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Go with the glow: fluorescent proteins to light transgenic organisms

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Once a biological novelty known for their role in bioluminescence, fluorescent proteins (FPs) from marine invertebrates have revolutionized the life sciences. Organisms from all kingdoms have been transformed with the *Aequorea victoria* green fluorescent protein (GFP), and biotechnology has been advanced by the use of FPs. This article reviews the current uses of FPs in whole transgenic organisms and genomics and looks beyond GFP to the complete color palette and spectral properties afforded by FPs from other marine organisms. Coupled with electronic devices for visualizing and quantifying FPs, recently cloned FP genes might be useful for the ecological monitoring of transgenic organisms in the environment. Therefore, this review also addresses the *in vivo* labeling of organisms with an emphasis on plants.

Introduction: out of the sea, into the laboratory, and onto land

Fluorescent proteins (FPs) are not new to science, nor were they always a hot topic; rather, it is biotechnology that has made FPs what they are today. Marine biochemists labored in relative obscurity for 30 years before the jellyfish *Aequorea victoria* green fluorescent protein (GFP) gene was first cloned [1] and transformed into the nematode *Caenorhabditus elegans* [2]. Since then FPs have become an essential tool in the production and analysis of transgenic organisms in basic and applied biology. The number of scientific articles on FPs in transgenic organisms is growing exponentially: 200 articles were published between 1994 and 2001, whereas between 2001 and 2004, more than 900 articles appeared. More than three-quarters of those published recently used *A. victroria* GFP and its variants, despite plenty of new FPs available for use. Although this review analyzes how FPs have been used, in conjunction with dedicated instrumentation, for macroscopic visualization and measurement, it also addresses the ideal spectral properties required for future applications of FPs. Essentially, how far can we glow?

Lit-up leaves and legs, fins and fronds

Model organisms were the first to be transformed with GFP. After *C. elegans* [2] came the fruitfly *Drosophila melanogaster* [3] followed by mammalian cell lines [4] and yeast [5]. Larger organisms, such as plants [6], mouse [7], zebrafish [8] and the frog *Xenopus laevis* [9] were transformed with GFP variants yielding visible green fluorescence. FPs quickly transcended science to be used in ornamental fish and even iconic works of art. The most infamous of these is 'Alba', the GFP bunny commissioned by the artist Eduardo Kac. The erstwhile albino rabbit was engineered with GFP to yield a striking fluorescent phenotype (Figure 1a), which was a focal point of the *Eighth Day* art exhibit, where art and biotechnology intersected (www.ekac.org/gfpbunny.html#gfpbunnyanchor). The transgenic rhesus monkey 'ANDi' [10] was also iconic (at least from a news perspective) not because it had a macroscopically fluorescent body but simply because it was the first reported GFP transgenic primate – obviously reporters and the public had a closer taxonomic identity with the monkey than the bunny. The most successful commercial ornamental transgenic organism to date has been 'GloFish™' (www.glofish.com), marketed by Yorktown Technologies (Figure 1b). Since 2003, transgenic zebrafish (*Danio rerio*) have been sold to aquarium enthusiasts for approximately US $5.00 each. *D. rerio* were transformed with several different FPs – GFP, yellow fluorescent protein (YFP) and the red FP from the coral *Discosoma* sp. (DsRed) – under the control of a strong muscle-specific (mylz2) promoter [11]. Illuminated with an ultraviolet aquarium lamp, GloFish™ appear brightly fluorescent red, and, in fact, the expression of the FP is great enough to color the fish red under room light. Apparently, more colors are on the way for pet zebrafish. FPs have revealed a lighter side of science than the public is usually accustomed to seeing, which might contribute to greater acceptance of genetically modified organisms.

Functional genomics and developmental biology

On a more serious note, FPs have become integral tools in functional genomics and development. This approach has been particularly useful in plant biology. FPs have been linked to numerous different proteins to monitor localization, and in functional genomics a transgenic approach using FPs might well be exploited to examine promoter activity and to clone regulatory elements [12]. For example, the constitutive CaMV 35S promoter, widely used in transgenic plants, is not really constitutive but has developmental and tissue specificity, as shown by differential GFP expression in tobacco [13], mustards [14] and cotton [15]. Novel promoters have been characterized by examining GFP fluorescence in living plants, as shown by a recent description of several promoters from the taro

