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Mini Review

The art of reporter proteins in science: past, present and future applications

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Starting with the first publication of *lacZ* gene fusion in 1980, reporter genes have just entered their fourth decade. Initial studies relied on the simple fusion of a promoter or gene with a particular reporter gene of interest. Such constructs were then used to determine the promoter activity under specific conditions or within a given cell or organ. Although this protocol was, and still is, very effective, current research shows a paradigm shift has occurred in the use of reporter systems. With the advent of innovative cloning and synthetic biology techniques and microfluidic/nanodroplet systems, reporter genes and their proteins are now finding themselves used in increasingly intricate and novel applications. For example, researchers have used fluorescent proteins to study biofilm formation and discovered that microchannels develop within the biofilm. Furthermore, there has recently been a "fusion" of art and science; through the construction of genetic circuits and regulatory systems, researchers are using bacteria to "paint" pictures based upon external stimuli. As such, this review will discuss the past and current trends in reporter gene applications as well as some exciting potential applications and models that are being developed based upon these remarkable proteins. [BMB reports 2010; 43(7): 451-460]

INTRODUCTION

Reporter genes along with their proteins are commonly used in many laboratories. For example, whenever a researcher or student performs a blue/white colony assay, they are relying upon the nature of the *lacZ* reporter gene to indicate which colony is carrying a cloned region of DNA. Furthermore, a search for articles containing the keywords "GFP fusion" and "reporter fusion" at NIH's National Center for Biotechnology Information (NCBI) website (1) retrieves 6,400 and 920 articles, respectively, suggesting that these remarkable proteins have far-reaching potential. This review will present just a few of the current uses of reporter genes as well as their application in several scientific fields and disciplines, including their conception and initial application to current research and ground-breaking studies.

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THE PAST

Introduction to reporter genes and proteins

The monitoring of transcriptional regulation via coupling with reporter gene expression has been used extensively to investigate various biological processes (2). Reporter genes, such as β-galactosidase (*lacZ*), firefly luciferase (*luc*), bacterial luciferase (*luxCDABE*) and green fluorescent protein (*gfp*), have been widely used as a rapid and convenient means of detecting and quantifying molecular and genetic events. The central concept of a reporter gene is simple: it is a gene that is attached to a regulatory sequence, which when introduced into a biological system, provides an easily measurable signal output upon modulation of its expression (2). As such, the literature abounds with reports featuring mostly three reporter systems and proteins-β-galactosidase, luciferases and fluorescent proteins - all of which will be the main focus of this review.

β-galactosidase (*lacZ*)

Although earlier studies focused on *lac* operon fusions (3), the first study to report *lacZ* gene fusion was in 1980 (4). Shortly after, Lis *et al*. (5) published an article in which the *lacZ* gene from *Escherichia coli* was fused with the *hsp70* gene from *Drosophila* in order to study the heat shock response within this organism. Within *E. coli*, the β-galactosidase protein (β-gal) is responsible for the hydrolysis of lactose to galactose and glucose. This enzyme is slightly promiscuous, however, and can hydrolyze substrates other than lactose, including the chromogens o-nitrophenol β-D-galactopyranoside (ONPG), 5-bromo-4-chloro-3-indolyl-β-D-galactopyranoside (X-gal) and 3,4-cyclohexenoesculetin-β-D-galactopyranoside (S-gal) (6), all of which produce yellow, blue and black products/precipitates, respectively. Other protocols and substrates that sig-

nificantly enhance the sensitivity of the β-gal assay are also available (7-9). Two major limitations of this reporter are the purchase of costly and potentially toxic chemicals for the assay and lysis of cells, both of which prevent its application in on-line and real-time detection systems.

Luciferases

Luciferases are proteins that generate biologically-based luminescence and are generally categorized as eukaryotic or bacterial, depending on their origin. Firefly luciferase (*luc*), cloned in 1985 (10), is one of the most commonly used reporters genes (11) for several reasons, including its high sensitivity, the tight coupling of the Luc protein concentration with luminescence output, and the fact that the protein requires no post-translational modifications and is active immediately after translation (10). It should come as no surprise, therefore, that the *luc* gene has been used to study gene expression patterns in numerous organisms (12-15). Similar to *lacZ* reporter systems, however, *luc* gene-fusion systems require the addition of a costly substrate, *i.e.*, luciferin, to monitor and measure reporter activity.

