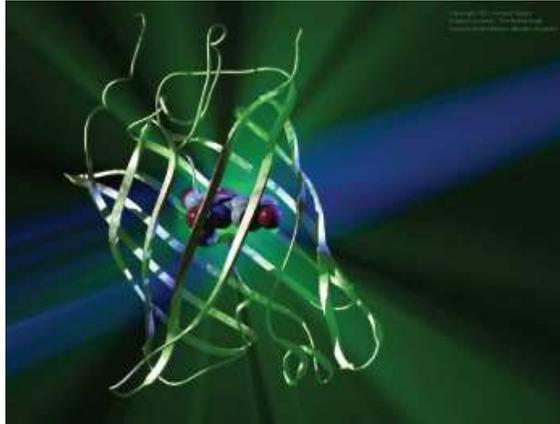


A green glow



Phantatomix molecular graphics

December evenings are the most brightly lit of the year. Town squares and streets glitter with decorations, shop windows sparkle with gold and silver, and Christmas trees shimmer with tinsel. Nature needs none of these artifices to shine; depending on the season, animals and plants startle us with their muted and flamboyant colours. More fascinating still is Nature's ability to produce light, a phenomenon known as luminescence. Luminescence in living organisms is caused by proteins - one of which has become an essential experimental tool for scientists: GFP or Green Fluorescent Protein.

In the twilight of the marine underworld

A number of living organisms emit light. The firefly is one. Other insects, worms, bacteria and even mushrooms also share this amazing faculty, but we have to go into the depths of the ocean to observe the extent and diversity of luminescent species. An inventory of over 700 of these organisms has been made, yet many are still unknown since they live in unexplored depths. Among these are phytoplankton, shrimps, squid, jellyfish, corals and other fish which have luminescent organs or cohabit with luminescent bacteria. Terrestrial luminescent species emit varied colours - blue, green, yellow or red - but marine species are limited to blue or green, simply because these are the light waves that propagate best in water.

The capacity that some creatures have to emit light is known as bioluminescence. Why do they need to shine in the first place? The diffusion of light in the darkness of the ocean depths or in the night is an asset, and those who own the faculty use it with great imagination. One of the roles of bioluminescence is obviously to give light, yet it is not the most common. Some nocturnal fireflies

certainly use their lighting capacities to illuminate the leaf on which they are about to land but bioluminescence can also be used as a means of communication during the mating season when the opposite sexes engage in enlightening conversation. On the whole though, bioluminescence is mainly used either to attract prey or, conversely, to keep predators at bay. Jellyfish flash "lightening" to dissuade predators. Male lantern fish carry a sort of bulb that sways above their head, which they use to attract competitors in order to draw their attention away from the females lying low in the dark. Some fish which float close to the surface of the water can hide their dark silhouette by lighting up their skin so as to merge into the daylight.

A stroke of luck

Aristotle (384-322 BC) was the first to mention bioluminescence when he observed the phenomenon on dead fish covered with luminescent bacteria. He called it cold light since it emitted no heat. At the beginning of our era, Pliny the Ancient continued Aristotle's work of observation and further described several luminescent

organisms. It was only towards the end of the 17th century though - a thousand years later! - that some scientists tried to understand this phenomenon, and proof that oxygen is essential for the production of light was demonstrated only in the following century. Finally, in 1887, Raphael Dubois discovered that a chemical reaction is at the source of bioluminescence, i.e. the oxidation of a compound called luciferin by a catalyser called luciferase results in the emission of light.

The characterisation of both factors proved to be difficult until 1960 when there was a sudden leap forwards. A young Japanese researcher, Osamu Shimomura, undertook to identify the luciferin and luciferase in *Aequorea victoria*, a luminescent jellyfish found along the coast of the North-East Pacific (Fig.1). Approximately 5 to 10cm in diameter, it is also known by the name of "crystal jelly" because of its transparency. This small jellyfish emits green rays of light from the rim of its umbrella, probably following mechanical stimulation such as contact or movements of the water. All that can be seen of this jellyfish is a thin luminous circle, visible only by night... and with a little bit of luck!