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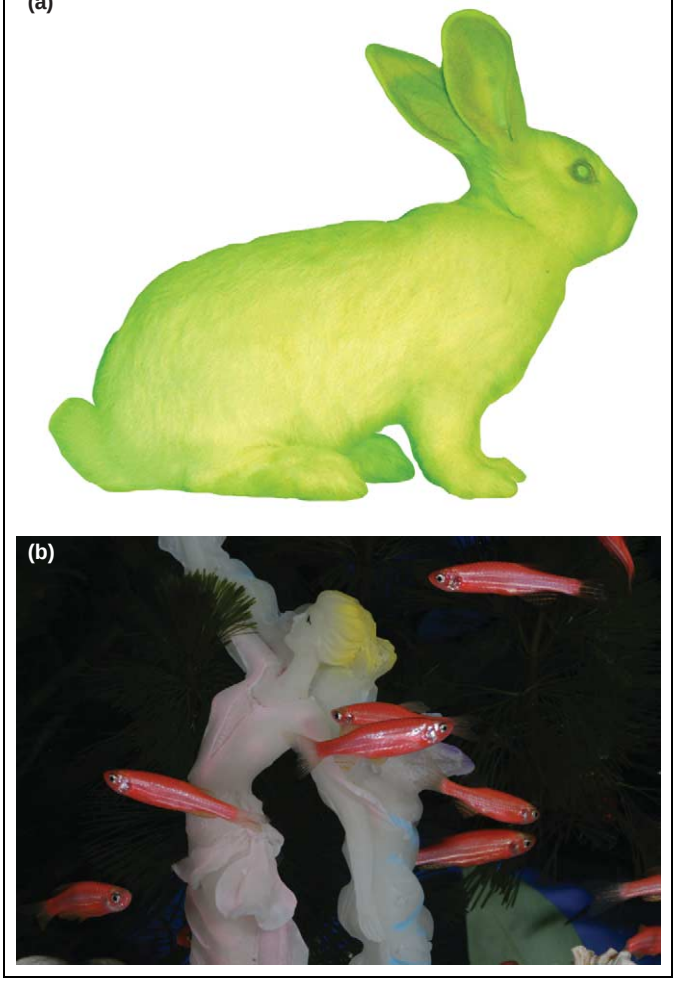

Figure 1. FPs in aesthetic works. **(a)** Alba, the fluorescent rabbit. Eduardo Kac, GFP Bunny, 2000, courtesy of Galerie J. Rabouan Moussion, Paris. **(b)** GloFish™, which contain a RFP. Courtesy of Yorktown Technologies, L.P.

bacilliform virus (TaBV), tested in transgenic banana and tobacco plants [16].

Several groups have used FPs to tag genes 'en masse' and examine fluorescence activity: Janke *et al.* [17] have described an effort in yeast and Cutler *et al.* [18] described a general approach to tag *Arabidopsis thaliana* cDNAs with GFP to identify subcellular structures. GFP has been a powerful tool in studying various genomic phenomena. One prominent example has been RNA interference, in which GFP transgenic plants have been infected with *Agrobacterium tumefaciens* containing various other GFP constructs to examine patterns of gene silencing [19,20]. Another recent example used GFP to monitor experimental gene targeting in transgenic *Arabidopsis* [21]. With the requisite help of a yeast chromatin-remodeling gene, which dramatically increased the efficiency of gene targeting to a specific locus, researchers were able to determine which *A. thaliana* seeds were transgenic for the targeted sequence, based on the green fluorescence of primary transgenics (i.e. when the gene was targeted to the intended site, the result was GFP seeds).

Animal functional genomics studies have also greatly benefited from FPs. Hendricks and Engelward [22] described 'recombomice' research, which specifically employed the use of YFP for the development of a fluorescent yellow direct repeat' (FYDR) mouse model for the detection of homologous mitotic recombination. In this system, the reconstitution of YFP, and subsequent yellow fluorescence, indicated a somatic recombination event had occurred – the YFP gene was split so that the two halves of the truncated gene came together during recombination. In other research, DsRed and derivatives have been shown to be effective in mammalian *in vivo* labeling [23]. It was found that a monomerized DsRed derivative, mRFP1, was developmentally neutral when overexpressed in mouse, where there was no observed detrimental effect [24]. However, when Hirrlinger *et al.* [25] labeled various cell populations in mouse brain with different Anthozoan fluorescent proteins, they found that FP precipitants, probably from tetramerization, hampered their development studies. Thus, it is clear that developmental biology will be greatly served by multicolored monomeric FPs.

