GFP on Land and Sea

Santa Clara County Biotechnology Education Partnership

As the early fog lifts off Friday Harbor on an island near Victoria, British Columbia, two scientists were seen scooping buckets full of small luminescent bags from the ocean. Looking into these containers you could see that these bags were actually jellyfish with a green fluorescence emitting from the bottom edge of their transparent umbrella shaped body. The two scientists, Drs. Frank Johnson and Osamu Shimomura, were collecting the jellyfish *Aequorea* because they were interested in isolating the source of the bioluminescence. They cut off the light-containing region from 20-30 jellyfish and then pushed this through rayon gauze. The liquid luminescent "squeezate" was collected and used for their further experiments. Little did they know that almost 40 years later this "bioluminescence substance" would become an essential tool for scientists throughout the world!

During the 1960s many scientists, including Shimomura, were studying the fluorescent properties of the purified green protein, named Green Fluorescent Protein or GFP, isolated from *Aequorea*. Their main interest was to learn more about bioluminescence in a variety of animals. In the 1980s, molecular biologists were using genes from a variety of organisms in their cloning methods. They realized that GFP would be an excellent molecular tool to follow the genes they were manipulating. By the mid 1990s the structure of GFP was elucidated by these molecular biologists. They found that GFP was composed of 238 amino acids, and forms an 11-stranded beta-can structure. This "can or barrel" structure is unique to GFP. In the center of this compact barrel is the region of the protein that is responsible for the chemical reaction that results in fluorescence, called the fluorophore.

What makes GFP so remarkable and such a powerful tool for scientists? First, GFP is a "loner", it does not require anything from the jellyfish to function; no other coenzymes, cofactors or products from the jellyfish. GFP only needs UV light at a wavelength of 395nm. GFP is also extremely stable in many experimental conditions and therefore can be used in a multitude of research applications. This protein is also very small, and is easily shuttled into and out of cells and cellular compartments, such as the nucleus, various organelles, membranes, and the cytoplasm.

In order for GFP to be used by various scientists for a variety of purposes, it needs to be easily obtainable in large quantities. What better way to do this than to clone GFP? In 1992, Prasher and his colleagues were the first to insert the DNA of GFP into a vector, for the purpose of cloning. The genie "GFP" was now out of the bottle, with researchers exploring the use of GFP in their investigations. These recent experiments have shown that GFP can be expressed in cell culture (*in vitro*) and in living organisms (*in vivo*), from bacteria to humans.

Scientists are not doing all these experiments only to study GFP expression. They also use GFP to study specific genes they are researching. It is almost like tricking the cell. When GFP is tagged onto another gene it is being used to "report" on the expression or activity of the gene of interest, thus it is called a "reporter gene". Many reporter gene systems have been created and are commonly used as biological tools. GFP is an optimal reporter gene because it is small, requires only UV light to be observed, and is very stable.

GFP is naturally expressed at moderate levels in the jellyfish. Molecular biologists had to manipulate different regions of the GFP DNA to obtain high expression in hosts that range in diversity from bacteria to humans. To obtain high levels of expression in some cases, the promoter region was altered. Changes were also made in the coding region of GFP. This resulted in a more stable protein, a higher yield of product, and a "greener" cell. Some of the most exciting changes to GFP are to the fluorophore region. Specific changes in this region have resulted in new colors being expressed, blue, yellow and cyan. Cells can now be labeled with a variety of fluorescent proteins allowing researchers to look at cascading events at the cellular level (i.e. protein-protein interactions, monitoring gene expression simultaneously from two different promoters, or analyzing mixed cell populations).

When "green fluorescent protein" is written in a search using just the journal *Science*, over 3,000 references are listed! The GFP reporter system is being used to study biological processes such as cell division, angiogenesis (blood vessel formation), apoptosis (programmed cell death), tumor formation, drug delivery systems, cancer therapies, and much more. GFP has not been limited to the cellular level of study. There exists a GFP bunny that glows fluorescent green when exposed to UV light and a rhesus monkey that contains the GFP DNA inserted into it's own genome. Before long you may be drinking green fluorescent beer on St. Patrick's Day.