In contrast, the substrate for bacterial luciferase (LuxAB) is produced by the LuxC, D and E proteins, all of which are encoded in the *lux* operon, *luxCDABE*. This feature has made LuxAB a prime reporter system for the on-line and continuous monitoring of gene expression patterns, particularly within bacterial biosensors (16, 17). Furthermore, the activity of these proteins is integrally connected with ATP production and the electron transport chain, enabling researchers to study the real-time effects of both biotic and abiotic effectors. An additional benefit is the combination of the LuxAB proteins with other reporters, as was reported in two recent publications where bacterial luciferase was combined with the green fluorescent protein (GFP) to study the transcriptional control of two independent genes simultaneously (18, 19). A major limitation of bacterial luciferase, however, is that it cannot be used within eukaryotic systems. To overcome this, one research group recently constructed a vector through which the luciferase proteins can be expressed within yeast (20).

Fluorescent proteins

The most well-known fluorescent protein is GFP. GFP protein was originally isolated from the jelly-fish *Aequoria victoria* (21, 22), but its gene was not cloned until two decades later (23). That monumental study showed that GFP was functional in both prokaryotic (*E. coli*) and eukaryotic hosts (*Caenorhabditis elegans*), a finding that eventually led to the 2008 Nobel Prize in Chemistry. Several studies over the next couple of years demonstrated that the *gfp* gene could be used to study transcriptional activities within a wide range of hosts, including plants (24) and zebrafish (25).

The benefits of using fluorescent proteins as reporters are numerous, including broad-host applicability and absence of cell lysis or substrate addition. Furthermore, over 10 different color variants are commercially available, including blue, yellow and red varieties (26). However, there are some drawbacks to using fluorescent proteins. As opposed to luciferase proteins, which are not functional within a non-viable host, fluorescent proteins are generally stable proteins and continue to emit fluorescence long after the host has died. Furthermore, the fluorophore within wild-type GFP protein needs to be generated through a natural process, which can take up to 2 hours, although mutants with assembly times as low as 5.3 minutes have been reported (27, 28).

THE PRESENT

Reporter genes and synthetic biology

Early application of reporter genes focused primarily on the analysis of *cis*-acting genetic elements in the regulatory regions of genes, such as promoters and enhancers (29, 30). Despite their simplicity, these reporter gene fusions were, and still are, pivotal for the study of gene expression patterns and the development of biosensors (16, 17), which rely on the expression of "stress-inducible" genes. Recently, advances in biotechnology have expanded the use of reporter systems to more advanced applications, including monitoring the performance of synthetic genetic circuits (31-33), genome-wide expression arrays (34), and *in vivo* protein localization and trafficking (35, 36).

Synthetic biology is an emerging field with the aim of designing and constructing complex artificial biological systems using standard biological parts in a fashion similar to that of an engineer designing an electronic or mechanical system. This field has the potential to make significant contributions to biology through the process of building and testing new systems and understanding their dynamic behaviors, such as how they respond to a variety of cellular needs and how life overcame all the complexities found within nature and biology. Unlike electronic systems, biological circuitry is often difficult or impossible to characterize since its components and architecture of its complex networks are not well described (37). Therefore, it is very important to quantitatively characterize biological components and their activities in order to develop mathematical algorithms that will help engineers to more effectively model and simulate biological behavior prior to actual physical testing (38, 39). Thus, the use of fusions between genetic regulatory elements and reporter genes greatly simplifies the screening and quantification of the dynamic behavior of standard biological components (38, 40).

