

The 2008 Nobel Prize in Chemistry: Osamu Shimomura, Martin Chalfie, and Roger Y. Tsien : the Green Fluorescent Protein

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Abstract: The 2008 Nobel Prize in Chemistry was awarded equally to the Japanese Osamu Shimomura and the Americans Martin Chalfie and Roger Y. Tsien “for the discovery and development of the green fluorescent protein GFP.” Relevant research by the laureates and others, especially Douglas Prasher, is discussed along with applications of their work.

On October 10, 2008 Svenska Kungliga Vetenskapsakademien (The Royal Swedish Academy of Sciences) awarded the Nobel Prize in Chemistry jointly to the Japanese Osamu Shimomura (b. 1929; Marine Biological Laboratory, Woods Hole, MA and Boston University Medical School, Boston, MA) and the Americans Martin Chalfie (b. 1947; Columbia University, New York, NY) and Roger Y. Tsien (b. 1952; University of California, San Diego, La Jolla, CA) “for the discovery and development of the green fluorescent protein GFP” [1–6] (Figure 1).

The history of the research crowned by the Nobel Committee extends over half a century and three acts. It began in 1955 with the description of a green fluorescent substance in jellyfish (Shimomura). This discovery was followed in the 1990s by its application as a luminous tracer molecule in biology and as a universal genetic marker (Chalfie) and later extended to a palette of colors beyond the green (Tsien) [7–10].

Osamu Shimomura, Discoverer of the Green Fluorescent Protein [7–11]

Osamu Shimomura was born on August 27, 1928 in Fukuchiyama, Kyoto Prefecture, Japan. His father was an army officer who, after serving in Manchuria and Osaka, moved with his family to Ishaya, a district of Nagasaki. On the day of the city’s bombing, August 9, 1945, young Osamu witnessed a flash of light, a strong pressure wave, and the fall of black rain about 12 km. (7.4 miles) from the epicenter of the nuclear blast (Figure 2). In 1951 Shimomura graduated from Nagasaki College of Pharmacy, where he received his B.S. degree. He later worked as a research student in organic chemistry at Nagoya University, and from 1955 to 1958, the year that he received his M.S. degree in organic chemistry, he was assistant to Professor Yoshihamasa Hirata (1915–2000) [12] (Figure 3).

Hirata was interested in bioluminescence—the phenomenon in which chemical reactions within living organisms produce light. He asked his assistant to find out why the crushed remains of a small crustacean type of mollusc, *Cypridina luciferin* (“sea-firefly,” in Japanese), emitted a green light when moistened with water. One may be surprised

that Hirata gave such an inexperienced assistant such a difficult task. A leading American research group had tried for a long time to isolate this material, so Hirata decided that he did not want to give the job to a student who needed to succeed in order to get his or her doctorate [8].

In 1956 Shimomura discovered that the emission of light came from a protein that glowed 37,000 times more brightly than the crushed mollusc. In 1960 he received his Ph.D. degree and was awarded a three-year Fulbright grant. He joined Frank Johnson (1908–1990) [13] (Figure 4) at Princeton University as a Research Associate in Biology. Together they investigated the bioluminescence of a medusa, the green jellyfish *Aequorea victoria* (or crystal jelly) (Figures 5 and 6), found on the coast of the Pacific Ocean outside Friday Harbor in Puget Sound in the state of Washington (Figures 7 and 8).

During the summer of 1961 Shimomura and Johnson collected about 10,000 jellyfish (Figures 9 and 10). The team extracted a few milligrams of a protein that they named *aequorin*, a blue bioluminescent protein [14–18] located in the outer part of the medusa (Figure 11). The isolation of sufficient amounts of aequorin from jellyfish was a formidable task. Each year, in one or two summer months, 50,000 species were collected, with a total of 850,000 jellyfish within 19 years.

