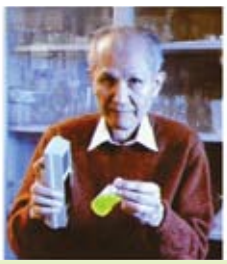
Glowing Proteins with Promising Biological and Medical Applications


By Linda Zajac

Under the lens of a microscope, a mouse is still from anesthesia. Every few seconds, a camera captures images of brightly glowing cancer cells moving through the mouse's blood vessels. Robert Hoffman, a professor of surgery at the University of California, San Diego, studies the images of spreading cancer. Each cancer cell glows red, while its nucleus glows green. Some cancer cells roll and crawl on blood vessel walls while others collide and drift through the bloodstream.

Hoffman has spent the past 12 years developing an imaging technique that allows scientists to see growing tumors in live mice. Thanks to this technique, scientists around the world are able to follow cancer cells wherever they go in the body of animals affected by cancer. What makes this possible is a molecule called a fluorescent protein. By making proteins and cells glow, it brought about a revolution in biology and medicine by allowing scientists to actually see inside cells and better understand how viruses and tumors work.

The use of fluorescent proteins has been such a success that it landed this year's Nobel Prize in Chemistry to three scientists who discovered the first fluorescent protein: Osamu Shimomura, a Japanese citizen based in the United States, and Americans Martin Chalfie and Roger Tsien.

Before this protein was discovered, very few techniques offered a way of seeing what was happening inside the cell. Proteins are so tiny they can't be viewed under an electron microscope. But by attaching fluorescent proteins to these proteins, it is possible to constantly track their whereabouts. What's more, these fluorescent proteins are now available in many colors, so scientists can study different types of proteins by labeling them with different colors.

Here is the story of the scientists who discovered this protein, realized how it worked, and ultimately won the Nobel Prize and how other scientists found ingenious ways to use it to study diseases, such as cancer, Alzheimer's disease, and crop diseases.

Discovery of the green fluorescent protein

It all started in July 1961 when Osamu Shimomura, a senior scientist at the Marine Biological Laboratory in Woods Hole, Mass., and Frank Johnson, a scientist at Princeton University, decided to study what made crystal jellyfish glow in the dark.

Before joining Johnson, Shimomura had shown that tiny egg-shaped crustaceans called ostracods produced light through a process called bioluminescence, which is the emission of light by a living organism as a result of a chemical reaction. In the ostracod, bioluminescence is produced by a chemical reaction that adds oxygen (O_2) to a chemical called luciferin. The reaction is catalyzed by an enzyme called luciferase and leads to the production of an oxidized form of luciferin, carbon dioxide (CO_2), and light:

$$\text{luciferin} + O_2 \rightleftharpoons \text{oxidized luciferin} + CO_2 + \text{light}$$

The two scientists were wondering whether crystal jellyfish emitted light in the same way. So they traveled to Friday Harbor Laboratories in Friday Harbor, Wash., to collect jellyfish and determine what chemicals made them glow. After many weeks of work, Shimomura identified a molecule called aequorin as the probable cause of the bioluminescence of jellyfish.

In 1962, Shimomura, Johnson, and colleagues showed that, in the presence of calcium ions (Ca^{2+}), aequorin emitted blue light after splitting into two molecules—called apoaequorin, coelenteramide—and carbon dioxide (CO_2).

But jellyfish emit green light, not blue light. The researchers thought that another molecule was probably involved as well. After a few months, they found another protein, which they called the green fluorescent protein (GFP).

Pictured above: Martin Chalfie, Osamu Shimomura, and Roger Tsien received this year's Nobel Prize in Chemistry for the discovery of the green fluorescent protein, which, according to the Nobel Foundation, is "one of the most important tools used in contemporary bioscience."

In 1974, the scientists discovered that the blue light produced by aequorin was immediately absorbed by GFP, which then emits green light through a process called fluorescence (which happens when a molecule absorbs light and emits light of a different color).