Transgenic ecology

Enabling ecological molecular genetics

Before transgenic organisms were available, ecological genetics typically focused on endogenous genes (usually unknown) using representative phenotypic traits or anonymous DNA markers. However, ecologically important individual traits can be manipulated through the introduction of transgenes, for example, insect resistance. FPs can be used to tag these transgenes and to monitor their movement and ecological effects, an ever important factor in biotechnology risk assessment [26]. At night, a low-tech UV spotlight can show exactly which plants are transgenic for a genetically linked trait such as insect resistance. This approach has been valuable in assessing gene flow from transgenic crops to wild relatives. Because transgenes hybridize and stably introgress into a wild and weedy crop relative they can be monitored non-destructively and in real time, thereby aiding ecological research [27]. Someday, this application might move from research to commercial monitoring. Additionally, FPs can enable *in vivo* fluorescence monitoring of the expression of a linked or fused gene such that quantitative measurements of FPs can yield correlated assessments of expression of a gene of interest in the field [28,29].

FPs are neutral markers

GFP has many properties that indicate it is a neutral, nontoxic, universal marker when expressed in transgenic organisms. GFP does not confer any measurable ecological host costs when expressed in transgenic plants [6,30,31] and, given their structural similarities to GFP, Anthozoan FPs are not expected to be any different in this regard. GFP also appears to be neutral with regards to oral toxicity and allergenicity. When GFP was fed to rats, it was found to be non-toxic when ingested either in a purified form or expressed in transgenic plants [32]. At physiologically relevant levels, GFP is completely digested and does not have any allergenic features; but when rats were fed extraordinarily high amounts (1 mg/day purified GFP) it altered the spectral properties of their feces (Figure 2). Aside from this novelty, the benign features of

Figure 2. GFP in rat feces. Under UV light, green feces are mixed with nongreen fluorescent feces recovered from rats fed 1 mg purified GFP earlier in the day [32].

FPs might pave the way for their use in ecological and physiological monitoring.

Indicating hetero- and homozygosity

One practical problem when working with transgenic organisms, particularly in large sample sizes, is the determination of the transgene zygosity of dominantly or semi-dominantly expressed traits. As demonstrated by Halfhill *et al.* [29], FPs can provide instantaneous data on homo- or heterozygosity. In this study, heterozygous (hemizygous) transgenic canola plants exhibited half the green fluorescence of homozygous plants (Figure 3). Furthermore, when GFP homozygous canola plants were hybridized with non-transgenic wild relatives, the progeny, which were heterozygous, had fluorescence measurements that were the same as the heterozygous parent crop, indicating that FPs might be a tool in analyzing hybridization and introgression status. This use is not exclusive to plants. Sato *et al.* [33] demonstrated

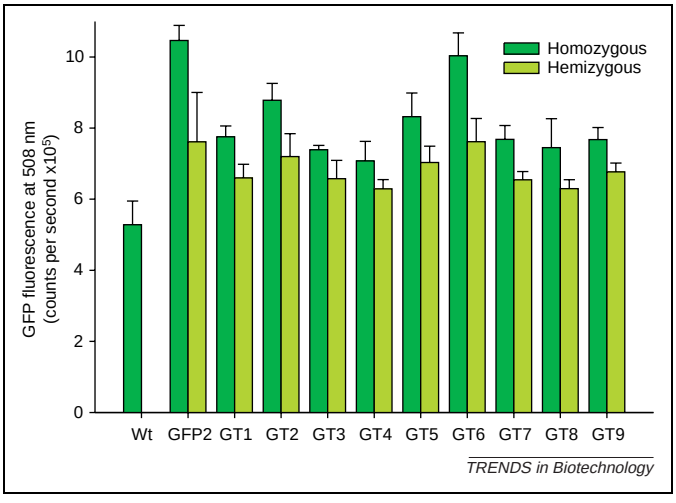

Figure 3. Additive GFP effects: homozygous plants fluoresce approximately twice as much as hemizygous (heterozygous plants). GFP fluorescence at 508 nm for homozygous versus hemizygous T_1 plants, in ten independent transgenic *Brassica napus* events, when excited at 385 nm. Non-transformed *B. napus* (Wt) was used as a control [29].

that GFP might be used to confirm the homozygous state of a mouse transgene at the preimplantation stage of embryo development. Animal population studies might also be facilitated by such an *in vivo* heterozygosity marker.

Keeping tabs of transgenic organisms

Innumerable transgenic plants have been grown in the USA during the past decade and, throughout the world, concerns have arisen about their environmental impacts [34]. Because transgenes can move from commercial crop hosts to wild relatives (and unintended hosts) [35], there are compelling reasons for monitoring the presence and expression of transgenes in plants [26]. It is not only plants that would be good candidates for FP-tagged environmental monitoring but insects, fish and livestock can also be monitored in this way.