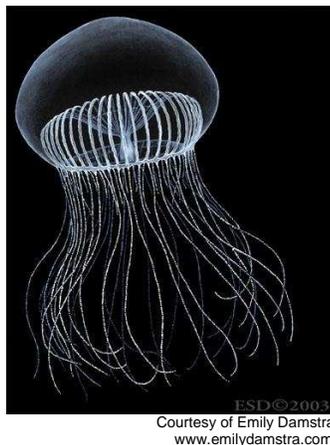


Fig.1. Drawing of *Aequorea Victoria*. The GFP is at the circular base of the umbrella from where protrude the tentacles.

O. Shimomura extracted a protein from a jellyfish - which contained both luciferin and luciferase - and named it aequorin. To his astonishment, he found that the isolated aequorin emitted a blue light and not a green one. Why? Because the cellular extracts contained yet another protein that was responsible for the green glow, i.e. green fluorescent protein or GFP.

So there were two luminescent proteins: one blue, the other green. And yet *Aequorea victoria* only emits green light. O. Shimomura discovered the reason why only years later when he demonstrated that GFP's role is to absorb the blue light emitted by aequorin and then to change it

into green light. What is the advantage? One explanation is that green wavelengths are a more powerful repellent for lurking predators.

From an accordion to a barrel

At the time of its discovery, the mystery around GFP aroused little interest and it was forgotten until 1992 when its gene was deciphered. Its structure was then resolved in 1996 which brought to light the existence of a fascinating new shape: a barrel. How can a protein acquire such a structure?

All proteins are composed of a succession of amino acids - called a sequence - just as a necklace is composed of a string of pearls. This particular chain of amino acids folds over progressively until it gives the protein its specific shape. How?

First, a number of neighbouring amino acids in the sequence are attracted to one another and, like magnets, they get closer, ultimately establishing very loose contacts known as hydrogen links. Such local links between amino acids create two types of structures: the alpha helix and the beta sheet. In the alpha helix, the attraction sustained by the amino acids twists the protein chain into the shape of a spring. As for the beta sheet, several tiny parts of the sequence - called strands - gather side by side, the end result of which looks like an accordion. (Fig.2).

Both of these local structures then join up to give the protein its global shape in space, i.e. its three-dimensional structure.

In GFP's case, the barrel shape is formed by way of an eleven-stranded beta sheet. Like a piece of paper rolled into a tube, the GFP sheet curls onto itself into a near perfect barrel shape, which is closed at either extremity by amino-acid loops. (Fig.2).

A fluorescent heart

Does the "barrel" structure play a role in GFP fluorescence? The answer is yes. The protein's compact form protects the alpha helix - which contains the light source - from chemical damage. The element responsible for the actual fluorescence is known as the chromophore which consists of a sequence of three amino acids: serine, tyrosine and glycine. To be functional, the chromophore must be subjected to a specific modification catalysed by GFP itself and with the sole aid of oxygen.

Once functional, the protein can diffuse light but it must first of all absorb some. This is the principle of fluorescence. Sunlight is composed of