Shimomura returned briefly to Japan from 1963 to 1965 as Associate Professor of Earth Science at Nagoya University before returning to Princeton University as a Research Biochemist in Biology. At Princeton he pursued the investigation and discovered that aequorin, responsible for the self-luminescence of *Aequorea victoria*, emits *blue* not *green* light. The phenomenon was due to a second, hereto unknown, protein. Several more years were required to recognize the mechanism of fluorescence resonance energy transfer (FRET) [19] involved in the phenomenon: the green light from the jellyfish was due to electronic excitation of the “green” protein by radiationless transfer of the blue self-luminescence from aequorin, followed by emission of the green fluorescence. Shimomura also found that *Aequorea* releases calcium ions, which bind to aequorin, which then releases blue light upon calcium binding (Figure 12). Shimomura and Johnson called it the “green protein,” but it was later called the “*green fluorescent protein*” (GFP), a protein composed of 238 amino acids (Figure 13).

According to the Royal Swedish Academy of Sciences,

* Series Editor contribution.



Figure 1. The 2008 Chemistry Nobel Laureates. (Courtesy, Nobel Foundation).



Figure 2. Ruins of the Medical College of Nagasaki, 1945. (Courtesy, Nobel Foundation).



Figure 3. Professor Yoshihamasa Hirata (1915–2000). (Courtesy, Nobel Foundation).



Figure 4. Professor Frank Johnson (1908–1990). (Courtesy, Nobel Foundation).



Figure 5. Jellyfish *Aequorea victoria*, in which Shimomura Discovered GFP. (Courtesy, Nobel Foundation).

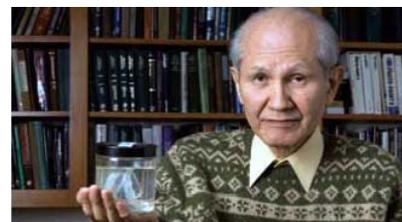


Figure 6. Osamu Shimomura Holding Some of the Hundreds of Thousands of Jellyfish Collected from Friday Harbor. (Courtesy, Nobel Foundation).



Figure 7. Friday Harbor, 1961. (Courtesy, Nobel Foundation).



Figure 8. Friday Harbor Laboratories, University of Washington, 1961. (Courtesy, Nobel Foundation).



Figure 9. Frank Johnson Collecting Jellyfish. (Courtesy, Nobel Foundation).

Without the pioneering research of Shimomura, mainly with classical methods for protein purification and spectroscopy, it is likely that the GFP revolution would have been delayed by decades or even remained one of the hidden secrets of the Pacific Ocean [7].

Shimomura acted as Research Biochemist until 1982. During the next two decades he was Adjunct Professor at the Boston University School of Medicine and Senior Scientist at the Marine Biological Laboratory (MBL) in Woods Hole, MA. Since his official retirement in 2003 at the age of 75 he maintains his private "Photoprotein Laboratory" in the basement of his home.

Shimomura received the Pearse Prize of the Royal Microscopical Society in 2004, the Emile Chamot award in 2005, and the Asahi Prize in 2006. In 2006 he published a comprehensive overview of the biochemical aspects of some 35 types of all the luminescent organisms known up to that time [20]. It was the first book to provide chemical information on all known bioluminescence systems in a single volume.

Shimomura is the 56th Nobel Prize scientist affiliated with the Marine Biological Laboratory since its founding in 1888, following Roger D. Kornberg (b. 1947), the 2006 Nobel Laureate in Chemistry [21]. Gary Borisy, the MBL director and Chief Executive Officer and a prominent cell biologist, declared,

GFP technology has revolutionized what we can see at the most fundamental levels of life. GFP is revealing, for example, how proteins move and interact in cells. Now that the human genome is sequenced, understanding protein function is one of the greatest scientific and medical challenges of our time [22].

Shimomura was awarded the Nobel Prize 40 years after his discovery of GFP. On receiving the news, he was surprised:

Since many years ago, I had been told that I might win the prize in medicine, but I hadn't been thinking about the chemistry prize....Back in 1979, when the chemical structure of GFP was defined, I thought that my job had finished. I still held an interest in it, but I felt that I had done what I had to do...[23]. I don't do my research for application or any benefit. I just do my research to understand why jellyfish luminesce and why that protein [GFP] fluoresces [24].