Using fluorescent proteins as tracers inside cells

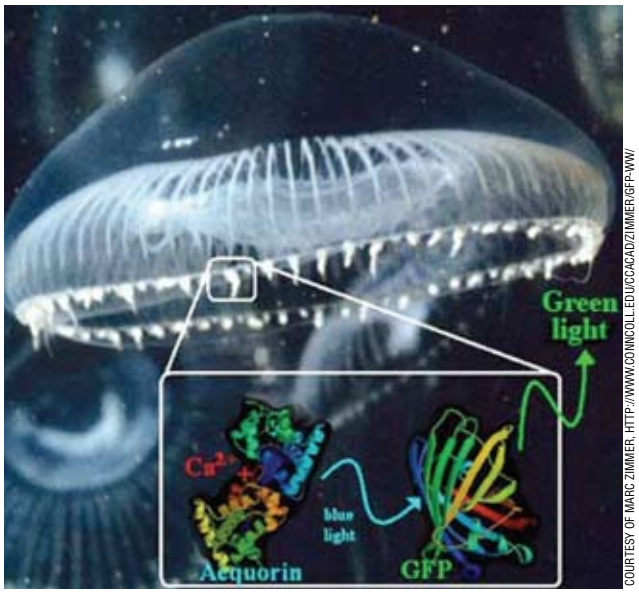
In 1987, Douglas Prasher, an assistant biochemist at the Woods Hole Oceanographic Institute, Woods Hole, Mass., thought it might be possible to attach GFP to a specific protein. If researchers could see cancer cells glowing, they could tell if the disease was spreading.

Prasher decided to look for the gene that produces GFP. He reasoned that after identifying this gene, he would insert it in the DNA of other organisms—including humans—to make proteins with GFP attached to them (Fig. 2). Then these proteins would be exposed to blue light that they would absorb to emit green light (in the same way as when GFP in jellyfish absorbs blue light from aequorin to emit green light). As a result, GFP would make proteins glow, allowing scientists to see them move through a cell.

In 1992, Prasher found the GFP gene and published his results in the journal *Gene*. Unfortunately, funding for his grant ran out before he could show that GFP could be used as a tracer, so he had to stop his research. But before leaving Woods Hole, Prasher made copies of the GFP gene that he distributed to other scientists.

Martin Chalfie, a biologist at Columbia University, New York, N.Y., read Prasher's article with great interest and called him up to receive a copy of the GFP gene. Then he inserted the GFP gene in bacteria. Within one month, Chalfie and colleagues saw a glow through the microscope that proved that GFP could indeed be inserted into a living organism.

"I was ecstatic!" Chalfie says. "The very first experiment gave us fluorescent bacteria. By putting GFP into bacteria, we demonstrated that it didn't need anything else from the jellyfish to make it work."



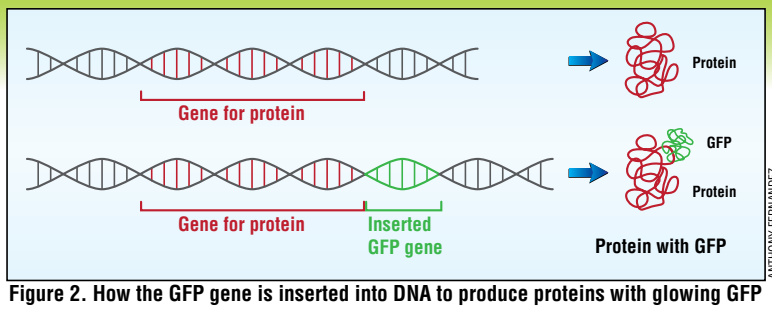
COURTESY OF MARC ZIMMER, HTTP://WWW.CONNCOLL.EDU/CCACAD/ZIMMER/GFP-WW/

Figure 1. Jellyfish contain an umbrella with light-emitting organs along the edge of the umbrella. Inset: In the presence of calcium (Ca^{2+}), the bioluminescent blue light produced by aequorin is absorbed by a green fluorescent protein (GFP), which then emits fluorescent green light.

