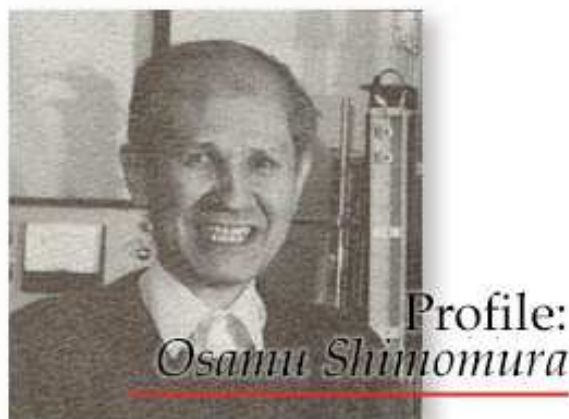


Discovery of the Green Fluorescent Protein

Abstract: Many scientists were fascinated by the ability of some organisms to give off energy in the form of light. This property, known as bioluminescence, was observed when ancient mariners were sailing the high seas at night and saw a green glow upon the waters. This glow was later attributed to the crystal jellyfish, *Aequorea*. Generations later, in the early 1960s, scientists began to unravel the bioluminescent properties of the jellyfish *Aequorea*. In 1962, Dr. Osamu Shimomura and his colleagues, identified the substance in the jellyfish that causes bioluminescence.

In the early 1960s, several scientists at Princeton University in Indiana were studying the luminescent properties of the jellyfish *Aequorea*. They were trying to identify the specific substance that caused these jellyfish to “twinkle like tiny stars in the moonlit waters.” One scientist, Dr. Osamu Shimomura, had just arrived from Japan’s Nagoya University to Princeton as a new research associate. He joined his colleagues that summer on a trip to the northwest coast off of Washington at Friday Harbor, to collect and examine hundreds of these comb jellies known as *Aequorea*.

The waters there were abundant with the comb jellies. In the early morning the researchers, including Dr. Shimomura, would row out from the harbor and scoop up buckets full of these jellyfish. Once at the lab, they would cut off the light-emitting region around the periphery of the umbrella from 20-30 of the comb jellies and push these through a piece of rayon gauze. The resulting liquid luminescent “squeezeate” was collected and served as the source of their further experiments since it contained the bioluminescent substance.



Osamu Shimomura, Ph.D.
 MBL Senior Scientist
 B.S. Nagasaki College of Pharmacology, 1951
 Ph.D. Nagoya University, 1960

As Dr. Shimomura was to discover, it took a lot of time not only performing experiments but also thinking about bioluminescence in a new way. He spent day after day trying out new chemicals, looking for just the right ingredients that would result in discovering the bioluminescent properties of *Aequorea*. After several weeks he felt he was no closer in discovering what made the comb jelly luminesce. Due to his frustration, he stepped away each afternoon from his lab and went on a trip alone on his rowboat in Friday Harbor...just thinking. He spent hours trying to figure out what was missing from his experiments.

“One afternoon a thought suddenly struck me,” he remembers. “It was a thought so simple that I ought to have had it much sooner!” He smiles. He went back to the lab and tested his new hypothesis. This led him on the right trail and the following year, in 1962, Shimomura had purified the mystery proteins involved in the bioluminescence of *Aequorea*. He named the two proteins involved in the reaction aequorin and Green Fluorescent Protein (GFP).

References:

<http://hermes.mbl.edu/publications/LABNOTES/5.1/Shimomura.html>

A nice historical perspective of Osamu Shimomura, complete with his photo.

<http://www.mbl.edu/publications/LABNOTES/5.1/weapons.html>

This is not directly related to GFP, but shows an interesting historical use for using bioluminescence in military use.

Glossary for History of GFP**aequorin**

A protein found in the jellyfish *Aequorea* that is activated by calcium and emits blue light through a bioluminescent reaction. This blue light is then absorbed by GFP, stimulates a chemical reaction which results in the emission of a visible green light. Both aequorin and GFP are found in high concentrations in the light producing cells on the periphery of the medusa of this jellyfish.

green fluorescent protein (GFP)

A protein found in jellyfish that fluoresces, or emits a green visible light when excited by UV light with a wavelength of 395 nanometers. It can function as a biological marker when co-expressed with other proteins. The structure of the protein is cylindrical with the glowing component, an amino acid complex called a *fluorophore*, in the middle of it.

bioluminescence

A complex chemical reaction that occurs within a living organism, in which the end product of energy is released in the form of light instead of heat. Some organisms which have this ability are glowworms, fireflies, jellyfish, fungi, and some deep-sea fish.

chemiluminescence

A general term for the production of light when the excitation energy comes from a chemical reaction. Bioluminescence is a type of chemiluminescence that occurs in a living organism.

electromagnetic waves or radiation

Are made up of oscillating electric and magnetic fields that can travel through many substances. Particles that make up the waves are called photons. They consist of many frequencies. At the high frequencies are the gamma rays and X rays, followed by ultraviolet, then the visible light. At the lower frequencies are the infrared radiation waves and microwaves. Within the lower frequencies are the radio waves.

fluorescence

A phenomenon shown by certain substances when they are hit by ultraviolet radiation. The substance absorbs high frequency wavelengths and emits it at a lower frequency light. This emission stops as soon as the high frequency radiation is removed. For example, GFP absorbs the higher frequency blue light emitted by aequorin, undergoes a chemical reaction, and emits the lower wavelength green light.

light

Consists of electromagnetic waves of specific frequencies and wavelengths. Light is often drawn as a ray. The ray shows the path taken by the light waves, the direction in which the energy is being carried.

lysis (to lyse)

The destruction of a cell's membrane or wall, which breaks open the cell, spills its contents, and kills it. The lysate is the contents that are collected from the lysed cell.

ultraviolet radiation (UV)

Electromagnetic radiation produced by the sun and or produced when an electrical current passes through ionized gas between two electrodes. It consists of wavelengths between 200 and 400 nanometers. Exposure to excessive amounts of UV radiation damages DNA and can cause health problems such as skin cancer and cataracts in the eyes.

visible light

Electromagnetic waves or radiation that are produced by the sun detectable by our eyes. It consists of wavelengths between 400 and 750 nanometers. Colors depend on the wavelength lengths; a short wavelength (the 400 nm side) looks blue and a long wavelength (the 750 nm side) looks red.