Asked about the achievements of his co-laureates Shimomura said,

Chalfie came up with the applications for GFP, and Tsien improved it. I think I was able to receive the prize because they were here [23].

The discovery of GFP set into motion researches that continue to grow with more than 100 papers published per month and more than 6,000 publications up to 2005 [16].

Shimomura's Family Life

Shimomura met his wife Akemi, an organic chemist, at Nagasaki University. She also became a member of the MBL staff and was her husband's assistant from 1982 to 2001. The couple lives in Falmouth, Massachusetts and have two children. Their son Tsutomu is a computer security expert, who helped the Federal Bureau of Investigation (FBI) to arrest the controversial computer hacker Kevin Mitnick (b. 1963). The couple's daughter Sachi is Associate Professor of English

at Virginia Commonwealth University, Richmond, VA and the author of a critically acclaimed book of literary criticism [25].

Douglas Prasher's Interlude in GFP Research [26–31]

Two decades after Shimomura's discovery, the American molecular biologist Douglas C. Prasher (b. 1951) [26, 27] (Figure 14) was the first to realize the potential use of GFP as a tracer molecule. With a Ph.D. degree in biochemistry from the Ohio State University in 1979, he was interested in the chemistry of how certain animals are able to glow [28, 29]. From 1979 to 1983 he carried out research on genetics and biochemistry at the University of Georgia, Athens, where he identified aequorin [27, 31]. He then joined the Department of Biology at the Woods Hole Oceanographic Institution in Woods Hole, Massachusetts, where he studied bioluminescence. In the late 1980s he applied to the National Institutes of Health for a five-year grant to discover the protein (GFP) that gives the jellyfish its glow. He said, "I knew it could serve as a genetic marker, and it would be really, really useful, which it has turned out to be" [29]. However, his application was rejected.

In 1988 Prasher was successful in obtaining a three-year, \$200,000 grant from the American Cancer Society to try to clone the gene for GFP. The time and funding were sufficient for him to isolate the gene but not enough to pursue any applications [29]. After Chalfie and Tsien had each contacted him, he shared his findings with them. Prasher's idea was to use GFP as a tracer for the investigation of a molecule to which it was attached, *i.e.*, a GFP from a jellyfish could be attached to a protein and used to visualize the labeled molecule by merely irradiating it with ultraviolet light. Furthermore, GFP was a small molecule and less likely to hinder the function of the investigated protein. Prasher reported the cloning of GFP and the sequence of its 238 amino acids [28].

Prasher did not secure tenure at Woods Hole. He worked as a population geneticist for the U.S. Department of Agriculture at its Otis Plant Protection Center in Cape Cod, Massachusetts but later transferred to its Plant Germplasm Quarantine & Biotechnology Laboratory in Beltsville, Maryland, where he developed methods for identifying pests and insects [27, 29]. When funding was cut there, he began to experience bouts of depression and moved to Huntsville, Alabama, where he worked for AZ Technology, a NASA subcontractor that was developing mini-chemistry laboratories to be used in a potential human flight to Mars, a job that he enjoyed. After funding for this project was eliminated, he remained in Huntsville but was unemployed for a year. With his life's savings exhausted and unable to find a job in science, he took a job driving a shuttle bus for Bill Penney Toyota in Huntsville for \$10 an hour [27, 29].

After the award of the 2008 Nobel Prize in Chemistry was announced, Prasher said that he would have been uncomfortable if he had been selected as one of the three winners by nudging out one of the other nominees:

There are other people who would have deserved it a whole lot more than me. They worked their butts off over their entire lives for science, and I haven't [29].

In a telephone interview of October 9, 2008 with National Public Radio, one of Prasher's former colleagues called his current predicament "a staggering waste of talent" [26].



Figure 10. Jellyfish Collectors, 1974. (Courtesy, Nobel Foundation).