Also, in these animals, the fluorescent proteins emitted a range of colors, including cyan, green, yellow, and red. "The key finding was a protein that emitted red fluorescent light, which we called the red fluorescent protein" Lukyanov says. "This protein opened up new possibilities for looking inside living cells and living animals, because red light penetrates through animal tissue much deeper than green."

Several years later, Lukyanov's team also found fluorescent proteins in tiny, nonbioluminescent crustaceans called copepods off the coast of South Carolina. Curiously, because of their well-developed eyes, copepods were the first known animals that can actually see their own fluorescent proteins

In 1994, Roger Tsien, a professor of pharmacology, chemistry, and biochemistry at the University of California, San Diego, and Howard Hughes Medical Institute investigator, modified the GFP gene to produce new GFP proteins called mutant GFP proteins that emitted brighter light. Then he created other mutant GFP proteins that emitted other colors, such as blue, yellow, and cyan. This allowed researchers to track more than one protein at a time by attaching different GFP proteins to different proteins inside the cell.



ANTHONY FERNANDEZ

Figure 2. How the GFP gene is inserted into DNA to produce proteins with glowing GFP attached to them.

In September 1998, Sergey Lukyanov, a molecular biologist at the Russian Academy of Sciences, and colleagues discovered fluorescent proteins in non-bioluminescent corals. Unlike ostracods and jellyfish—which first emit bioluminescent light, which is then converted into fluorescent light—these corals only emit fluorescent light.

Preventing plant diseases

Scientists can track plant diseases with fluorescent proteins. Jeanmarie Verchot-Lubicz, a plant virologist at Oklahoma State University, Stillwater, and colleagues are using fluorescent proteins to understand how viruses infect plants. By looking at how a virus called potato virus X infects potatoes, they expect to find ways to prevent viruses from infecting other crops and better understand how viruses cause animal and human diseases in general.

Verchot-Lubicz and her team attached a fluorescent gene to viral genes, so that viral proteins were produced with a fluorescent protein attached to them. Then, by using a microscope, the scientists tracked the movement of the viral proteins as they moved inside plant cells.

"Using fluorescent proteins has been extremely useful," Verchot-Lubicz says. "This is the only way we can see viral proteins moving inside plant cells while they are causing infection. If we don't use fluorescent proteins, we can only look at cells after they have been infected. Here, we can see live everything from the moment the virus enters a cell to how it spreads throughout neighboring cells."

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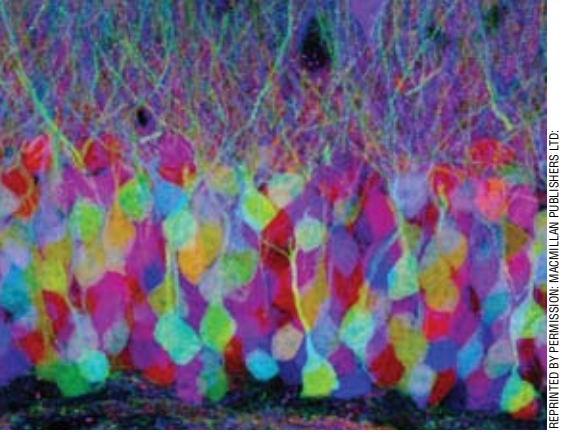

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Figure 3. Section of the brain of a "Brainbow mouse" in which cells produce different amounts of fluorescent proteins.

This research has led to the first evidence that, after a potato virus X enters a cell, it uses small cavities called vesicles to carry proteins to the surface of the infected cell. Then, the proteins move across tiny tunnels that connect the infected cell to its neighboring cells, causing these cells to become infected as well.

Verchot-Lubicz's team is now trying to determine what is inside the vesicles and how these vesicles are carried to the cell surface. This information could help scientists develop antiviral drugs that would destroy the vesicles' viral proteins or prevent the vesicles from leaving an infected cell.