Muir and Howard [36] have proposed models that suggest faster growing fish might be ecologically disruptive if they mated with wild-type fish – the so-called 'Trojan gene' hypothesis. Thus, it would seem reasonable to use FPs to monitor any transgenic fish released into the environment through aquaculture. As evidenced by GloFish™, fish can be engineered to be brightly fluorescent and can, therefore, be tagged with FPs: transgenic medaka [37] and rainbow trout [38], among others, have been produced in the laboratory.

Furthermore, transgenic insects also require imminent monitoring using FPs. The agricultural and environmental release of transgenic insects has been discussed widely, and made feasible by more efficient transformation methods. Indeed, insect transformation has been enabled by FPs [39–41] and FPs might be used to help monitor insects released into the environment, particularly how they spread from a release point. For example, there is interest in introducing transgenic, fitness-altered insects that damage economically important crops to breed with members of their own species as a potential biocontrol measure. The weakened gene pool might be transmitted in such a way that the general population becomes less fit, thereby decreasing its virulence. If FP-tagged transgenic insects were used, the efficacy of the control, in addition to the population dynamics, might be monitored.

Farm animals might also be monitored by FPs, which would probably be more important for the farmer as an available *in vivo* tag for identity purposes rather than for environmental purposes. Might FPs become the kinder and gentler branding? Transgenic FP-chickens [42], pigs [43] and cattle [44] have been produced with the FP serving as a marker for transgenicity.

Environmental monitoring

Not only might FPs be used to monitor the fruits of biotechnology but they might also have an important role in monitoring a variety of environmental conditions. Oofusa *et al.* [45] fused a metallothionein promoter with GFP, and introduced the construct into the germline of *Xenopus laevis*. They showed that low concentrations (micromolar) of zinc and cadmium were detectable by fluorescence in transgenic tadpoles.

Most research on potential environmental monitoring applications has focused on plants, given their potential uses for covering a wide area. Yoshimoto *et al.* [46] produced transgenic *Arabidopsis thaliana* plants containing a sulfate transporter promoter fused to GFP, which indicated the patterns of sulfate accumulation in whole plants. Researchers interested in space biology have also made use of FPs in plants. In one study, two different GFP constructs were made using general stress-inducible promoters fused to the FP reporter gene, and introduced into *Arabidopsis*; these plants were then used to report low atmospheric pressure, mimicking that occurring on a space flight [47]. To alert to an attack by chewing insects, Perera and Jones [48] fused a peroxidase promoter to GFP and found that fluorescence was easily detectable in tobacco after two different caterpillar species had fed on the leaves. Several research groups have investigated the production of transgenic plants to report early infection of plant diseases. One method entailed the subcellular localization of several GFPs in plants to examine the perturbations of fluorescence patterns caused by fungal pathogen attack [49]. Kooshki *et al.* [50] demonstrated that a salicylic acid-inducible promoter fused to GFP might be used for the detection of a fungal pathogen but GFP expression was low because of the weak promoter, resulting in a GFP signal that was not discernable above background fluorescence. Similarly, Rookes and Cahill [51] used another pathogen-inducible promoter fused to GFP and also observed low-to-moderate levels of fluorescence in response to pathogen attack. Although it seems feasible to use FPs to monitor the incidence of plant disease in real time, it is clear that inducible FP expression must be increased *in planta*. In addition, it is clear that the spectral properties of GFP are suboptimal for this and other environmental reporting purposes and other FPs might be better suited. A combination of FPs can be used in integrated pest management and/or precision farming systems to indicate the need to treat for insects, pathogens or even fertility problems.

Instrumentation for FP detection

Observing FPs in whole organisms is possible because of their unique fluorescence signatures, which can be monitored by simple observation or through the use of

Table 1. Properties of FPs, with published extinction coefficients and quantum yields, that appear bright enough to be useful in transgenic organisms. Most are untested in transgenics