A major problem with most reporter systems is the relatively long half-life of the reporter proteins, which prevents real-time monitoring of dynamic changes during gene regulation. Furthermore, when reporter proteins are used as components in synthetic gene circuits, their long-term stability may significantly degrade circuit performance. For example, the half-life of wild-type GFP is longer than 24 hours (41), and this will extend over many bacterial generations, complicating studies of gene regulation in which *gfp* expression decreases over time

or upon various input. To solve the problem of long half-life proteins, Andersen *et al*. (41) constructed unstable *gfp* variants with half-lives of approximately 40, 60 or 110 min by adding a degradation tag to the C-terminal end of wild-type GFP. Likewise, short half-life variants of LuxA and LuxB protein from *Photorhabdus luminescens* were constructed in *Escherichia coli* by inclusion of an 11-amino acid C-terminal tag that is recognized by endogenous tail-specific proteases (42).

Another major problem is background expression, which refers to preexisting reporter molecules present within the cells before the actual experiment that cause a low signal-to-noise ratio. Balancing a low background with a broad dynamic range is important, especially when studying synthetic gene circuits in which dynamic signal performance is critical (42). Therefore, the use of a destabilized reporter system is preferred since it decreases the background signal, as the vast majority of pre-existing reporter molecules (the background) are degraded very rapidly. In some cases, however, it is difficult for reporter molecules inside the cell to remain active long enough for them to be measured, which leads to an extremely limited dynamic range and low signal intensity.

Current applications of reporter genes

Whereas many past studies that featured reporter proteins were ground-breaking and important to the pursuit of scientific knowledge, nearly all used freely suspended cultures of the particular bacteria being studied. It is increasingly clear to the scientific community that this is in contrast to that which occurs in nature, wherein bacteria are often found within biofilms or microcolonies. Furthermore, as mentioned above, each of the reporter proteins has benefits and limitations when applied to simple transcriptional fusions. To overcome these limitations, various genetic tools have been applied, such as the introduction of mutations that reduce protein stability (41, 42). For a complementary approach, current researchers are currently seeking applications in which these limitations are minimized or actually become beneficial to some aspect of the study. Therefore, reporter genes and their proteins are now being used extensively to study bacterial populations within smaller scale systems such as biofilms, micro-patterned colonies and microfluidic systems.

Biofilm studies

Biofilms are an important bacterial construct found throughout nature. They are biologically and medically important since they increase bacterial resistance to antibiotics as well as the immune response. Furthermore, biofilms are persistent and once formed are very difficult to remove. For these reasons, many groups are studying biofilms to determine how to block their formation (43-46). Often, these studies use fluorescent proteins due partly to the stability and diversity of fluorescent reporters available.

Usage of a fluorescent reporter protein provides striking results when studying biofilm structures and architectures, especially when coupled with confocal scanning laser microscopy (CSLM). Several recent publications by Thomas Wood's lab at Texas A&M include images (and videos on their website) from their CSLM analyses that clearly show the 3-D structures and channels present within the biofilms formed by *E. coli* and select mutants (47-49). Other studies are expanding on this concept and are exploiting the differences between the excitation and emission spectra of different fluorescent proteins, which allows them to study the activity of more than one bacterial species within a mixed biofilm (50-52).

Micropatterning and microfluidics

As mentioned above, bacteria within nature are commonly found within microcolonies. However, the standard cell culture tools for centuries have been the dish and the flask. Although still very useful, these tools typically only provide homogeneous environments under static or constantly stirred conditions; they do not resemble the conditions found in nature. In contrast to the largely uniform and non-dynamic nature of these conventional culture tools, the rapid progress in reporter system technology now routinely enables real-time spatio-temporal readout of cellular responses at sub-micrometer length scales with sub-second timescale resolution. To take full advantage of the information obtainable using these advanced readouts, investigators are increasingly turning to new culture tools in order to increase spatio-temporal control over cell cultures within various microenvironments. This section will provide just a few recent examples of microtechnologies useful for enhancing spatio-temporal control of the cell culture microenvironment. They are categorized roughly as microtools for spatial control and as microtools for temporal control.

