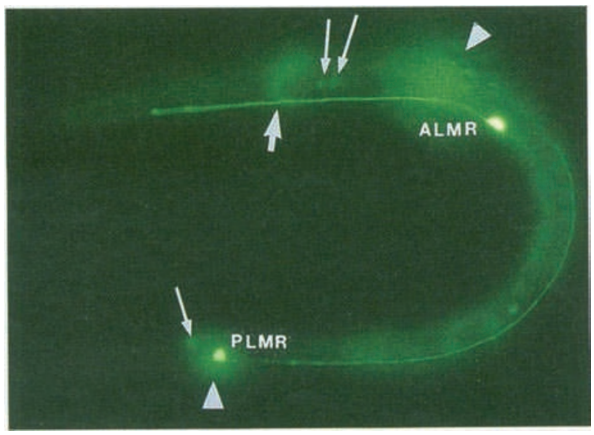
MILESTONE 18

GFP: the green revolution



Cell bodies of two touch-receptor neurons from the worm *Caenorhabditis elegans* are labelled with green fluorescent protein expressed from the gene encoding β-tubulin. Image is reproduced, with permission, from M. Chalfie *et al.* © (1994) American Association for the Advancement of Science.

In 1994, Chalfie *et al.* published a report in *Science* showing that the green fluorescent protein (GFP) from the jellyfish *Aequorea victoria* could be used as a marker for protein localization and expression in living bacteria and worm cells, in the absence of any auxiliary factors from *A. victoria*. This demonstration of GFP as a tool to study proteins *in vivo* fundamentally altered the nature and scope of the issues that could be addressed by cell biologists.

GFP was first discovered fortuitously in 1962 by Shimomura and colleagues during the purification of the bioluminescent protein aequorin from *A. victoria*. Subsequent purification, crystallization and reconstitution of energy transfer *in vitro* from aequorin to GFP by Morise and colleagues in 1974 provided insight into the fluorescent properties of GFP, which was shown to emit green light on energy transfer from aequorin.

Whether GFP would need aequorin and possibly other factors from the jellyfish to fluoresce in heterologous systems remained an open question for many years. In 1992, 30 years after its discovery, Prasher *et al.* cloned the gene encoding GFP, paving the way for experiments to assess its utility as an *in vivo* tag for proteins. Two years later, Chalfie *et al.* showed that GFP could fluoresce when expressed in bacteria and worm cells. In the worm, GFP was expressed from the promoter of a gene that encoded β-tubulin. Its spatial and temporal expression in specific neurons of the worm mimicked that of the endogenous β-tubulin gene, thus proving that GFP could be a faithful marker for monitoring gene-expression patterns. Soon thereafter, Roger Tsien's laboratory engineered native GFP to become brighter, more photostable and excitable at a wavelength that matched that of conventional microscope filter sets, increasing its practical usability (as summarized in his 1998 review). The next breakthrough in GFP technology came with the development of GFP variants to produce blue, cyan and yellow fluorescent proteins (also described in Tsien's review), thus enabling imaging experiments that could follow multiple tagged proteins in cells and organisms.

> "This development opened the floodgates to countless uses of GFP…
> *Vladislav Verkhusha*"

Almost half a century after GFP was discovered, the 2008 Nobel Prize in Chemistry was awarded to Osamu Shimomura, Martin Chalfie and Roger Tsien "for the discovery and development of the green fluorescent protein, GFP", acknowledging the seminal nature of this discovery.

Sowmya Swaminathan, Senior Editor,
Nature Cell Biology

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