The Arabinose Operon and the GFP gene

Our bodies contain thousands of different proteins which perform many different jobs. Digestive enzymes are proteins; some of the hormone signals that run through our bodies and the antibodies protecting us from disease are proteins. The information for assembling a protein is carried in our DNA. The section of DNA which contains the code for making a protein is called a gene. There are over 30,000–100,000 genes in the human genome. Each gene codes for a unique protein: one gene, one protein. The gene that codes for a digestive enzyme in your mouth is different from one that codes for an antibody or the pigment that colors your eyes.

Organisms regulate expression of their genes and ultimately the amounts and kinds of proteins present within their cells for a myriad of reasons, including developmental changes, cellular specialization, and adaptation to the environment. Gene regulation not only allows for adaptation to differing conditions, but also prevents wasteful overproduction of unneeded proteins which would put the organism at a competitive disadvantage. The genes involved in the transport and breakdown (catabolism) of food are good examples of highly regulated genes. For example, the sugar arabinose is both a source of energy and a source of carbon. *E. coli* bacteria produce three enzymes (proteins) needed to digest arabinose as a food source. The genes which code for these enzymes are not expressed when arabinose is absent, but they are expressed when arabinose is present in their environment. How is this so?

Regulation of the expression of proteins often occurs at the level of transcription from DNA into RNA. This regulation takes place at a very specific location on the DNA template, called a promoter, where RNA polymerase sits down on the DNA and begins transcription of the gene. In bacteria, groups of related genes are often clustered together and transcribed into RNA from one promoter. These clusters of genes controlled by a single promoter are called operons.

The three genes (*araB, araA* and *araD*) that code for three digestive enzymes involved in the breakdown of arabinose are clustered together in what is known as the arabinose operon.These three proteins are dependent on initiation of transcription from a single promoter, P_{BAD}. Transcription of these three genes requires the simultaneous presence of the DNA template (promoter and operon), RNA polymerase, a DNA binding protein called *araC* and arabinose. *araC* binds to the DNA at the binding site for the RNA polymerase (the beginning of the arabinose operon). When arabinose is present in the environment, bacteria take it up. Once inside, the arabinose interacts directly with *araC* which is bound to the DNA. The interaction causes *araC* to change its shape which in turn promotes (actually helps) the binding of RNA polymerase and the three genes *araB*, *A* and *D*, are transcribed. Three enzymes are produced, they break down arabinose, and eventually the arabinose runs out. In the absence of arabinose the *araC* returns to its original shape and transcription is shut off.

The DNA code of the **pGLO** plasmid has been engineered to incorporate aspects of the arabinose operon. Both the promoter (P_{BAD}) and the *araC* gene are present. However, the genes which code for arabinose catabolism, *araB, A* and *D*, have been replaced by the single gene which codes for **GFP**. Therefore, in the presence of arabinose, *araC* protein promotes the binding of RNA polymerase and **GFP** is produced. Cells fluoresce brilliant green as they produce more and more **GFP**. In the absence of arabinose, *araC* no longer facilitates the binding of RNA polymerase and the **GFP** gene is not transcribed. When **GFP** is not made, bacteria colonies will appear to have a wild-type (natural) phenotype—of white colonies with no fluorescence.

This is an excellent example of the central molecular framework of biology in action:

DNA→ RNA→ PROTEIN→ TRAIT

RED FISH, BLUE FISH, GLOW-IN-THE-DARK FISH

Want to add some pizazz to your aquarium? A Taiwanese scientist has devised a way to make otherwise colorless fish glow neon green in the dark. Professor H.J. Tsai at National Taiwan University works this biological magic by injecting a protein extracted from jellyfish into the fertilized eggs of rice fish. He also uses a protein from coral to make fish glow a vibrant reddish pink. Opponents of genetic engineering fear that these creatures could crossbreed with wild species, creating glowing schools of Frankenfish. To keep them from spreading their shining DNA, the distributor, Taikong International, sterilizes them all.

INVENTOR H.J. Tsai
AVAILABILITY Now, $7.50 each (in parts of Europe and Asia but not in the U.S.)
TO LEARN MORE www.azoo.com.tw

Taken from the Student Manual pGLO Transformation, Available at http://biosci.usc.edu/courses/2002-fall/documents/bisc300-lab_bactrans.pdf