Spatial patterning of bacterial colonies

Spatially patterned cultures of bacteria on agar have been utilized for decades, although typically not in microscale. Perhaps one of the oldest and still useful techniques for spatially patterned cultures includes the replica plating technique developed in the 1950s by Lederberg (53). Here, velvet pads or another transfer material is used to pick up parts of bacterial colonies from one plate and transfer them to another plate. Typically, tens up to hundreds of colonies are transferred from plate to plate with maintenance of relative position of each colony (Fig. 1A left). Although not completely confining, the semi-solid nature of agar is sufficient to retain segregation of colonies at sub-centimeter resolution. Traditionally, replica plating is used to isolate or find colonies that are still viable when transferred to plates containing different selective agar media. With the ability to genetically engineer bacteria that express reporter proteins in response to different biochemical stimuli, one can reverse this concept. In this manner, multiple colonies composed of different bioluminescent bacteria can be arrayed in defined positions within the agar media and then exposed to water samples containing unknown biochemical

analytes (54, 55). By reading and monitoring the bioluminescence pattern, one can deduce what the unknown analyte is or what effect has on the bacterial cells.

Although useful, the spatial resolution obtained from a patterned culture on or in agar is limited due to bacteria motility, dispersion through the soft media, and the lack of high resolution bacteria plating tools. Therefore, there is a need for higher resolution, higher density bacteria culture techniques since finding bacterial strains with ideal genetic traits can easily require the screening of millions of colonies. Biosensor applications using bacteria would also benefit from the ability to array the required reporter colonies in as small a footprint as possible. Recently, microtechnological tools, custom surface chemistries, and bacterial tethering have enabled the higher resolution patterning of bacteria on cellulose ester membranes (56), nanoporous aluminum oxides (57), and silicon surfaces (58) (Fig. 1A right). Consequently, the size of microbial colonies that can be patterned has been decreased from the sub-centimeter to sub-100 micrometer scale with increased precision of positioning. This two order of magnitude decrease in linear colony dimensions allows for a four order of magnitude increase in the number of colonies that can be cultured per unit area of culture substrate. Due to this advance, one can theoretically analyze 1,000,000 colonies in the same area in which one could previously only screen 100 colonies using conventional methods. Other notable methods for the micropatterning of bacteria include the use of stencils to generate arrays of biofilms (59). We have also developed the use of aqueous two phase systems (60, 61) to spatially micropattern bacteria suspensions and stably maintain cell localization for extended periods without dispersion (unpublished results).

Perhaps the ultimate demonstration of high resolution arraying and sensing of reporter bacteria has been reported by Kuang *et al.* (62). The investigators constructed a high-density living bacterial cell biosensor array by inserting reporter protein-expressing bacteria into microwells formed on one end of an imaging fiber bundle. The size of each microwell allowed for only one cell to occupy each well. Since each microwell has an optical fiber and sensor coupled to it, thousands of single cell responses could be recorded simultaneously with high spatio-temporal resolution.

Temporal patterning of biochemical stimuli using microfluidics

With the development of reporter proteins, it is now possible to visualize in real-time how cells respond to dynamic biochemical conditions. Therefore, it has become increasingly important to find convenient and reliable ways to provide temporally patterned biochemical stimulations in a user-friendly format. Typical dish cultures, which are static, typically can only receive a new chemical stimulus as a bolus in which the chemical concentration is increased suddenly once and remains constant (Fig. 1B left). For some applications, however, it is useful to periodically increase and decrease the chemical concentration (e.g. Fig. 1B right). Microfluidic systems can again be helpful in this regard by enabling efficient exchange of solutions that cells are exposed to (63). For example, periodic biochemical exposure can mimic rhythms in the body