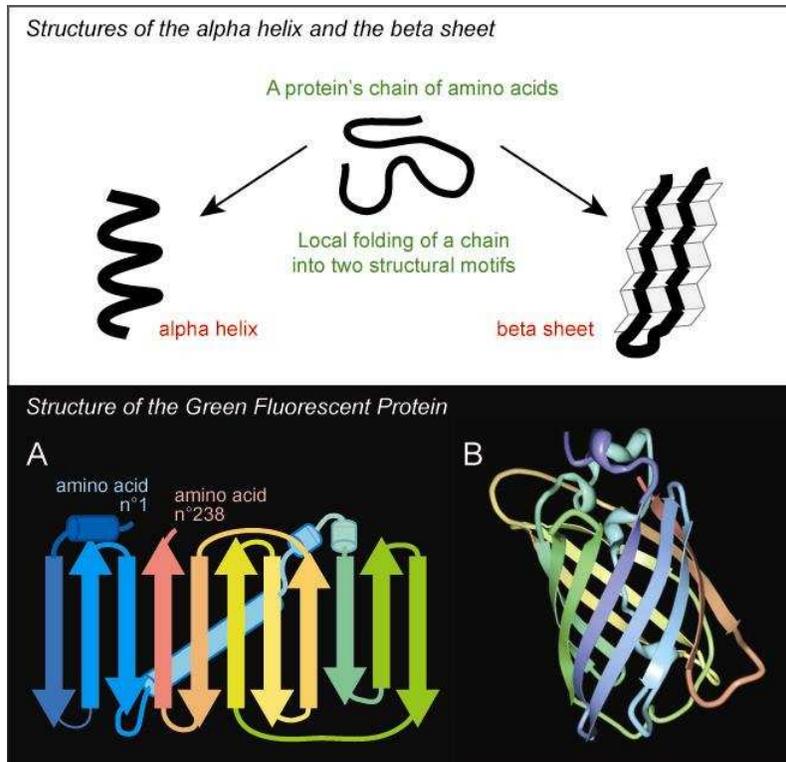


Fig.2 Structures. *Above:* Schematic illustration of the alpha helix and the beta sheet. *Below:* **A.** A two-dimensional representation of the local motifs in GFP: the alpha helices are represented by cylinders and the beta sheet by the group of arrows. **B.** Three-dimensional structure of GFP, which is the result of the folding in space of the alpha helices and the beta sheets.

six colours visible to the human eye (violet, blue, green, yellow, orange and red) and of two imperceptible ones (ultraviolet and infrared). GFP absorbs the ultraviolet and the blue light. The energy captured in the form of light destabilises the protein whose only wish is to revert to its initial state. To do this, it liberates a speck - or photon - of green light.

A radiant career

The extraordinary thing about GFP is that it acquires its own fluorescence. Or almost... It doesn't need an enzyme or anything, just a little bit of oxygen. This is what was so tantalising for biologists. All you need to do is introduce the GFP gene into a cell for it to produce green fluorescence when bathed in blue light. Not only is the protein harmless for the cell but the fluorescence it provides has no effect on the cell's lifespan. This is why GFP has become such a choice experimental tool in the study of biological processes in cells and living organisms.

Of what use are fluorescent cells? Not only does fluorescence make cells visible to the naked eye

but you can also follow their movements and development. If, for example, GFP is inserted into cancerous cells, scientists can follow the progress of tumours in laboratory animals. In the same way, "labelling" neurons with GFP in young mice shows both their migration and their evolution in the brain, thus giving an indication on cerebral development.

GFP is also used to visualize something even smaller than cells: proteins. Several techniques have been developed to study their function. One such technique is used to find out where and when a particular protein is produced - in the brain, the lower limbs or the digestive system for instance (Fig.3). More detailed questions as to the protein's role in the cell, for example, can be answered by way of a second method known as "protein fusion". In practice, the GFP gene is "hitched" to a protein's gene much in the same way as a caravan is to a car. The cells then produce a "fusion protein" in which GFP and the protein of interest are linked, without the latter being altered. As a consequence, the protein can be observed thanks to the fluorescence of GFP which acts as a minute bulb. Such a process gives unlimited information on the protein. For instance, it is possible to

observe exactly when and where a protein is synthesized, if and where it relocates, if it is secreted out of the cell, and where, or if it interacts with other proteins (Fig.4).

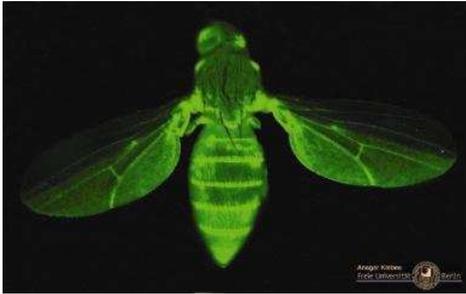


Fig.3 Tracking the "engrailed protein" in the *Drosophila melanogaster* fly. The GFP gene has been modified in such a way that its product is expressed at the same time and in the same place as the "engrailed protein". Thus, by deduction, the green fluorescence indicates that the engrailed protein is normally located at the back of the wings and in certain segments of the abdomen.

