ILLUMINATING LIFE

Since its discovery 50 years ago, Green Fluorescent Protein has become one of the most useful tools in biology. **Zoe Cormier** shines a spotlight on this glorious, glowing molecule

Japanese scientist Osamu Shimomura reported in 1962 that he had extracted a glowing protein from the jellyfish *Aequorea victoria* (pictured). Since then, researchers have used green fluorescent protein (GFP) to shed light on every corner of biology, from the brain's complex circuitry to the spread of cancer.

"We knew that, if it worked, it would be very powerful," says Professor Roger Tsien of the University of California, San Diego. "It was surprising how easy the jellyfish made it for us." In 2008, Shimomura, Tsien and another biologist Martin Chalfie (see 'Reporter Gene', p48) were awarded the Nobel Prize in Chemistry for their work on GFP.

To isolate the protein, Shimomura collected over 10,000 jellyfish (each 5cm in diameter) from the Pacific Ocean, trimmed away their glowing organs, and filtered the marine goo to extract the glowing molecule. Then in the 1980s, his team would gather 50,000 jellyfish to extract a measly 150 milligrams of the molecule. As Shimomura recounted in his Nobel acceptance speech, "Our laboratory looked like a jellyfish factory and was filled with the jellyfish smell."

Understanding the chemistry of GFP became Shimomura's life's work, but it remained useless for 30 years until researchers managed to read the DNA sequence of the jellyfish gene and create a copy through cloning. Biologists could then genetically modify organisms to produce the glowing protein, inserting cloned GFP genes into their DNA. When the GFP gene is inserted next to another gene, it's switched on at the same time, producing the glowing protein. The gene therefore acts as a 'reporter', producing a protein that glows green to indicate when and where its neighbouring gene is active.

Tsien earned his share of the Nobel Prize by mutating the GFP gene to create a palette of colourful proteins. "I didn't appreciate how many colours would be developed," he says. "But once they became available, people used them beyond anything I could have thought of." ▶

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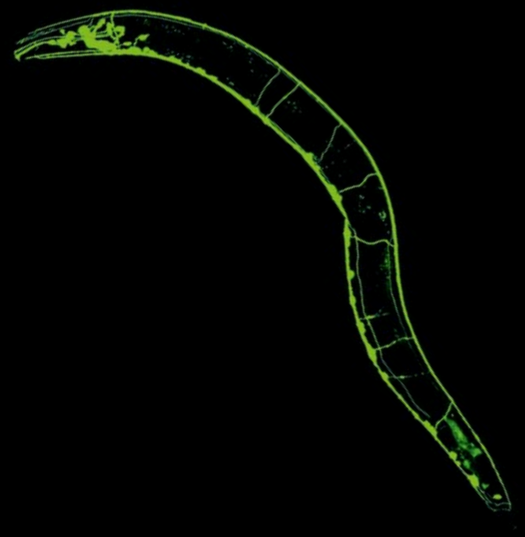

Highlighting genes

Green fluorescent protein glows bright green under blue and UV light. Biochemist Douglas Prasher, who cloned the gene that encodes the protein in 1992, was the first person to recognise GFP's potential to revolutionise biology as a 'reporter gene' that illuminates the activity (expression) of neighbouring genes.

Once the jellyfish gene had been cloned, it could be implanted into new species and used to light up specific groups of cells – enabling biologists to monitor the mechanics of life in real-time. Martin Chalfie showed this was possible by highlighting nerve cells in the nematode worm *Caenorhabditis elegans* (pictured).

Inside the cell

Biologists can not only harness the headlights of GFP to tag specific kinds of cells within an organism, they can also illuminate the fine structures of cellular machinery. In the root of this thale cress, genes active in the membrane (cell wall) of the plant's cells are tagged with a green glow, while a red fluorescent protein marks the nucleus.

MARTIN CHALFIE, SCIENCE PHOTO LIBRARY, FERNAN FEDERICI

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Adding red to the mix

Although Roger Tsien managed to develop new forms of GFP in an array of colours – allowing researchers to paint different parts of an organism in a variety of hues – he couldn't produce red. Russian scientist Sergey Lukyanov helped complete the palette by isolating a fluorescent protein from coral, DsRed, pictured here glowing in mosquito larva.