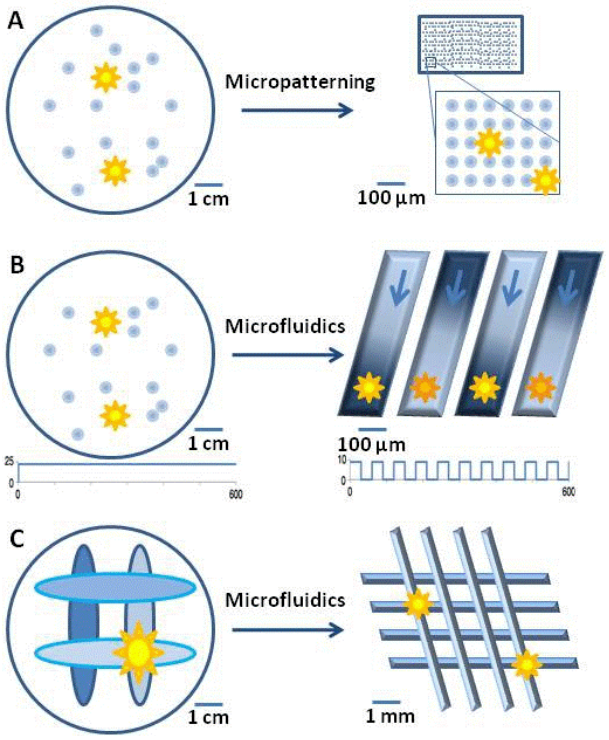

Fig. 1. (A) Spatial patterning of bacteria. (left) Replica plating has been the standard for decades. Colonies of interest can be rapidly identified when engineered with reporter functionality. Small circles represent bacterial colonies, and "sun"-shaped spots represent cells with positive signals from reporter proteins. (right) Microtechnologies can be used to pattern microcolonies of cells several orders of magnitude smaller in diameter and cell number, which increases the throughput and efficiency of screening and for reporter protein-based sensing. (B) (left) In conventional cell cultures, reagents are added only once as a bolus, instantly increasing the reagent concentration. The bottom graph depicts how cells in such cultures are exposed to such a step increase in reagent concentration. (right) Microfluidic cultures can be designed to allow rapid exchange of reagents by switching the reagent solutions that are allowed to flow through the channels. The schematic depicts a time sequence (4 steps out of many) in which cells are exposed periodically to a reagent that increases the reporter protein signal and then to a reagent that decreases the reporter protein signal. By changing the reagent exposure frequency and analyzing the resulting changes in reporter protein signal frequency and amplitude, one can obtain insights into the intracellular signaling pathways architecture. The bottom graph depicts how cells in such microfluidic cultures can be exposed to periodic increases and decreases in reagent concentration. (C) Spatial patterning of both bacteria and reagents. (left) Cross-streak assays can be performed on conventional agar plates at the macroscopic scale to identify the combination of cells and reagents that gives a positive signal from reporter proteins. (right) Use of criss-crossing microfluidic channels can restrict cell-reagent interactions to channel intersections by greatly increasing the density with which cell-reagent interactions can be screened.

(64, 65) or simulate cyclic natural events such as water waves in intertidal zones near the beach (66).

Several recent papers analyzing the response of yeast to temporally patterned chemical stimulations provide excellent examples of how real-time imaging of reporter proteins combined with microfluidics and computer simulation of intracellular signaling pathways can provide unique mechanistic insights (67-69). In these studies, cells are exposed to a different frequency of biochemical stimulation and the cellular responses are observed. The observed experimental cellular responses are then compared with mathematical models of the relevant pathway based on existing information. Discrepancies between the experiment and the model in terms of how the reporter protein signals manifested themselves, allowing identification of missing components and interactions in the signaling pathway. The key point here is that dynamic variation in the biochemical environment provided a type of new information previously unobtainable using conventional static or uniformly mixed cultures in which the chemical microenvironment is largely unchanging or changed only by an instantaneous increase in the concentration of an added reagent.

Spatial and temporal control of both bacteria colonies and biochemical stimuli

For some applications, one may need to test the exposure of different bacteria colonies to various biochemical stimuli. Here, it becomes necessary to not only spatially pattern the location of bacterial colonies but also to localize the biochemical stimuli to select groups or parts of bacterial colonies. Macroscale assays of this type have again been developed using agar plates. A representative assay is the "cross-streak assay" (70) (Fig. 1C left) in which biochemical reagent solutions of interest are allowed to drip across a vertically held plate in lanes, after which the resulting solution streaks are soaked into the agar. Next, suspensions of different bacterial strains are allowed to drip across the same plate, but only in a direction perpendicular to the reagent streaks. Bioluminescence or the reporter protein readout is observed only when the appropriate reagent stimulation is combined with the suitable bacterial colony. The advantage of this type of technique is that it allows simultaneous testing of all combinations of reagents using any combination of bacterial strains. Due to diffusion and dispersion of both the reagents as well as the bacteria, however, the resolution of this technology is low and on the centimeter scale.