Species of origin and designation(s)	Excitation max. (nm) and (extinction coefficient) (10^3 M^{-1} cm^{-1})	Emission max. (nm) and (% quantum yield)	Oligomerization (M=monomer, D=dimer, T=tetramer)	Refs
Aequorea victoria GFP	395 (27)	504 (79)	M	[64]
A. victoria GFP S65T	489 (55)	510 (64)	M	[64]
A. victoria EGFP	488 (56)	508 (60)	M	[64]
A. victoria GFP Emerald	487 (58)	509 (68)	M	[64]
A. victoria GFP YFP Topaz	514 (94)	527 (60)	M	[64]
A. victoria GFP YFP Venus	515 (92)	528 (57)	M	[65]
Zoanthus sp. ZsGreen	497 (36)	506 (63)	M	[56]
Zoanthus sp. ZsYellow	528 (20)	538 (2042)	T	[56]
Anemonia majano AmCyan	458 (40)	486 (24)	T	[56]
Heteractis crispa t-HcRed1	590 (160)	637 (4)	T	[66]
Discosoma sp. DsRed	558 (75)	583 (79)	T	[62]
Discosoma sp. mRFP1	584 (50)	607 (25)	M	[61,62]
Discosoma sp. dimer2	552 (69)	579 (29)	D	[61,62]
Discosoma sp. mOrange	548 (71)	562 (69)	M	[62]
Discosoma sp. dTomato	554 (69)	581 (69)	D	[62]
Discosoma sp. tdTomato	554 (138)	581 (69)	D (tandem)	[62]
Discosoma sp. mStrawberry	574 (90)	596 (29)	M	[62]
Discosoma sp. mCherry	587 (72)	610 (22)	M	[62]
Discosoma sp. mPlum	590 (41)	649 (10)	M	[63]
Galaxeidae sp. Azumi Azami Green (AG)	492 (72)	505 (67)	T	[67]
Galaxeidae sp. mAG	492 (72)	505 (81)	M	[67]
Cerianthus membranaceus cmFP512	503 (59)	512 (66)	T	[68]
Entacmaea quadricolour eqFP611	559 (78)	611 (45)	T	[69]
Trachyphyllia geoffroyi Kaede	508 (99)	518 (80)	T	[70]
Acropara sp. MiCy	472 (27)	495 (90)	D	[71]
Fungia concinna KO	548 (110)	561 (45)	T	[71]
Fungia concinna mKO	548 (52)	559 (60)	M	[71]
Phialidium sp. phiYFP	525 (115)	537 (60)	D	[72]
Pontellina plumata pplu GFP2	482 (70)	502 (60)	M	[72]
Pontella meadi pmeaGFP1	489 (99)	504 (74)	Unknown	[72]
Pontella meadi pmeaGFP2	487 (98)	502 (72)	Unknown	[72]
Unidentified pdae1GFP	491 (105)	511 (68)	Unknown	[72]
Lobophyllia hemprichii EosFP	506 (72) 571 (41)	516 (70) 581 (55)	T	[73]
Lobophyllia hemprichii d2EosFP	506 (84) 569 (33)	516 (66) 581 (60)	D	[73]
Lobophyllia hemprichii mEosFP	505 (67) 569 (37)	516 (64) 581 (66)	M	[73]

more sophisticated detection devices. In many ways the instrumentation needed for wide-scale detection has lagged behind the biotechnology of FP discovery and modification. Although one can simply use a UV spotlight [6], or flashlights [52,53], in the dark with certain FPs, more sophisticated techniques are needed for commercial applications. OptiSciences (www.optisci.com) has recently developed a GFP meter that uses a fiber-optic-facilitated leaf clip to sample spectra on intact leaves [54]. This portable spectrofluorometer performs in a similar way to laboratory-sited instruments and can take fluorescence measurements every few seconds. Although this instrument is currently used for research, it is envisaged that it might be used in field surveys or mounted on a tractor-driven accessory for GPS-interfaced monitoring of commercial fields for precision agriculture. For detection from a distance, a laser-induced fluorescence imaging device has been developed and tested in GFP transgenic plants [55]. Similar to the GFP meter, it can be used in the daylight but has a broader measurement capability, which can interrogate entire plant canopies from a distance. Unlike the GFP meter, laser-based instruments are expensive and not commercially available for detecting fluorescence. However, these kinds of instruments might be deployable in aircraft or on towers to survey large geographical areas relatively quickly, and might be useful for environmental monitoring, particularly for strategic purposes where general detection over a wide area is required.

The best FPs for transgenic organisms?

Following the respective cloning and characterization of the genes encoding GFP in 1992 and 1994 [1,2], and DsRed in 1999 [56], a large number of FP genes and proteins have been discovered. The most promising source of FPs is from non-bioluminescent Anthozoan species, the subject of several reviews [57–59]. There is no need to provide an exhaustive review of all the available FPs for transgenic organisms but Table 1 lists many of the best candidates based on their spectral qualities. The only FPs addressed here are those for which the extinction coefficient and quantum yield data are available – parameters indicative of brightness. Many other properties of FPs are important, such as folding dynamics, monomerization, excitation–emission matrices and resistance to photobleaching; however, brightness is the parameter most often considered for determining which FP to use in any given application.

