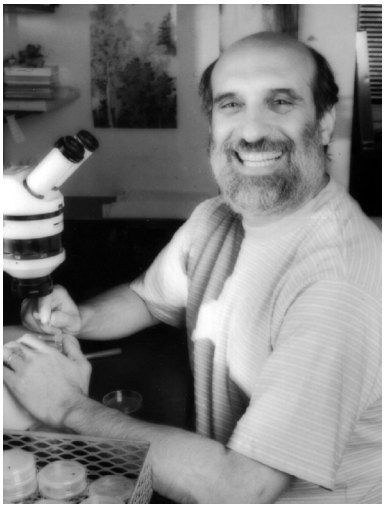
The 2008 Nobel Prize for Chemistry



L-R:Professors Osamu Shimomura, Martin Chalfie and Roger Tsien*

*Images used with permission from Boston University (Shimomura: *www.bu.edu/today/world/2008/10/08/bu-prof-wins-nobel-prize-chemistry*), Martin Chalfie (*www.columbia.edu/cu/biology/faculty/chalfie*), and UC San Diego (*http://ucsdnews.ucsd.edu/newsrel/science/NobelPrize08.asp* courtesy UC San Diego).

The 2008 Nobel Prize for Chemistry was awarded for the discovery and development of the green fluorescent protein (GFP). It rewards the initial discovery and a series of important developments that have led to its use as a tagging tool in bioscience. The Laureates were Osamu Shimomura, an Emeritus Professor of the Marine Biological Laboratory and Boston University Medical School, Boston; Martin Chalfie, Professor of Biological Sciences at New York's Columbia University; and Roger Y. Tsien, who is Professor and Investigator at the Howard Hughes Medical Institute at UC-San Diego.

The remarkable, brightly glowing, green fluorescent protein was first observed in the jellyfish, *Aequorea victoria* in 1962 by Shimomura. Since then, it has become one of the most important tools used in contemporary bioscience as it provides a means to monitor processes that were previously invisible, such as the development of nerve cells in the brain or how cancer cells spread. By using DNA technology, researchers can now connect GFP to other interesting, but otherwise invisible, proteins. The glowing marker allows them to watch the movements, positions and interactions of the tagged proteins. They can also follow the fate of various cells with the help of GFP, such as nerve cell damage during Alzheimer's disease or how insulin-producing β cells are created in the pancreas of a growing embryo. In one spectacular experiment, researchers succeeded in tagging different nerve cells in the brain of a mouse giving a kaleidoscope of colours.

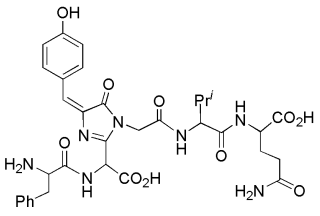
Green fluorescent protein is now a standard tool for thousands of researchers the world over. The story of its discovery has its origins in Japan in the years after WWII. Osamu Shimomura's education was disrupted by the war and the devastation caused by the atom bomb. Nonetheless, in 1955 he was employed as an assistant by Professor Yashimasa Hirata at Nagoya University and put to work on a seemingly impossible project – to discover what made the remains of a crushed mollusc, *Cypridina*, glow when it was moistened with water. That Hirata gave the inexperienced assistant such a difficult task may seem strange as leading American researchers had tried for a long time to isolate the material.

In 1956, and against all odds, Shimomura had the material in his hand. It was a protein that glowed 37,000 times more brightly than the crushed mollusc. After publishing his results, Shimomura was recruited to Princeton University by Frank Johnson. Professor Hirata was generous in seeing to it that Shimomura, despite not being a doctoral student, was awarded a PhD from Nagoya .

In Princeton, Shimomura began to study another naturally luminescent material. This time it was from the beautiful jellyfish *Aequorea victoria* (Fig. 1) that lives in the sea off the Washington coast of NW America and whose outer edge glows green when the jellyfish is agitated. During the summer of 1961, Shimomura and Johnson gathered jellyfish in San Juan Island's Friday Harbor. They cut off the edges of the jellyfish and pressed them through a filter to get what they termed a *squeezate*. One day when Shimomura poured some of the squeezate into the sink, it flashed brightly. He realised that there was seawater in the sink and that it was the ions present that had caused the chemical reaction. Strangely enough, the flash of light was not green like the edges of the jellyfish, but blue. The raw material gathered that summer was taken back to Princeton and within a few months they had isolated a few milligrams of the blue luminescent material from the liquid. It was a protein that they named *aequorin*.