Figure 11. Shimomura and Johnson extracted GFP from the edges of the jellyfish's "umbrella." (Courtesy, Nobel Foundation).

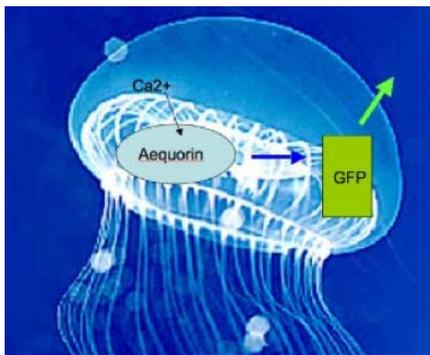


Figure 12. Intermolecular Energy Transfer in the Jellyfish *Aequorea victoria*. The protein aequorin emits blue light upon bonding with calcium. The blue light is then totally absorbed in the GFP, which in turn gives off the green light [9]. (Courtesy, Nobel Foundation).



Figure 13. The amino acids of GFP are linked together in a long chain, which folds up into the shape of a beer can. The amino acids inside the can absorb ultraviolet and blue light, and they fluoresce green. (Courtesy, Nobel Foundation).

Chalfie and Tsien expressed their gratitude to Prasher by sponsoring his and his wife's attendance at the Nobel Prize ceremony in Stockholm.

Martin Chalfie: GFP, a Luminous Genetic Tag [7–9, 32–38]

Martin Lee Chalfie (Figure 15) was born on February 1, 1947 and grew up in Chicago, Illinois. He entered Harvard University, Cambridge, Massachusetts in 1965 intending to major in mathematics, but he eventually became "attracted to biochemistry because he could do a little bit of everything: chemistry, math, and biology. The subject also seemed new and exciting" [32]. He spent the summer after his junior year working in Klaus Weber's laboratory, but "it was so disheartening to completely fail that I decided I shouldn't be in biology" [38]. He completed his major with courses in law, theater, and Russian literature. He competed on the swimming team, was named captain for his senior year, and was awarded the Harold S. Ulen Trophy, which is "given annually to the senior who best demonstrates those qualities of leadership, sportsmanship and team cooperation as best exemplified by Harold S. Ulen," who was Harvard's head swimming coach from 1930 to 1959 [38].

After graduating in 1969 and still uncertain about a career in research, Chalfie accepted several short-term jobs such as selling dresses for his parents' Chicago dress manufacturing business and teaching high school at Hamden Hall Country Day School in Hamden, Connecticut [33, 38]. He eventually applied to work at the laboratory of José A. Zadunaisky, M.D. (1932–2005) at Yale University, New Haven, Connecticut. He returned to Harvard for graduate studies, and in 1977 he received his Ph.D. degree in neurobiology. That same year he moved to Cambridge University in the United Kingdom to work as a postdoctoral fellow with Britons Sydney Brenner (b. 1927) and John Sulston (b. 1942), who were to share the 2002 Nobel Prize in Physiology or Medicine with the American H. Robert Horwitz (b. 1947). After five productive years in England ("My time as a postdoc made me the scientist I am today" [32], Chalfie joined the Department of Biological Sciences of Columbia University, where he continued his studies on the glow-worm *Caenorhabditis elegans* [39] (Figure 16).

In 1988 or 1989 (Chalfie does not remember the exact date [36]) at a Columbia University seminar Chalfie heard of the fluorescent protein for the first time and immediately realized that it could be useful for his research on *Caenorhabditis elegans*:

I work on this transparent animal, this is going to be terrific!
I'll be able to see the cells within the living animal [36].

It would act as a glowing green for various reactions in the worm.

When Prasher finally found the gene for GFP after years of research, Chalfie was on sabbatical at the University of Utah. According to Prasher, "At the time, he was never at the phone. He had a girlfriend [Tulle Hazelrigg] out there" [27]. Prasher published his description of the GFP gene [28]. When the two finally made contact, Prasher sent Chalfie a copy of the sequenced gene. Chalfie had the idea of fusing it with other proteins, beginning with *Escherichia coli*, which fluoresced green upon irradiation with ultraviolet light [40]. This showed that GFP glows without any additives unlike aequorin and



Figure 14. Douglas Prasher (b. 1951). (Courtesy, Nobel Foundation).