Unlike *A. victoria* GFP, which is a monomer (except in high concentrations, where it dimerizes), Anthozoan FPs are obligate homotetramers. Tetramer formation results

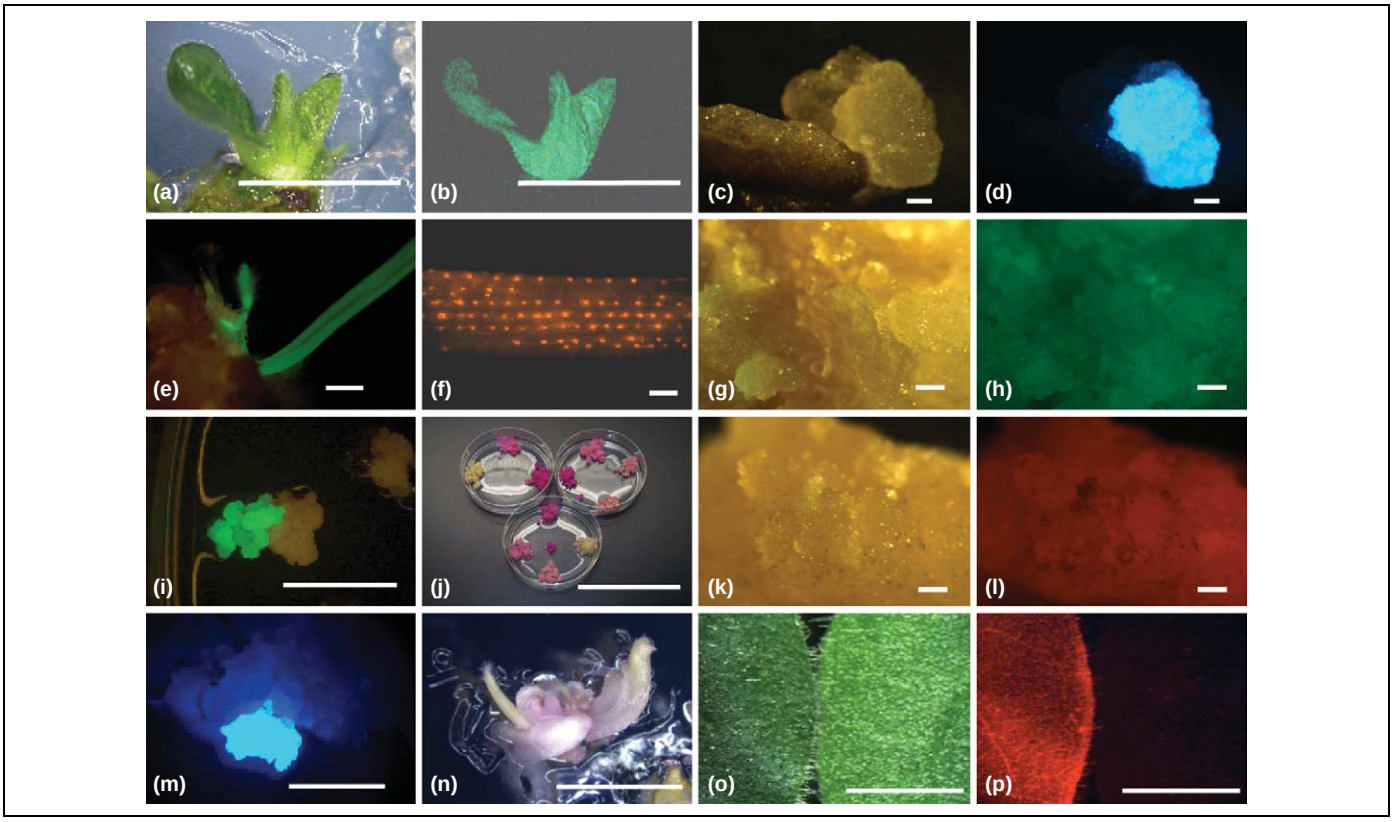

Figure 4. . Expression of the various fluorescent proteins in mannose-, kanamycin- or hygromycin-selected transformants. **(a)** Soybean under white light (*bar*=1 cm). **(b)** Soybean under fluorescent light – ZsGreen (*bar*=1 cm). **(c)** Cotton callus under white light (*bar*=1 cm). **(d)** Cotton callus under fluorescent light – AmCyan (*bar*=1 cm). **(e)** Barley shoot under fluorescent light – AmCyan with green filter (*bar*=2 mm)]. **(f)** Wheat leaf under fluorescent light – DsRed (*bar*=2 mm). **(g)** Cotton callus under white light (*bar*=1 mm). **(h)** Cotton callus under fluorescent light – ZsGreen (*bar*=1 mm). **(i)** Rice callus under fluorescent light – ZsGreen (*bar*=0.5 cm). **(j)** Rice callus under white light – AsRed (*bar*=10 cm)). **(k)** Cotton callus under white light (*bar*=1 mm). **(l)** Cotton callus under fluorescent light – AsRed (*bar*=1 mm). **(m)** Maize callus under fluorescent light – AmCyan (*bar*=0.5 cm). **(n)** Maize callus under white light – AsRed (*bar*=1 cm). **(o)** White light– DsRed transformed tobacco on left, untransformed on right (*bar*=1 cm). **(p)** Fluorescent light – DsRed transformed tobacco on left, untransformed on right (*bar*=1 cm). Figure is reproduced courtesy of Allan Wenck [60].

in aggregation and the general inability to produce heterologous translational fusion proteins. Nevertheless, tetramerization does not exclude the use of Anthozoan FPs for transgenic labeling because several Anthozoan FPs have been altered to form dimers or monomers. In addition, mutants with altered spectral properties have been selected, which should be at least as useful as GFP in transgenic organisms.

Some of the first Anthozoan FPs, reported by Matz *et al.* [56], were transformed into several plant species [60]. Samples of the transgenic plant tissues were examined for fluorescence, and Wenck *et al.* [60] reported that AmCyan, AsRed, DsRed, ZsGreen and ZsYellow all displayed fluorescence (Figure 4). One of the most interesting results was found in rice callus: AmCyan1 transgenic material appeared to be yellow–green and AsRed appeared to be red under white light. This is significant because, unlike GFP, these FPs provided passive altering of tissues under room light. Furthermore, DsRed1 in transgenic, but otherwise green, tissue appeared reddish under white light [60]. It is also of interest that these FPs had relatively low extinction coefficients and quantum yields, compared with more recent derivatives (Table 1). Thus, these results, which were much more impressive