In the 1962 publication that described the process by which aequorin was obtained,[1] Shimomura and Johnson also mentioned that they had isolated a protein that was slightly greenish in sunlight, yellowish in the light from a light bulb, and fluorescent green under UV light. This was the first time that anyone had described GFP. Shimo-

mura and Johnson called it the *green protein*, later to be named the *green fluorescent protein* by Morin and Hastings.[2] GFP contains 238 amino acids[3] and residues 65-67 (Ser-Tyr-Gly) spontaneously[4] form the fluorescent *p*-hydroxybenzylideneimidazolinone chromophore (shown below) in the primary structure.[5] The excitation spectrum has a dominant maximum at about 400 nm with a much smaller absorbance *ca*. 470 nm, while the emission maximum is a sharp at *ca*. 505 nm with a shoulder at 540 nm, it is green.[6]



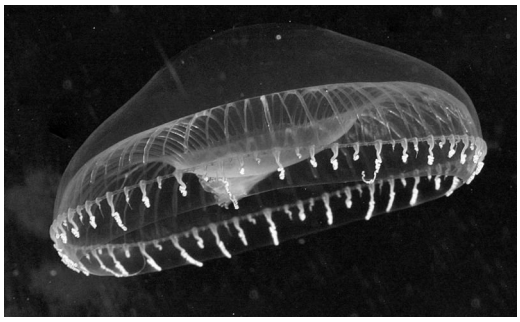


Fig. 1. *Aequorea victoria* from *http://en.wikipedia.org/wiki/Image:Aequorea4.jpg*; copyright S. S. Blakely

In jellyfish, the GFP chromophore simply transforms blue light from aequorin into green allowing it (and aequorin) to glow in different colours. GFP is revolutionary in that it does this alone in contrast to aequorin and other bioluminescent proteins, which require a continuous supply of energy rich-molecules. Irradiation of GFP with UV or blue light activates the GFP directly inside a cell where it glows green.

Chalfie had spent some time studying the millimetre-long roundworm *Caenorhabditis elegans* (Fig. 2), one of the most frequently studied organisms in the world. Although it consists of only 959 cells, it has a brain, grows old and mates. In addition, a third of the roundworm's genes are related to human genes. Last but not least, *C. elegans* is transparent making it easy to study its organs under an ordinary microscope. Chalfie heard about GFP in a 1988 seminar dealing with bioluminescence and he recognised that it would be a fantastic tool for mapping the roundworm. It would act as a glowing green signal for various activities in the roundworm's cells.

Fig. 2. Caenorhabditis elegans from *http://en.wikipedia.org/wiki/Image:Adult_Caenorhabditis_elegans.jpg*

To test his ideas, Chalfie needed to locate the gene for GFP in the genome of *Aequorea victoria*; he found that Douglas Prasher (Woods Hole Oceanographic Institution, Falmouth, MA) had already started the search. Prasher was asked if he would get in touch if he succeeded in cloning the gene, which he did. The GFP gene was sent to Chalfie who, in turn, had a graduate student attempt to get *E. coli* to produce GFP. Success came in about one month and the bacteria glowed green when irradiated with UV light.[7]

This discovery forms the basis of today's revolutionary use of GFP. However, the discovery was quite unexpected at the time as it was generally accepted that naturally fluorescing molecules and pigments were produced in several steps in the cells with each step requiring a protein to control the chemical production. Many believed that several proteins were needed to produce the chromophore in GFP, but Chalfie's experiment showed that this premise was wrong. The only protein needed is GFP. In the next step, Chalfie placed the gene behind a promoter that is active in six touch receptor neurons in roundworm *C. elegans* and saw the neurons glowing bright green. The image was displayed on the cover of *Science* in February 1994.[7]

GFP is generally non-toxic and can be expressed to high levels in different organisms with minor effects on their physiology.[7] Furthermore, when the gene for GFP is fused to the gene of a protein to be studied in an organism, the expressed protein of interest retains its normal activity; moreover, GFP retains its fluorescence. Thus, the location, movement, and other activities of the studied protein can be followed by microscopic monitoring of the GFP fluorescence.[8] Taken together, the remarkable and unexpected properties of GFP from *Aequorea victoria* are essential to its usefulness in studies of dynamic processes in living cells at the molecular level.

