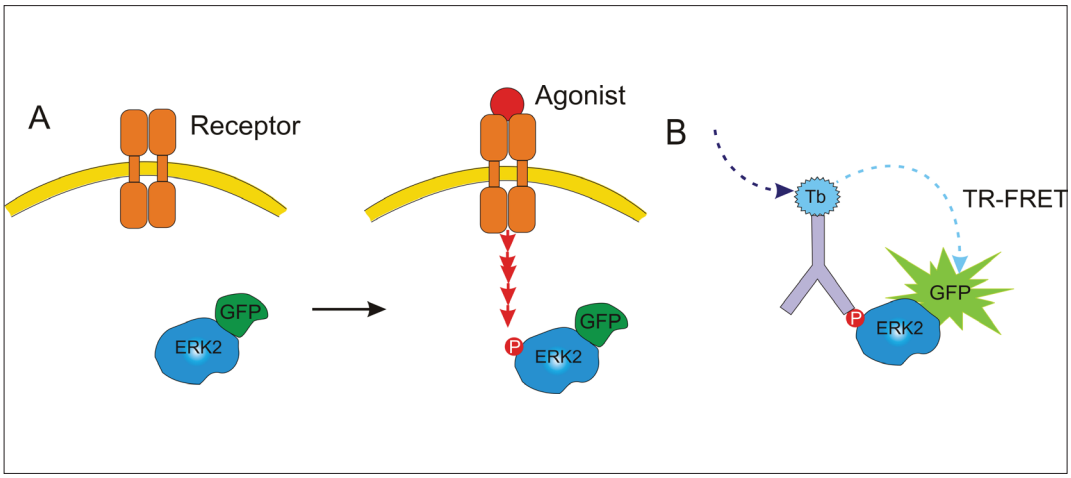

Figure 5. **Schematic depiction of agonist-mediated Receptor activation leading to ERK2 phosphorylation.** Live cells expressing GFP-ERK2 fusion protein are stimulated to promote ERK phosphorylation. (B) Following stimulation, a LanthaScreen cellular lysis solution containing a Tb-labeled anti-phospho-ERK2 [pThr185/pTyr187] antibody is added to the cells. Binding of the antibody provides the close association necessary for energy transfer from the excited donor fluorophore Tb to GFP, leading to an increase in TR-FRET signal. The result is a lysate-based immunoassay in which GFP serves as a FRET acceptor for the Tb-labeled phospho-ERK2 antibody donor [18].

FRET is a well established system for studying protein interactions and cellular screenings but, like any method, has its limitations. In order to overcome some of these limitations, other methods such as bioluminescence resonance energy transfer (BRET), have been utilized. BRET uses enzyme-catalyzed luminescence rather than the fluorescence used by FRET/FLIM type screenings [19]. It is a form of radiation-free energy transfer that can occur between energy donor and an expressed GFP (energy acceptor), but does not require excitatory light. BRET provides an assay readout that is amenable to high-throughput screening applications (Figure 6). The two target molecules can be tagged with a luciferase and a fluorescent protein allowing BRET to serve as a sensor that detects interactions between pairs of cellular proteins [19]. This eliminates many of the problems that can be found with FRET/FLIM such as autofluorescence and photobleaching. BRET is useful because it can detect protein interactions easily, *in vivo*, in real time and also in a signal-dependant manner [19].