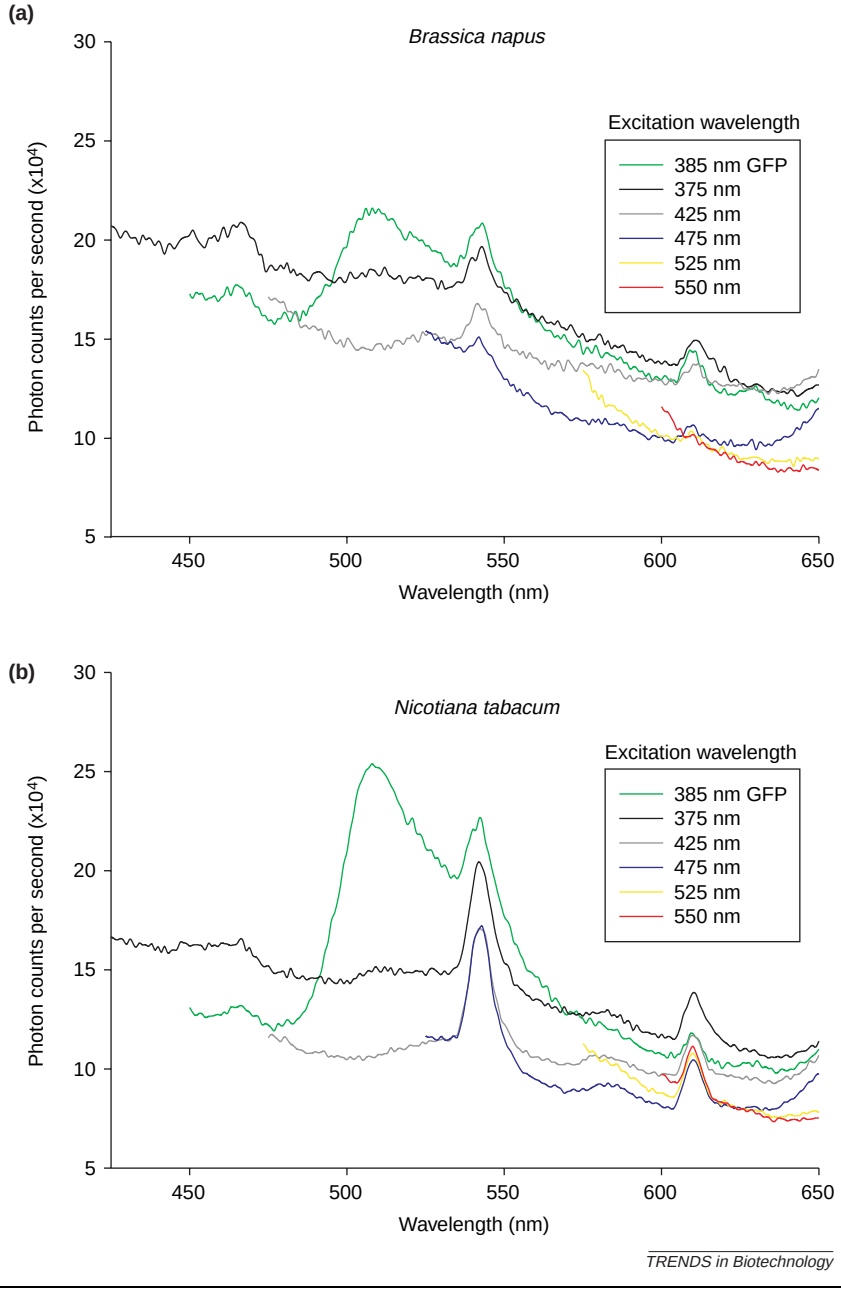

Figure 5. Standardized scanning fluorescence measurements of GFP- and non-transgenic plants. One transgenic event from each species was used and compared with its non-transgenic parental variety. **(a)** *Brassica napus* and **(b)** *Nicotiana tabacum* plants grown in a growth chamber. A plant leaf from each transgenic plant that expressed **(a)** low and **(b)** moderate amounts of GFP, along with leaves from their non-transgenic counterparts, were assayed for fluorescence under different excitation wavelengths using published methods [54]. Background fluorescence varies among species and growing conditions but similar patterns are observed. In general, higher excitation wavelengths produce lower background fluorescence. These data also show spectral 'windows of opportunity' for ideal engineered fluorescence signatures in plants.

than those using *Aequorea* GFP, seem to put us at the beginning rather than the end of the rainbow, and the FP color palette seems ever-expanding.

DsRed is the Anthozoan FP that has garnered the most attention in transgenic organisms, which is why much effort has been made to create a monomeric form (e.g. mRFP1) [61] and shift its spectral properties. Shaner *et al.* [62] and Wang *et al.* [63] have been successful in increasing the brightness of mRFP1 and altering its emission spectrum through mutagenesis. DsRed, originally emitting at 583 nm, and mRFP1, at 607 nm, now have variants that range from yellow (537 nm) to far red (649 nm) emission. The red FPs are of special interest in transgenic plants because there is little natural reflectance in green tissue in the red wavelengths, thus various red FPs should be easier to visualize compared with GFP.

Customized colorization and detection

It is no longer truly novel to clone, mutagenize or express a new FP-encoding gene in transgenic organisms. For the myriad biological applications, it is desirable to have the entire range of FP colors but, beyond that, the specific needs of a particular application might drive a mutagenesis criterion to obtain the desired