Roger Tsien's greatest contribution to the GFP revolution was to extend the palette by providing new colours that glowed longer and with increased intensity. To begin with, he charted how the GFP chromophore is formed chemically in the 238-amino-acid-long GFP protein. It was known that the three amino acids in position 65–67 of GFP react with each other to form the chromophore. He accounted for the reaction by showing that oxygen but no other protein is needed.[6] With the aid of DNA technology, Tsien then exchanged various amino acids in different parts of GFP to generate proteins that absorbed and emitted in other parts of the spectrum. By altering the amino acid composition, Tsien was able to develop variants of GFP that emit more strongly and with different colours such as cyan, blue and yellow.[6,8] Use of these now allows researchers to mark different proteins in different colours to see their interactions. Despite these advances, Tsien was unable to produce a red colour. Since red light is the best for penetration of biological tissue, it is especially useful in the study of cells and organs inside the body. Mikhail Matz and Sergei Lukyanov became involved in the GFP revolution by finding GFP-like proteins in fluorescent corals. They identified six more proteins; one red, one blue and the rest green.[9]

The desired red protein was designated DsRED from the coral *Discosoma*.[9] Unfortunately, it was larger and heavier than GFP. It consists of four amino acid chains instead of one, thus making it of less use as a fluorescent tag.[10] Tsien redesigned DsRED such that the protein is now stable and fluoresces as a single amino acid chain that can easily be connected to other proteins.[11]

From this smaller protein, Tsien's group also developed proteins with mouth-watering names like mPlum, mCherry, mStrawberry, mOrange and mCitrine, according to the colour they glowed.[12] Several other researchers and companies have also contributed new colours to this growing palette. Today, some forty-six years after Shimomura first wrote about the green fluorescent protein, there is a kaleidoscope of analogues that transmit all the colours of the rainbow.

Three of the proteins have been used recently in a spectacular experiment. Mice were genetically modified to produce varying amounts of yellow, cyan and red within the nerve cells of their brain. The result was a mouse brain that glowed with the colours of the rainbow. The researchers could follow nerve fibres from individual cells in the dense network in the brain. The experiment was termed *the brainbow*.[13]

One mystery yet remains to be solved. Why does the jellyfish *Aequorea victoria* shine? Many organisms living in the sea use light from biofluorescent proteins to confuse their enemies, to attract food or to tempt a partner. But no one yet knows which has caused *Aequorea victoria* to evolve aequorin and GFP.

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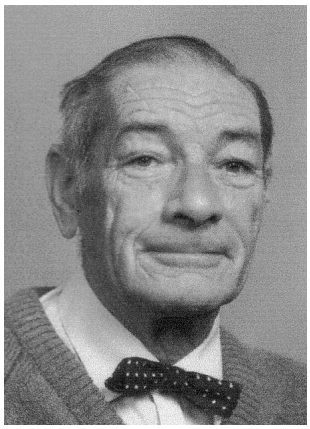
Brian Halton

(Compiled from material freely available; see: *htpp://nobelprize.org*)

Obituary

William Edward (Ted) Harvey 1925-2008

October 20 saw the passing of an icon in New Zealand Chemistry and the Institute. Ted Harvey was known nationally and internationally as the face of Chemistry in NZ from the nineteen years that he served as our Honorary General Secretary, his bowtie a hallmark. He became Assistant Secretary in 1956 and Secretary from 1957-1975. His worldwide dealings with our sister societies and their officers, and the chemistry visitors hosted by NZIC were paper-based, occasionally by telephone (e-mail and fax were not available then), and in person. Indeed, whenever he returned from an overseas trip he would tell us that he had met up again with … – and the list of distinguished chemists would roll off his tongue. On completion of his secretarial service in August 1975 he became 2nd Vice-President and subsequently President for the 1977-78 year, a role that he fulfilled with distinction. Subsequently he was elected to Honorary Fellowship.



Ted was born in Auckland in 1925 and attended Epsom Normal School and Auckland Grammar, gaining third place nationally in his Scholarship examinations after John Ziman and Bob Tizard (formerly Professor of Physics at Bristol University, and politician, minister and Deputy Prime-Minister, respectively). His MSc degree was with Professor Lindsay Briggs at Auckland University after which he moved to Cambridge for PhD study with Alexander (Lord) Todd. He then took a postdoctoral position with Holger Erdtman in Stockholm that lasted for two years and this was his most prolific research period generating five publications. His return to NZ was