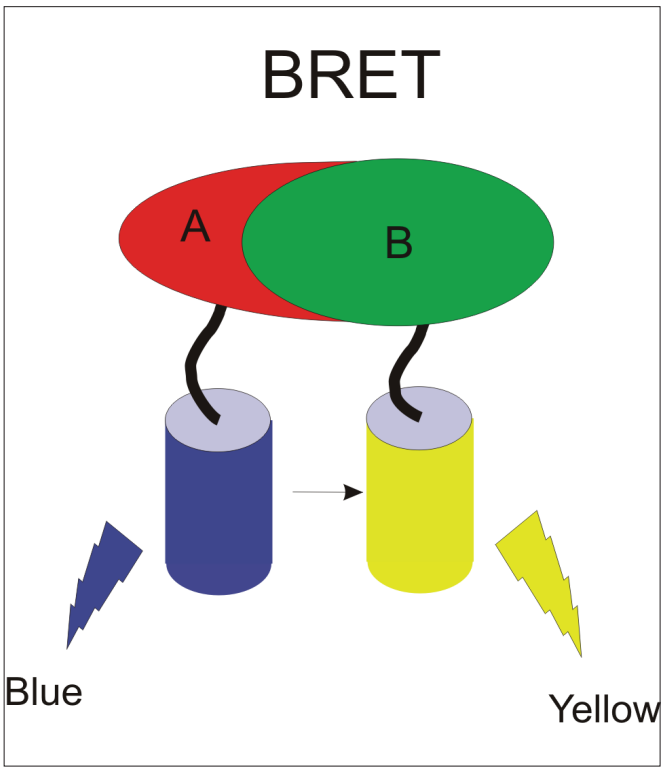

Figure 6. **Schematic depicting the BRET system.** A candidate protein is labeled with a bioluminescent luciferase and the target protein is labeled with a GFP mutant. When the two proteins are arranged closely together, the interactions between them cause a resonance energy transfer [23].

Imaging Assays

GFP and its variants are particularly useful for live cell imaging applications allowing for the monitoring of kinetic responses of physiological process. Indeed, GFP is a workhorse for high content screening applications using imaging microplate readers. A common assay used for G Protein-coupled Receptor assays is the Transfluor™ assay, which uses a β-Arrestin-GFP fusion protein to track the internalization of the GPCR receptor after agonist treatment. This assay has been used for screening of small molecule compounds using imaging microplate readers to visualize the internalization event at the level of a single cell [75].

GFP and some of its variants have been used in numerous studies to determine localization of specific proteins by analyzing fusion proteins. For example GFP has been used to localize Rho GTPases in living cells [86]. The use of Ras and Rho family GTPase fusions with different fluorescent proteins has allowed for breakthroughs in the understanding of how CAAX proteins are targeted to specific cell membranes [91]. The utility of GFP to be used for membrane protein localization and degradation was demonstrated in the yeast Saccharomyces cerevisiea using a GFP fusion with hydroxymethylglutaryl-Co reductase (HMGR) [90]. One caveat to this approach for nuclear localization is the finding that due to its small size, GFP can diffuse through nuclear pores into the nucleus. Only through the careful analysis of the fusion protein integrity can nuclear localization be confirmed [85]. Large databases such as the Yeast GFP Fusion Localization Database [87] the mammalian gene LIFEdb protein database [88] and the plant Arabidopsis [89] database have been developed providing searchable archives of protein localization.

Besides conventional and confocal microscopy for subcellular localization a new approach using two-photon dual-color microscopy has recently been explored using a blue and a red fluorescent protein. Two-photon microscopy is an imaging technique that allows imaging of living tissue with little scatter and can be an alternative to confocal microscopy. This method can be used for the simultaneously studying the expression, localization and trafficking of two colors in tissues or cells up to a very high depth [20]. Traditionally, fluorescent molecules with similar excitations wavelengths, but with large differences in their Stokes shift have been used. By screening a number of orange and red fluorescent proteins spectra for optimal two photon characteristics, the protein tagRFP was identified [21].

This protein was then paired with a number of blue, teal and green proteins, of which mKalama1 was found to be optimal. This method, which has been developed using simultaneous excitation of the lowest-energy electronic transition of a blue fluorescent protein and the higher-energy electronic transition of a red fluorescent protein does not require large differences in Stokes shifts and can also be used with many fluorescent proteins pairs with two-photon absorption efficiency and better imaging properties like other GFP mutations and color variations [21].

Stem Cells

The discovery of embryonic stem cells (ESCs) and the reprogramming of somatic cells into induced pluripotent stem cells (iPSCs) have opened numerous new avenues of research. The use of fluorescent proteins as a fluorescent tracer to monitor stem cells is almost a matter of routine. Early human ESC work focused on the use of fluorescent proteins as non invasive markers. Embryonic stem cell lines were established with EGFR constitutively expressed under control of the Elongation factor-alpha promoter [83]. These cells lines would produce EGFP as undifferentiated ESC, as well as after they were induced to differentiate [83]. In addition to their use as a simple tracer, fluorescent proteins can be used to monitor ESC expression specific to undifferentiation. ESC permanently transfected with EGFP under control of the Oct4 promoter demonstrate expression for long periods of time. The induction of differentiation of these cells or targeted knockdown of Oct4 expression results in a reduction of EGFP fluorescence.