Performing this type of cross-combination assay with higher resolution requires the direction and locations of the reagent and bacterial dispersion to be limited. For example, this can be achieved using a multi-layered network of intersecting microfluidic channels (Fig. 1C right). Tani *et al*. (71) utilized an optically transparent, biocompatible silicone rubber called poly (dimethylsiloxane) (PDMS) to generate parallel arrays of microchannels. Two layers of these channels were stacked perpendicular to each other with a third layer comprised of through-holes that connect the two channel layers sandwiched in between. The channel array of one PDMS layer was filled with different luciferase-expressing sensor strains while the channel array of the other PDMS layer was filled with different concentrations of biochemical stimuli, luciferin, and ATP. Similar to the macroscopic cross-streak assays, the bioluminescence signal is observed only when the appropriate reagent stimulation is combined with the suitable sensor bacteria strain. The major difference is that with diffusion being spatially restricted by the PDMS channels and silicon layer, the resolution with which different lanes of bacterial colonies and biochemical stimuli can be applied is on the millimeter rather than centimeter scale. This allowed the investigators to test 25 combinations of conditions in an area in which only one combination can be tested using conventional agar-based cross-streak assay.

Reporter gene system for quantitative biology

One of the most compelling challenges in current systems biology is to understand how the functional repertoire of cells is connected to the system-level properties of cellular information processing networks. The suggestion that cellular networks can be decomposed into functional modules, like their electronic counterparts, has led the way for large-scale studies of their organizing principles. A particularly important class of functional modules is the genetic switch in which genes are turned on or off in response to environmental cues. The genetic switch is also an important "circuit" of which fluorescent reporter gene systems are indispensible components. Guet *et al*. (72) built genetic networks composed of genes encoding the transcriptional regulators LacI, TetR, and lambda CI, as well as the corresponding promoters in a combinatorial manner. This lends a basis for diverse information processing capability through changes in network connectivity.

As appropriately pointed out by the authors, one interesting observation was that the connectivity of a network does not uniquely determine its behavior. Even in such a general model as the Boolean regulation model, which allows not only any possible combination of parameters but also any functional dependency within the monotonicity constraint, the reporter system shows irreconcilable differences between the model and the real experiments (Fig. 2). Kim and Tidor (73) brought up this issue and proposed, by relaxing the modeling assumptions one by one, that the discrepancy is linked to the C-terminal ssrA-tags that were designed to degrade the regulatory proteins by the Clp system (74). Despite the broad use of C-terminal ssrA-tags for the control of protein turnover in synthetic genetic circuits, the exact reaction kinetics of ssrA-tags have yet to be characterized.

In addition, many of the transcription factors form homo-oligomers whose half-lives may well be different from those of monomers (75). Hence, it is important to find if the ssrA-tag enhances the degradation of monomers and oligomers equally or if it preferentially enhances one of the forms. Since the cyto-

solic concentrations of ClpX and ClpP are low, the regulatory proteins in the circuit may compete for binding to ClpX with limited copy number. That is, one species of protein becoming dominant may lead to slow degradation of other proteins by outcompeting them for binding to ClpX. This saturation effect implies that the degradation of one protein species depends on the concentrations of the others, which eventually could alter the characteristics of the combined logic gates.

The ultimate goal of synthetic biology is to restore determinism in controlling the behavior of an engineered cell. A predominant source of deviation from such deterministic behavior is stochastic fluctuation, especially in the case of low molecular copy numbers (76). A potentially effective way to evade low copy numbers and/or the saturation of the Clp system may be the overexpression of Clp system components (74) or protein oligomerization (77). In addition to the efforts made in the discovery of novel reporter gene systems, parallel efforts for the quantitative characterization of their components, particularly those of protein degradation systems, make the reporter gene system an ideal tool for monitoring the dynamics of gene expression.