Like GFP, the DsRed gene can be inserted into DNA as a 'proof-of-concept' – to show that an organism could be genetically engineered to activate a gene it wouldn't normally have. For instance, instead of inserting DsRed or GFP, you could add a gene that would stop a mosquito carrying the malaria parasite.

Brainbow

One often-quoted fact is that there are more possible connections between the 100 billion neurones in the human brain than the number of atoms in the Universe. "Each neurone has contact with not just one, but hundreds or even thousands of other neurones," explains Professor Jeff Lichtman of Harvard University. "In order to understand how information flows in the brain, we need to be able to see where the connections lie."

But for decades, neuroscientists had been stymied in their efforts to visualise these dense networks of neurones. Lichtman himself had tried many different approaches, using various chemical stains and dyes in what he describes as 'imaginative but mostly failed ways'. It was Roger Tsien's palette of GFP-like proteins that finally provided the solution needed to crack the biochemical puzzle: with just three different genes for fluorescent proteins – red, green and blue – he could create a huge variety of visible colours.

Similar to the way in which a TV screen seemingly creates any hue from a combination of red, green and blue lights, Lichtman's team developed a breed of genetically-engineered mice whose neurones were programmed to produce a random combination of red, green and yellow fluorescent proteins. The result is a 'brainbow' (pictured), where every neurone of the mouse has a different shade in a technicolour spectrum – more than 90 colours in all.

Thanks to this technique, first publicised in 2008, Lichtman has not only been able to map the connections in the mouse brain, he can also observe when specific neurones fire electrical signals to each other. "I can't imagine how I'd be able to look at the patterns of nerve-firing activity without the brainbow," says Lichtman. "I can see patterns in nature I wouldn't otherwise see. GFP is a bit like the Hubble Space Telescope: you use it to look out there, and you see things that you don't understand. But you just have to try and figure out what they mean."

LICHTMAN/SANES/WEISSMAN/HARVARD UNIVERSITY

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Disease detection

Genetically modified animals carrying the GFP gene can be spotted at a glance, without the need for dissection or expensive genetic analysis. They can also be used to study human diseases with a view to treating them. Masaru Okabe's team at Osaka University was the first to engineer fluorescent mice, which are now widely used to study cancer.

Monkey markers

Primates are often used as a model for studying human disease, such as this glowing macaque created by geneticist Anthony Chan to investigate neurodegenerative diseases like Huntington's.

In 2009, Erika Sasaki developed a strain of marmoset monkeys that could pass on the genes for fluorescence, dispensing with the need to produce new genetically modified animals through IVF.

PENN STATE UNIVERSITY, MAYO CLINIC, ANTHONY CHAN/EMORY UNIVERSITY

From colour to cure

For some HIV researchers, a cure for the disease is no longer an unattainable ideal. "It is a realistic goal," says Dr Eric Poeschla, a physician and virologist at the Mayo Clinic in the US, whose work grabbed headlines last September when his lab announced that it had created a breed of genetically modified glowing green cats designed to be immune to the cat equivalent of HIV, feline immunodeficiency virus (FIV).

There are many variants of the virus in the animal kingdom, including versions that infect horses, cattle and monkeys. But only the feline virus causes AIDS in the same way as in humans, and GFP has allowed Poeschla to breed FIV-resistant cats far more quickly and efficiently than is possible through the arduous methods of traditional cloning. "GFP is easy to monitor and it's remarkably non-toxic – that's the really wonderful thing about it," he explains.

The research is deadly serious: a global pandemic of FIV is affecting millions of cats. "You don't do this kind of work frivolously, you do it because there are worldwide epidemics killing both people and cats."

The next step will be to determine whether the cats are resistant to infection from FIV, with the ultimate goal of understanding if other animals can be rendered immune to their own AIDS viruses. If so, Poeschla hopes the results could be used to design gene therapies that work in humans. ■

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