Induced pluripotent stem cells (iPSCs) are somatic cells that have been reprogrammed from their differentiated state back to being a pluripotent cell capable of differentiating into cells of different germ lines. During the initial reprogramming, fluorescent proteins are monitored using imaging systems as compared to standard microplate readers. Because most reprogrammed cells do not result in pluripotency, the use of traditional microplate readers to make measurements on the average signal across a population cannot be used to identify positive iPSC clones, but time-lapse imaging has been used in a number of different ways to prove as well as monitor the pluripotency of induced stem cells. For example iPSCs were generated using a lentivirus reprogramming system where the four defined factors (Klf4, Oct4, Sox2, and c-Myc), termed KOSM necessary for reprogramming were fused in-frame into a single open reading along with a green fluorescent protein (GFP) marker. True proof of iPSC formation is the ability to produce cells from multiple germ layers when injected into bastocysts. The non invasive use of imaging of GFP signal to track cell lineage has been used to confirm the ability of the single Lentiviral cassette to produce viable iPSCs in mice [84]. Cultured Tail-tip fibroblasts (TTFs) that expressed GFP were infected with a third-generation Lentiviral system that contained the original Oct4, Klf4, Sox2 and cMyc cassette that is currently routinely used to generate iPSCs. The GFP expression was also used to determine transduction efficiency as well as demonstrate multiple germ layer derivatives in embryos [84].

Fluorescent proteins have been also been used to identify key cellular attributes of successful reprogramming. Live GFP or YFP-expressing reprogrammed mouse embryonic cells were imaged over time. A rapid shift in proliferative rate and a reduction in cellular area were identified as indicators of successful reprogramming with a retrospective analysis of the cells that formed iPSC colonies [80]. Once pluripotency is established, it is maintained by a core regulatory network of transcription factors which can then be monitored using fluorescent proteins, whose expression parallels that of endogenous proteins [76]. Stable Rat iPSCs lines were developed using FACS analysis and cell sorting based on GFP expression of cells induced using a Lentiviral system that encoded EGFP, as well as Oct4, Sox2, Klf4, and cMyc [82]. GFP expression has been used as a means to demonstrate the utility bacterial artificial chromosomes (BACs) in generating transgenic clones of embryonic stem cells. The use of BACs in the generation of transgenic clones rather than small plasmids minimizes insertion location silencing of the transgene. BACs from a GFP transcriptional fusion library (GENSAT) specific for neuronal cell lines were introduced into embryonic stem cells and selected by G418 resistance. Only when the ESCs were induced to differentiate was GFP expression evident, indicating that BACs functioned correctly in ESCs [79].

Interestingly, not all fluorescent proteins are created equal with regard to ESCs. Several ES cell lines have been established that are capable of transgene expression of fluorescent proteins ECFP, EYFP and EGFP, in vitro as well as in vivo. However, difficulty in obtaining similar cell lines using DsRed-1 suggests that this fluorescent protein in not developmentally neutral [77]. Stepwise mutagenesis of DsRed to the monomer mRFP1 has resulted in a viable red-wavelength marker for ESC research [78].

Reporter technology

The versatility of fluorescent reporter proteins has resulted in many uses, such as their use in assays involving gene expression in living cells. Currently, fluorescent proteins are being used for identifying and isolating cell populations of embryonic system cells. There are numerous selectable genetic markers using fluorophores. These are typically some sort of fusion protein linked to a small promoter fragment and usually a number of different combinations are required before a suitable marker is found. Scientists are trying to reduce the upfront experimentation with a more ubiquitous genetic marker and produce a reporter that preserves the endogenous regulatory sequences upstream of the ATG start site. A series of plasmids with multiple modular genetic markers that have an independent reporter, a bacterial selection and a eukaryotic selection have been developed that are compatible with bacterial artificial chromosome (BAC) technology [11]. A self-cleaving peptide links the emerald GFP reporter to the native open reading frame (ORF) and the gene for an early cardiac marker (NKX2-5) a marker for embryonic system cells. The use of large BACs served to reduce any localized integration effects with this genetic reporter, while the cleaving peptide reduced artifacts caused by the protein fusion. This marker was expressed and used to detect differentiating mouse embryonic system cells. These results denote that the NKX2-5 cell reporter line is a good line to use for studies involving the early processes in cardiomyocyte formation. [11].

