Box 16-1 GFP and the road to Gold

The jellyfish Aeq*uorea victoria* [*Murbach & Shearer, 1902*] is found off the coast of North America, and as a defence mechanism emits bioluminescence from the photocytic cells around the margin of its umbrella. When the Victorian naturalist PH Gosse (who developed and popularised the aquarium) tapped a glass jar containing the jellyfish it quickly lit up with a ghostly greenish-blue light. This is the story of the men who won the Nobel Prize for work on GFP.

Aequorin is a calcium ion-activated photoprotein which in conjunction with the luciferin coelenterazine produces blue light of around 470 nm. This blue light is taken up by green fluorescent protein (GFP) and re-emitted as green light at around 504 nm. The transfer of energy from aequorin to GFP is non-radiative by FRET, so no blue photons are seen. The gene for GFP encodes its own fluorophore, which is what makes it so useful. What is the story behind this protein that has revolutionised modern biological science?

Osamu Shimomura (above) certainly didn't have an easy start, and only dogged determination and a brilliant mind changed his fortune for the better. Caught up in wartime Nagasaki his schooling was severely disrupted, yet in post-war Japan he managed to isolate coelenterazine from *Cypridina* (*vargula hilgendorfii*), a purification which had defeated an American group at Harvard. As a result Shimomura was invited to America to work on the bioluminescent reaction of *Aequora*. There he worked out the bioluminescent production of blue light when aequorin was activated (at will by the jellyfish) using calcium ions in seawater. The protein giving off green light was crystallized in 1974 and was named - rather pragmatically and uninspiringly - Green Fluorescent Protein. The fluorophore was determined in 1979.

For the next 15 years little happened concerning GFP until Martin Chalfie, a molecular biologist heard about it during a lunchtime seminar and wondered if it could be expressed in another organism: *Caenorhabditis elegans* a thin, transparent nematode worm amenable to study by light microscopy, and whose cell fate was being worked out for which a fluorescent marker would make a superb tool. Chalfie was looking for a dynamic marker that could be introduced non-invasively into living organisms, to supercede the existing static antibody and enzyme-substrate staining protocols then in current use. His ignorance of fluorescent proteins kept him unaware of the difficulties most people - including Shimomura – considered there would be to get GFP to fluoresce in isolation outside of its parent organism. Eventually, in 1993 Chalfie succeeded in introducing the cDNA of GFP heterologously into, first the bacterium *E. coli*, then into *C. elegans*. The GFP fluoresced without requiring any co-factors, enzymes or other proteins. It was an ideal reporter molecule.

(Glo-fish: fluorescent zebrafish)

Roger Tsien had previously worked on calcium signaling. His interest was in identifying fluorescent markers to dissect and analyse the 'cellular anthropology' of higher organisms. By 1992 Tsien was micro-injecting fluorescent dyes conjugated to protein into cells. With the announcement of Chalfie's work, Tsien realised that the native GFP signal - "dim, fickle and spectrally impure"- could be engineered to glow much brighter. From his work, and that of others, we now have a complete palette of fluorescent proteins that span the visible spectrum and into the infra-red. In 2008, Shimomura, Chalfie and Tsien shared the Nobel Prize in Chemistry.

Who was the fourth man? Someone had identified and had gone on to isolate the cDNA sequence of GFP and clone the gene, which was crucial to the success of the GFP story. That man was Doug Prasher. After his work on GFP and before publication of the cDNA sequence, Prasher's funding dried up and he eventually left science. Only two people requested cDNA samples from him: Chalfie and Tsien.

References:

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