Helping to cure brain diseases

One promising application of fluorescent proteins is in research to understand brain diseases. By using fluorescent proteins, scientists at Harvard University have recently developed a way to understand how brain cells interact with one another.

Jeff Lichtman, a professor of molecular and cellular biology at Harvard University, Cambridge, Mass., and colleagues inserted yellow, cyan, and red fluorescent proteins inside the DNA of brain cells in mice, so that each cell produced enough of these proteins to glow. "If you look at a brain tissue under a microscope, it looks like a jumble of cells that cannot be easily distinguished from one another," Lichtman says. "So we decided to use fluorescent proteins to give each cell a different color."

The scientists inserted the genes for the yellow, cyan, and red fluorescent proteins inside the DNA of brain cells in mice, so that each cell produces enough of these proteins to glow. Each cell glowed in a different color based on how many yellow, cyan and red fluorescent proteins were produced in that cell. This way, the scientists were able to produce a mouse brain in which cells glowed in nearly 90 different colors. The scientists called these mice "Brainbow mice" (Fig. 3).

The distinct colors of Brainbow mice can help researchers see individual cells and sort out how they connect with one another. By comparing brain samples from healthy mice with those of mice in which diseases are induced, the scientists hope to better understand what goes wrong in people with debilitating diseases such as Alzheimer's and Parkinson's diseases.

Scientists at the Albert Einstein College of Medicine of Yeshiva University, New York, N.Y., are using fluorescent proteins to study a form of dementia that occurs in about 20% of people infected with HIV, the virus that causes AIDS. The scientists, led by Harris Goldstein, director of the Einstein-Montefiore Center for AIDS Research, bred mice in which cells from

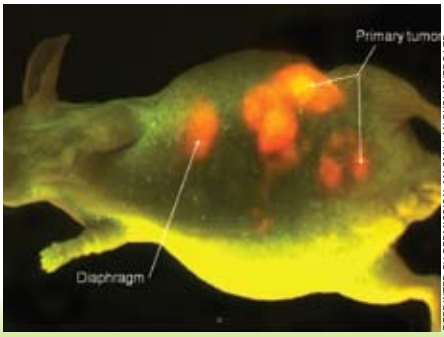

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Figure 4. Cancer cells that express red fluorescent proteins are visible in this mouse as they spread from the pancreas to other parts of its body.

their immune system contained both the gene that makes HIV and the GFP gene. This way, not only were the immune cells infected with the HIV virus, but they also glowed green.

By following the movement of these cells in the mice, the scientists showed that these cells went through the brain, thus infecting it too. This discovery was unexpected since these immune cells cannot go through the brain when they are not infected with HIV. These findings shed some light on why some HIV patients develop dementia and may lead to the discovery of drugs that prevent HIV- infected immune cells from getting into the brain.

Battling cancer

Perhaps the most important medical application of fluorescent proteins is its use in the study of cancer. Hoffman, the professor of surgery working on cancer cells in mice, is also the founder and CEO of AntiCancer Inc., a company that develops imaging technology and imaging equipment based on fluorescent proteins. AntiCancer Inc. also sells this equipment to scientific laboratories around the world, making the use of fluorescent proteins widely available.

Thanks to his technique, Hoffman and colleagues have seen how cancer cells migrate in blood vessels and how they grow aggressively to form colonies. The scientists also observed with some detail how cancer cells bind to healthy cells—maybe to infect them or to protect themselves from the immune system—and how cancer cells exchange DNA.

Hoffman and colleagues also discovered that a cancer chemotherapy drug called cyclophosphamide could stimulate the growth of cancer cells in the mice, which was opposite to expectation and suggests that certain approaches to chemotherapy should be modified.

"With this technology, we can expect to discover new classes of drugs for cancer," Hoffman says. "Not only can we see cancer cells move and spread in the body, but we can see how drugs affect them or neighboring cells, which allows us to design better drugs that target cancer cells more efficiently.

In research labs all around the world, fluorescent proteins are glowing brightly, casting a rainbow of light and a glimmer of hope for people suffering from diseases.

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