THE FUTURE

Future of reporter proteins and synthetic biology

As mentioned above, synthetic biology is an immature but rapidly developing area involved in the research of novel, engineered, purpose-built biological components, devices and organisms and is described by Craig Venter as "moving from reading the genetic code to writing it" (78). The ultimate goal of synthetic biology is to construct biological systems that can solve current issues in human health, energy, the environment, and other areas that cannot be solved by naturally existing biological systems. Using pre-synthetic biological technologies, Keasling and co-workers engineered bakery yeast into a drug factory by introducing *de novo* metabolic pathways that produce a naturally occurring, effective anti-malaria drug extracted from plants, but they were able to decrease the cost and improve efficiency (79).

In 2009, ExxonMobil announced a $600 million collaboration with Synthetic Genomics founded by Craig Venter for the research and development of next generation biofuels. In 2010, Craig Venter's group (80) succeeded in creating a synthetic bacterial genome and using it to boot a new life form. The synthetic genome described in his paper contains only

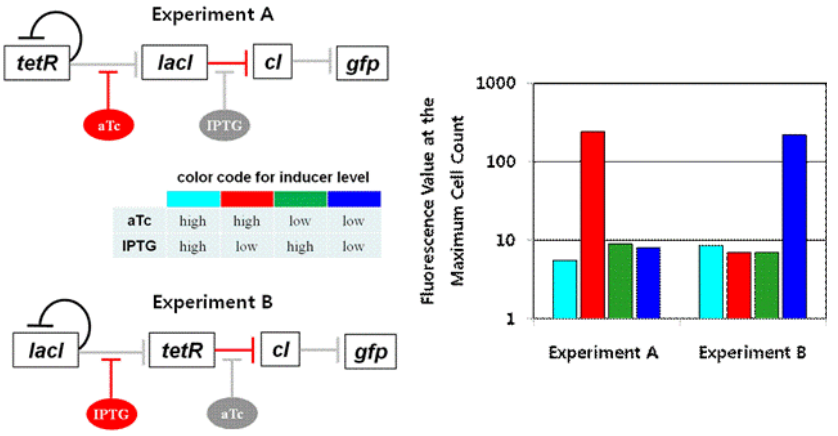

Fig. 2. Topology of combinatorial gene circuits (left) and their *gfp* expression levels (right) as studied by Guet *et al*. (72). The two circuits have the same topology as *lacI* and *tetR* interchanged (Thus, the roles of aTc and IPTG in the upper circuit are the same as those of IPTG and aTc in the lower circuit, respectively). The upper circuit shows its highest fluorescence at a high level of aTc and low level of IPTG, which corresponds with naïve Boolean logic. However, the lower circuit does not show its highest *gfp* expression at a low level of aTc and high level of IPTG. The figure on the right is modified from the original shown in Ref. (72).

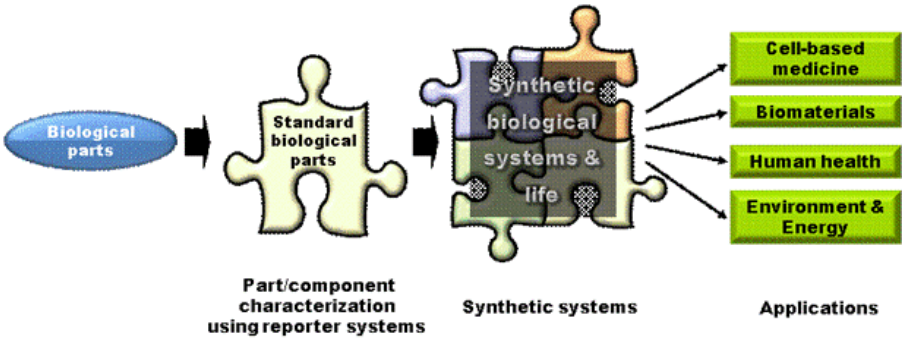

Fig. 3. Illustration showing the process of characterizing and combining different biological parts and components by means of reporter systems, with the final aim of applying these synthetic systems within a broad range of applications and scientific disciplines.

limited modifications compared to the naturally occurring genome of *Mycoplasma mycoides*. With the current progress in genome design, it will soon be possible to make real synthetic microbes that efficiently produce food, drugs, chemicals or energy (81).

