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Enlightening biology

This year's Nobel Prize in Chemistry recognized the researchers whose work literally illuminated biological processes.

The 2008 Nobel Prize in chemistry has been jointly awarded to a neuroscientist and two biochemists. The Royal Swedish Academy of Sciences has selected Osamu Shimomura (Marine Biological Laboratory at Woods Hole and Boston University Medical School), Martin Chalfie (Columbia University) and Roger Y. Tsien (University of California at Irvine) for their contributions to "the discovery and development of the green fluorescent protein, GFP."

GFP has become one of the most commonly used reagents in our ever-growing biochemical toolkit. But its usefulness as a means to monitor processes such as gene expression, cell division, cellular transport pathways, and protein localization and interaction networks emerged only in the past 15 years and was a discovery that was more than 30 years in the making.

The story begins with Shimomura, who used his experience working on the bioluminescent properties of the mollusk *Cypridina* to investigate the green bioluminescence of the jellyfish *Aequorea victoria* in the early 1960s. He identified a protein named aequorin as the active bioluminescent component, but surprisingly it emitted blue, not green, light in a Ca^{2+}-dependent manner. Further work resulted in the isolation of a second protein that emitted a strong green fluorescence when exposed to UV light and would come to be known as GFP. Shimomura and co-workers suggested that, on the basis of the spectral properties of aequorin and GFP, a fluorescence resonance energy transfer (FRET)–type reaction was occurring, with aequorin acting as donor and GFP as acceptor, explaining the jellyfish's green bioluminescence. Additional work from Shimomura in the 1970s suggested that the GFP chromophore was formed from a chemical reaction between three residues of the protein's peptide chain, a fact that was later confirmed by others.

Although the identity of the chromophore was now clear, it was still unknown what, if any, enzymatic processing steps were needed to promote its formation, and the assumption was that any recombinantly produced GFP would not fluoresce. However, Chalfie saw the potential of using GFP in a gene expression reporter system, similarly to what was being done with *lacZ* at the time. After the 1992 cloning of the GFP gene by Douglas Prasher and colleagues at the Woods Hole Oceanographic Institution, Chalfie obtained a copy of the clone and expressed it in *Escherichia coli*. The protein fluoresced in the presence of blue light, suggesting that it could indeed be expressed in heterologous systems without any specific auxiliary factors. In addition, Chalfie found that putting *gfp* under the control of the β-tubulin gene promoter in *Caenorhabditis elegans* allowed visualization of β-tubulin expression patterns during worm development, providing proof that GFP could be used effectively as a gene expression marker.

Later work, some of which originated in the Tsien laboratory, demonstrated expression of a fluorescent form of GFP in the model systems *Saccharomyces cerevisiae* and *Drosophila melanogaster*. Importantly, Shengxian Wang and Tulle Hazelrigg at Columbia University demonstrated that GFP could be fused to a protein of interest and that the activity of both proteins in the fusion construct could be kept. But Tsien's major contributions would come from the characterization of chromophore maturation and genetic engineering of GFP and GFP-like proteins. By expressing GFP anaerobically in bacteria, Tsien's group revealed that the only cofactor necessary for chromophore formation was molecular oxygen, and they were able to suggest a chemical scheme for it. By extensively tinkering with the GFP sequence, Tsien altered the brightness of GFP and its spectral properties, yielding variants that emitted colors ranging from blue to yellow. His development of several variants of red fluorescent proteins, based upon the DsRed protein from the coral *Discosoma*, helped to expand the color palette of this tool, making it possible to simultaneously monitor expression patterns of several different genes and track protein movement within the cell.

The discovery and subsequent manipulation of GFP represent technical breakthroughs in how we visualize living systems, allowing spatiotemporal monitoring of previously invisible processes. They strongly echo the accomplishments of earlier Nobel prize winners, among them George Palade (1912–2008). Palade, who passed away in early October, shared the 1974 Nobel Prize in Physiology or Medicine with Albert Claude and Christian de Duve for pioneering the field of modern cell biology through work done at the Rockefeller Institute for Medical Research (now Rockefeller University) in the late 1940s and 1950s. Palade developed techniques used to isolate cellular components, but he was most recognized for his ability to combine cell fractionation with the nascent technology of electron microscopy, making great strides in the improvement of this imaging technique. His technical developments contributed to a new spatial resolution in examining cellular compartments. Among the many biological insights that arose from his work were the discovery of small cytoplasmic granular components that would later be identified as ribosomes and the identification of the protein-secretory pathway that connects the endoplasmic reticulum to the Golgi apparatus.

Although awarded more than 30 years apart, the research upon which these Nobel prizes were based provided tremendous insight into cellular processes at the time the work was performed. The pictures that have been produced as a result have provided an invaluable understanding of fundamental biology. ■