Imagine you want to find out how long bacteria can survive in a particular river’s waters. Where would you begin? You could add some bacteria to a sample of the water. Then every so often you could drip some of the water on culture medium, let it incubate, and see if any bacterial colonies develop. But that technique demands lots of waiting, and you might prefer a quicker technique—say, one that allows you to watch the bacteria grow in real time. Laura Leff of the department of biological sciences at Kent State University in Ohio does just that—watches bacteria grow right before her eyes—by labeling them with green fluorescent protein (GFP). After introducing GFP into bacteria through standard genetic engineering techniques, she shines ultraviolet light on a sample of the bacteria, which stand out as green spots under a fluorescence microscope. Leff calls GFP an excellent marker for that purpose. GFP also works very well for biomarking a variety of other living organisms, as well as single cells and even cellular organelles.

The original purpose of GFP revolves around members of the phylum Cnidaria, which includes hydrions, sea anemones, and jellyfish. GFP plays a role in bioluminescence, giving these creatures a greenish glow. In the Pacific jellyfish (Aequorea victoria), for example, green light comes from points along the edge of its bell, in between the tentacles. That light radiates out when a pulse of ionic calcium activates a photoprotein called aequorin, which passes energy to the GFP.

Luckily for molecular biologists, GFP works just as well outside jellyfish. If blue or ultraviolet light is shone on GFP all by itself, it still gives off its green glow. Better still, when GFP is incorporated into an entirely different organism, such as a bacterium, it glows green just the same under blue or ultraviolet light.

GFP is a protein composed of 238 amino acids folded into a shape resembling a soda can with a curlicue running inside of it. Getting the most out of GFP required cloning its gene in order to provide a ready supply of the protein for those who might wish to use it. Douglas C. Prasher, a molecular biologist with the U.S. Department of Agriculture branch at Otis Air National Guard Base in Massachusetts, and his colleagues accomplished that in the early 1990s. Thinking back on that work, he said, “I was very lucky to [find] the gene, because I made several cDNA [complementary DNA] libraries and, as it turned out, the best library that I made, which was something like 1.6 million recombinants, had only one GFP clone in all of that. And I was lucky that the entire coding sequence was present. But you only need one.” Then in 1994, Martin Chalfie, a professor of biological sciences at New York’s Columbia University, and his colleagues first reported that a cDNA for GFP could be expressed in a prokaryote (Escherichia coli) and cells from a eukaryote (Caenorhabditis elegans). Those fundamental achievements—cloning and expression—triggered a green revolution.

In large part, that revolution was the result of GFP’s two primary advantages over other biomarkers. First, GFP requires no cofactor, as far as anyone knows. In other words, nothing but GFP needs to be added to a cell or organism in order for the protein to shine green under blue or ultraviolet light. Second, GFP can be used in living cells and organisms. Chalfie said that capability allows investigators to “monitor a variety of cellular processes, or monitor the presence or absence of something, by using living cells. In many cases, that is difficult or impossible to do in other labeling systems because you either have to fix the tissue or [make it permeable] to get these substrates in, and so on.”

Getting the Glow

Paul Kitts, a research scientist at Clontech Laboratories, Inc., in Palo Alto, California, said, “With GFP, you can, using the fluorescence microscope, look inside living cells and actually follow the GFP fluorescence in real time . . . If you tag the protein of inter-
est or you’ve tagged an organelle that you’re interested in, you can actually watch as it moves around inside the cell as the cell divides, moves along a surface, is infected, or goes through some other changes. This has really given cell biologists a new way of following what goes on inside a cell.” Kitts added that “the most frequent use of GFP is to make a fusion between the GFP protein and some other protein of interest. By following the GFP fluorescence, you can see how the marked protein redistributes inside the cell under different stimuli or different processes.” Surprisingly, attaching GFP to another protein rarely interferes with the normal processes of the other protein.

In fact, GFP seems to coexist easily with a variety of cellular neighbors. It has been expressed in many organisms, including bacteria, yeast, slime mold, many plants, fruit flies, zebra fish, many mammalian cells, and even viruses. Moreover, many organelles, including the nucleus, mitochondria, plasma membrane, and cytoskeleton, have been marked with GFP. Chalfie said, “Every time people have found any problems with it, other people have been able to circumvent those problems. At first people said it wasn’t going to work here or it wasn’t going to work there. Now, it seems to be working in virtually every system that people have looked at.” He continued, “The disadvantage at the moment for something like a gene-expression system is that it does take some amount of time for the expression of a fluorescent product to occur. But again, variants have been developed that make this actually pretty quick. But if you want to follow gene expression, you certainly, at the moment, won’t have minute-by-minute resolution that one would like to have.” Leff added that work needs to be done on “the stability of the marker-gene vector and the persistence of the GFP.”

Although the method for using this marker is dependent on its intended task, the general procedure revolves around one challenge: getting the GFP to the right place. In essence, that requires a vector system that delivers the cDNA into the right organism or even the right cells, where the cDNA gets expressed, thereby producing GFP. Chalfie said, “Basically, what we’re doing is transforming the organisms with DNA that will encode GFP. . . For example, in C. elegans, which is the worm we work on, it’s relatively easy to microinject the cDNA into the gonads. It becomes incorporated into the germ cells, and the subsequent progeny will express GFP. Then you just simply look under a microscope, shining UV light or blue light under it, and see what cells express. It’s just like adding any marker, but this is one that produces a fluorescent product.”

Today, you can purchase a variety of GFP-based products from several companies. Kitts and colleague Steven Kain formulated E-GFP, an enhanced version of the original gene isolated by Prasher that incorporates mutations that occurred in other laboratories. Kitts said the E-GFP vector “gives about two orders of magnitude greater sensitivity in human and mammalian cells than the original jellyfish gene.” Another product on the market is E-BFP, a version of E-GFP that gives off a bluish light. By using both E-GFP and E-BFP, two different proteins could be marked simultaneously in the same experiment.

Environmental Spotlights

Despite GFP’s relative youth in the molecular biology arena, it promises many applications in environmental health. Leff imagines lots of potential uses for it. She said, “GFP could be used to monitor survival of a variety of microorganisms in soil and water . . . [and it] may also play a role in the future of environmental monitoring of contaminants.” In addition, Leff said, “Reporter genes in selected bacterial species could someday be used to monitor the expression of genes induced by environmental stressors, such as heavy metals or organic pollutants. Such monitoring approaches could be used to develop pollution indices that may be less costly and more sensitive than conventional chemical or biological indicator measurements.”

At the Scottish Crop Research Institute in Dundee, Simon Santa Cruz and his colleagues used GFP to monitor potato virus X in a variety of plants, including peppers, tomatoes, and relatives of tobacco. In describing GFP’s performance, Santa Cruz called it “absolutely amazing. The ability to detect virus-infected cells noninvasively has opened up a whole range of experimental possibilities that were previously impossible.

The fact that the virus we use accumulates to high levels helps because we get high expression levels of GFP and hence easy detection. We’ve also used GFP-fusion proteins to look at protein trafficking and localization in plant cells, and here, too, we are able to perform experiments that would have been impossible without GFP.” For instance, with the help of GFP, Santa Cruz’s team showed that the virus needs its coat protein to move from cell to cell.

Even more applications lie just over the horizon; investigators are developing GFP-based diagnostic tests for drugs, food additives, herbicides, pesticides, carcinogens, and other chemicals. In the next few years, GFP will probably give a green light to many new forms of research and technology.

Mike May

Suggested Reading

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