However, many existing parts have not been quantitatively characterized. As a result, it is difficult to accurately predict the behavior of a biological system composed of such parts. Thus, standard biological parts should be well-characterized by testing and fine-tuning before more efficient engineering of increasingly complex and novel biological systems is performed (Fig. 3). The existing reporter systems used to characterize biological parts have both strengths and weaknesses. Drew Endy (82) suggested four features common to next generation reporters in synthetic biology: "First, reporters should function in chassis commonly used by synthetic biologists. Second, reporters should exhibit wide dynamic range, meaning their activity should be quantifiable when they are either weakly or strongly expressed. Third, the dynamic range of reporters should be characterized across a range of commonly used expression levels, and should be correlated with measurement reference standards if available. Fourth, reporters should enable analytical flexibility, ranging from high-throughput screening to quantitative single cell assays" (82). Bifunctional reporters that combine both enzymatic and fluorescence activities within a single protein have been suggested by his group as useful tools for the characterization of biological components in terms of their wider dynamic range and analytical flexibility.

Reporter genes and …art?

"After a certain high level of technical skill is achieved, science and art tend to coalesce in esthetics, plasticity, and form. The greatest scientists are always artists as well."- Albert Einstein

Nowhere in the scientific literature is this quotation probably more apparent than in the use of reporter genes. Several web-sites highlight some of the recent art made using reporter proteins, including LacZ (83) and fluorescent proteins (84), as well as some art by Erich Schopf that will be displayed at the Natural History Museum in Vienna (85). There is also a website devoted solely to art made using bacteria or other microbes (86), with one even showing art prepared using microfluidics (87). Although these websites may display some creativity and interesting pictures, many of the pieces do not embody Einstein's statement fully and are simply a form of art, not science.

However, some current publications and research are in the vein of Einstein's thought. For example, scientists have recently "taught" bacteria to photographically develop an image based upon exposure to light (88). To achieve this, they constructed a chimeric protein, consisting of the CphI light receptor from the blue-green algae *Synechocytis* and the EnvZ histidine kinase region from *E. coli*, which regulates the expression of the *ompC::lacZ* fusion gene. Accordingly, when these bacteria are exposed to light, the expression of the LacZ protein is turned off, and vice versa. Using media containing S-gal, the bacteria develop an image based upon the light pattern to which they are exposed. Some examples of the images can be found at their website (89). A subsequent study by the same authors furthered the capabilities of the so-called "photographic" bacteria using synthetic biology approaches (39). In their study, they developed a genetic circuit within *E. coli* which responds only when near the edge of light, *i.e.*, the interface between the exposed and unexposed regions. Using this synthetic gene circuit, only the bacteria present at this interface express LacZ and, subsequently, produce a black precipitate from the S-gal substrate.

A more practical scientific, and potentially medical, application of this technology was also recently published in which a light-gated protein-protein interaction allowed the researchers to control the translocation of reporter proteins in the cell as well as to reshape mammalian cell morphologies through a controlled actin reaction (90). To monitor these intracellular processes, the authors used target proteins fused to yellow fluorescent protein (YFP) or mCherry, another commercially available fluorescent protein (26).

CONCLUSIONS

The history of reporter genes and proteins began with the advent of molecular genetics and has grown progressively diverse and colorful ever since. As reviewed in this article, reporter genes are used within a wide-range of scientific fields and disciplines, including in studies of synthetic biology, biofilms, microfluidics, and modeling. These topics, however, are just the tip of the iceberg, and this review is far from comprehensive on this topic. However, as we enter the fourth decade of using transcriptional fusions to reporter genes, research into their potential applications is clearly still advancing, with some of the current applications being in the spirit of Einstein's quote as they are both scientifically noteworthy while also appealing to our aesthetic and artistic natures. To conclude, although advances in science and technology are not always clear, one can be certain that reporter proteins will continue to play a role in biological studies for many years to come.

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