This "fusion protein" method is very useful to check whether a particular protein has been correctly inserted into an organism. When an organism is bathed in a blue light, GFP fluorescence shows up and so confirms the presence of the protein in question. Such a technique has been tested in tobacco plants, for example, in order to verify the insertion of a gene which protects the plants against herbicides.

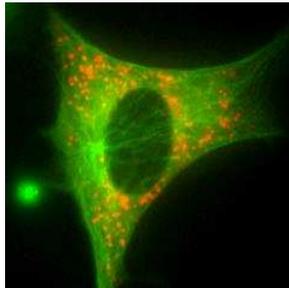


Fig.4 Visualization of tubulin fused with GFP in a mouse cell. Tubulin is a protein involved in cellular architecture. The green fluorescence shows tubulin fibres that stretch across the cell. The dark area denotes the nucleus where the DNA is located.

Besides its obvious qualities, GFP fluorescence is very sensitive to experimental conditions and especially to the variations of cell acidity. However...what could have turned out to be GFP's soft spot has actually revealed to be a novel way of using the protein. The variations of intensity of wild-type or modified GFP fluorescence can be used to indicate cellular fluctuations of

infinitesimal variations such as the evolution of acidity or changes in calcium concentration.

The revolutionary "GFP tool" has stirred the imagination of many a biologist. Would it, for instance, be possible to visualize several phenomena simultaneously? To do so, scientists would have to produce proteins which give off different coloured types of fluorescence. How? Fluorescence also exists in corals and sea anemones which express the fluorescent proteins - FP - that are similar to GFP but glow blue, yellow or orange-red. Consequently, thanks to genetically-modified GFP, scientists can now reproduce all the colours of the rainbow...

Multitudes of modified GFP now exist. Some are resistant to heat while others change colour with time. Among them, one has recently revealed an amazing property: it flashes. And as the temperature increases, the flash becomes slower. This discovery led to what was called a "molecular thermometer" which became a means of controlling an increase or a decrease in temperature in the most delicate experiments.

Biologists are not the only ones to have been seduced by GFP. The beauty of its structure inspired the Swiss sculptor Julian Voss-Andreae who created a major work-of-art in steel. The American "transgenic" artist, Eduardo Kac, created a genetically-modified rabbit: Alba. In 2000, Alba was the first rabbit born with GFP and, when placed under a blue light, she glows fluorescent green! According to Kac, the whole point of the exercise was to discuss the relationship between Science and Art, but also to question the ethics of biotechnology. Other less involved artists have dreamed of fluorescent ornamental plants or Christmas trees whose branches twinkle without tinsel... Experimental instrument or gadget, GFP certainly seems to have a bright future!

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For further information

On the Internet:

- GFP: http://en.wikipedia.org/wiki/Green_fluorescent_protein
- Bioluminescence: <http://en.wikipedia.org/wiki/Bioluminescence>
- Website of Eduardo Kac: <http://www.ekac.org>
- Website of Julian Voss-Andreae: <http://www.julianvossandreae.com>
- About the GFP, Protein Spotlight, "The greenest of us all":
http://www.expasy.org/spotlight/back_issues/sptlt011.shtml
- About Bio-Art, Protein Spotlight, "Bio-Art":
http://www.expasy.org/spotlight/back_issues/sptlt053.shtml

Illustrations:

- Fig.2 A, From Ormö M. et al., "Crystal structure of the Aequorea victoria green fluorescent protein" *Science* 273:1392-1395(1996) PMID: 8703075
- Fig.2 B, Source: PDB ID: 1EMA, M., Ormo, A.B., Cubitt, K., Kallio, L.A., Gross, R.Y., Tsien, S.J., Remington, Crystal structure of the Aequorea victoria green fluorescent protein. *Science* 273 pp. 1392 (1996)
- Fig.3, Source: Ansgar Klebes, Freie Universität, Berlin :
http://genetik.fu-berlin.de/institut/flychip_06.html
- Fig.4, Source: Dr. Ray Truant :
<http://www.science.mcmaster.ca/biochem/faculty/truant/movie.htm>

At UniProtKB/Swiss-Prot:

- Green fluorescent protein, Aequorea victoria (jellyfish): P42212

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