The ability of fluorescent proteins to provide information in living cells makes them ideal for research involving cancer. For example, ZsGreen fluorescent protein has been used to study cancer stem cells in lung cancer [22]. Cancer stem cells (CSC) are a subset of tumor cells capable of self-renewal as tumor spheres. One hypothesis is that by eliminating the CSCs, the source of tumor origin, one could "cure" lung cancer. CSCs were originally believed to exist in side population (SP) of cultured lung cancer cells, but new data suggests that some non SP cells are capable of self renewal. Determination of SP cells is based on their ability of lung cancer cells to efflux Hoechst dye. Lung tumor spheres from human cell lung carcinoma lines A549 and H1299 show morphological differences and increased expression of stem cell markers when compared to corresponding cells in monolayer cultures [22]. Proteosome activity was tested using ZsGreen fluorescent protein chimera with the C-terminus of the ornithine decarboxylase. Using a ZsGreen-cODC reporter assay, which is a target for proteosome degradation, one can identify cells lacking proteosome activity by green fluorescence. Lung tumor spheres were demonstrated to have decreased 26S proteosome activity, compared to the monolayer cells. This assay was used with Non small cell lung cancer (NSCLC) cell lines to identify and enrich for cells lacking proteosome activity, where it has been shown that less than 1% of the NSCLC monolayer cells were positive, while spherical cells were greatly enriched for fluorescence. [22].

Fluorescent proteins can be used to indicate gene promoter activity in bacteria in tumors. For example, *Salmonella enterica* prefers to infect solid tumors compared to normal tissue. Identifying Salmonella gene promoters that are preferentially activated in solid tumors would help elucidate this phenomenon. Researchers cloned a genomic library of S. *enteric typhimurium* 14028 upstream from a promoter-less gene encoding the fluorescent protein TurboGFP [23]. This library was injected into tumor-free nude mice and human PC3 prostate tumors which were also growing in nude mice. After two days, cells from spleens or tumors were sorted using fluorescence activated cell sorting to identify and enrich for cells expressing GFP. Hybridization with an oligonucleotide array of the Salmonella genome showed eighty-six intergene regions to be enriched in tumor samples but not spleen. Twenty of these candidate promoters were also found in 100 random clones from a library that was enriched for expression in bacteria growing tumors. Three candidate promoter clones were tested in vivo. And increased GFP expression in bacteria growing in tumors rather than spleen was observed. Two of those clones are known to be induced in hypoxic conditions similar to those in tumors [23]. While many of the other candidate promoters' regulatory mechanisms may not be related to hypoxia, these findings have potential to improve the targeting of drug delivery.

Green fluorescent proteins and their multiple variations are an extremely valuable tool in cellular assays and molecular imaging. They have helped to further our knowledge in many fields including signaling applications, proton transfer between proteins, in FRET/FLIM microscopy and in many other areas helping to understand cellular and protein structure and function. There are a few drawbacks, as no single GFP variant is ideal for every application, but each version offers different advantages for quantitative imaging in living cells. Because GFP doesn't require an outside stimulus in order to fold into the fluorescent structure, it is extremely stable even in the presence of denaturing substances or proteases as well as through a wide range of pH and temperature [24]. These characteristics along with the GFP's ability to report without interfering with the protein's regular function and movement make it a great tool for future projects that involve medical applications, eliminating cancerous tumors for example.

Table 1. Fluorescent proteins wavelength maxima and suggested filter combinations.

Blue Fluorescent Proteins	Excitation max (nm)	Emission max (nm)	Extinction coefficient (€)	Ex Filter	Em Filter	Mirror (cut off)	Ref#
Azurite	384	450	26,200	380/20	460/40	435	13
EBFP	383	445	29,000	380/20	460/40	435	16
EBFP2	383	448	32,000	380/20	460/40	435	25
mTagBFP	399	456	52,000	400/30	460/40	435	26
Y66H	382	459	25,000	380/20	460/40	435	27

Cyan Fluorescent Proteins	Excitation max (nm)	Emission max (nm)	Extinction coefficient (€)	Ex Filter	Em Filter	Mirror (cut off)	Ref#
ECFP	439	476	32,500	420/50	485/20	455	28
AmCyan1	458	489	44,000	440/40	500/27	455	29
Cerulean	433	475	43,000	420/50	485/20	455	30
CyPet	435	477	35,000	420/50	485/20	455	31
mECFP	433	475	32,500	420/50	485/20	455	32
Midori-ishi Cyan	472	495	27,300	440/40	500/27	455	33
mTFP1 (Teal)	462	492	64,000	440/40	500/27	455	34
TagCFP	458	480	37,000	440/30	485/20	455	35