spectral properties. For example, a pulsed Nd:YAG (neodymium-doped yttrium aluminum garnet) laser with a tripled frequency to 355 nm can be used to excite the mGFP5 variant in plants from a distance [55] but 355 nm is far from the optimal excitation wavelength of 390 nm. Excitation at 355 nm introduces about a ten-times lower signal-to-noise ratio for GFP fluorescence than excitation in the range of 385–390 nm [55]; furthermore, there is considerably more general plant background autofluorescence at lower excitation wavelengths (Figure 5). An Nd:YAG laser with doubled frequency (532 nm) will have twice the power and yield much less background autofluorescence in plants (Figure 5). In addition, the specific FP emission should fall, optimally, in the valleys of plant autofluorescence. GFP meets this criterion (Figure 5) but there is a substantial autofluorescence peak at 540 nm that seems to be correlated with general plant stress (Stewart *et al.*, unpublished data). Chlorophyll fluorescence is another spectral obstacle, with a minor peak at 610 nm (Figure 5) and a major peak at 680 nm (data not shown). We look forward to the day when a monomerized FP with a 532 nm excitation wavelength and an emission wavelength in the range 560–600 or 620–650 nm, together with a high extinction-coefficient and quantum yield, is available for use in transgenic plants. In fact, there is plenty of room in the plant fluorescence spectral landscape for dual labels in green and red fluorescence (and possibly beyond).

Conclusions

FPs have opened the world of *in vivo* labeling and monitoring to biotechnology. Until recently, FP biochemistry and molecular biology research has performed independently from the science and technology of spectroscopy and detection. We now have a rainbow of FPs, many of which are bright and monomerized. The time is now right for increased multidisciplinary collaboration between biochemists, engineers and biotechnologists to tailor proteins and instruments, in tandem, for monitoring applications: time to go with the glow.

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