Green Fluorescent Proteins	Excitation max (nm)	Emission max (nm)	Extinction coefficient (€)	Ex Filter	Em Filter	Mirror (cut off)	Ref#
AcGFP	480	505	50,000	460/40	516/20	510	36
Azami Green	492	505	55,000	485/20	516/20	510	37
EGFP (S65T/F64L)	484	507	56,000	485/20	516/20	510	38
Emerald	487	509	57,500	485/20	516/20	510	11
GFP (wt)	395	509	21,000	395/25	508/20	435	
	475	509	21,000	460/40	516/20	510	
GFP (uv)	395	509	21,000	395/25	508/20	435	7
GFP-S65T	488	509	56,000	485/20	516/20	510	39
mWasabi	493	509	70,000	485/20	516/20	510	40
Stemmer	395	509	27,000	395/25	508/20	435	
sfGFP (Superfolder GFP)	485	510	83,300	485/20	516/20	510	41
TagGFP	482	505	58,200	485/20	510	510	15
T-Sapphire	399	511	44,000	395/25	508/20	435	42
TurboGFP	482	502	70,000	475/20	508/20	510	23
ZsGreen	493	505	43,000	485/20	516/20	510	22

Yellow Fluorescent Proteins	Excitation max (nm)	Emission max (nm)	Extinction coefficient (€)	Ex Filter	Em Filter	Mirror (cut off)	Ref#
EYFP	514	527	83,400	500/27	540/25	525	43
mBanana	540	553	6,000	528/20	560/15	545	44
mCitrine	516	529	77,000	500/27	540/25	525	45
PhiYFP	525	537	124,000	516/20	550/10	525	46
TagYFP	508	524	64,000	485/40	540/25	525	47
Topaz	514	527	94,500	500/27	540/25	525	48
Venus	515	528	92,200	500/27	540/25	525	49
YPet	517	530	104,000	500/27	540/25	525	50
ZsYellow1	529	539	20,200	516/20	550/10	525	51

Orange Fluorescent Proteins	Excitation max (nm)	Emission max (nm)	Extinction coefficient (€)	Ex Filter	Em Filter	Mirror (cut off)	Ref#
DsRed/RFP	558	583	75,000	540/35	590/20	570	52
DsRed2	563	582	43,800	540/35	590/20	570	53
DsRed-Express	555	584	38,000	540/35	590/20	570	54
DsRed-Monomer	556	586	35,000	540/35	590/20	570	55
Tomato	554	581	69,000	540/35	590/20	570	56
tdTomato (tandem dimer)	554	581	138,000	540/35	590/20	570	57
Kusabira Orange	548	559	51,600	530/25	570/10	555	58
mKO2 (Kusabira Orange2)	551	565	63,800	540/25	575/10	555	59
mOrange	548	562	71,000	530/25	570/10	555	60
mOrange2	549	565	58,000	540/25	575/10	555	60
mTangerine	568	585	38,000	540/35	600/40	570	10
TagRFP	555	584	100,000	540/35	590/20	570	21
TagRFP-T	555	584	81,000	540/35	590/20	570	61

Red Fluorescent Proteins	Excitation max (nm)	Emission max (nm)	Extinction coefficient (€)	Ex Filter	Em Filter	Mirror (cut off)	Ref#
AQ143	595	655	90,000	590/20	645/40	595	62
AsRed2	576	592	56,200	560/20	596/15	570	63
dKeima-Tandem	440	620	28,800	440/40	620/40	550	64
HcRed1	588	618	20,000	590/20	635/32	595	65
tHcRed (tandem)	590	637	160,000	590/20	645/40	595	66
JRed	584	610	44,000	575/15	620/15	595	67
mApple	568	592	75,000	540/35	600/40	595	30
mCherry	587	610	72,000	585/10	620/15	595	69
mPlum	590	649	41,000	590/20	645/40	595	70
mRaspberry	598	625	86,000	590/20	645/40	595	71
mRFP1	584	607	50,000	575/15	610/10	595	72
mRuby	558	605	112,000	540/35	620/40	595	73
mStrawberry	574	596	90,000	560/20	620/40	595	74

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