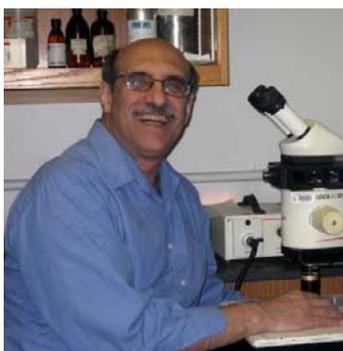


Figure 15. Martin Chalfie (b. 1947). (Courtesy, Nobel Foundation).

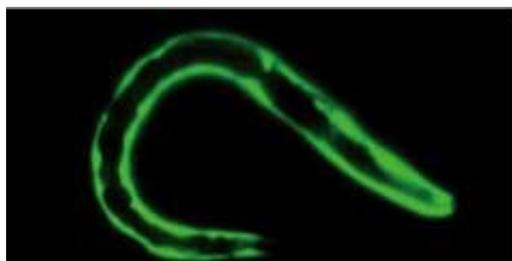


Figure 16. *Caenorhabditis elegans* is a small (about 1 mm. long) transparent soil nematode (earthworm) found in temperate regions. (Courtesy, Nobel Foundation).

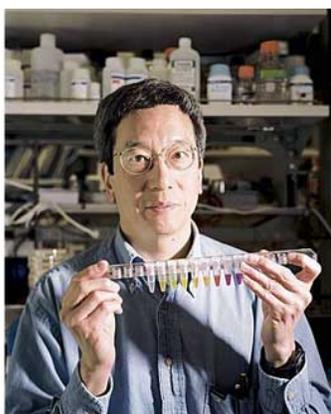


Figure 17. Roger Y. Tsien with a Palette of Fluorescent Biomolecules. (Courtesy, Nobel Foundation).

other bioluminescent proteins that require a continuous supply of energy-rich molecules. For the first time a protein could be located and followed in a living organism. This discovery marked the first use of GFP as a fundamental tool of cell biology, developmental biology, genetics, neurobiology, and the medical sciences.

Chalfie is the William R. Kenan, Jr. Professor of Biological Sciences at Columbia University. In 2006 he was elected to the U.S. National Academy of Sciences. He married Tulle Hazelrigg, the geneticist and noted fruit fly researcher who completed the first successful GFP fusions. She is a professor and research scientist at Columbia who is also pursuing research with GFP markers [27, 41, 42]. According to Roger Y. Tsien, the first fusions to GFP ever were made by Marty Chalfie's wife, Tulle Hazelrigg [43].

Chalfie's learning about his selection as Nobel laureate presents an amusing incident that has elicited considerable interest around the world and will probably become an anecdote in the lore surrounding the Nobel Prize. He slept through the early-morning notification call, but in his later telephone conversation of October 8, 2008 with Adam Smith, Editor-in-Chief of Nobelprize.org, Chalfie explained:

This is a sort of ridiculous situation, but sort of funny. I woke up at ten after six, and I realised that they must have given the Prize in Chemistry, so I simply said, "Okay, who's the schnook that got the Prize this time?" And so I opened up my laptop, and I got to the Nobel Prize and I found out I was the schnook! [36, 44].

Roger Y. Tsien, Polychromatic Genetic Tags [7–9, 47–50]

Roger Yonchien Tsien (Figure 17) was born in New York City on February 1, 1952 of parents from the Zhejiang Province of China. His father, Hsue-Chu Tsien, was a mechanical engineer, and his mother's brothers are professors at the Massachusetts Institute of Technology. His brother, Richard Tsien, is Professor of Molecular and Genetic Medicine at the Stanford University School of Medicine. His father's cousin, Tsien Hsue-shen, was a co-founder of the Jet Propulsion Laboratory (JPL) at the California Institute of Technology and later director of the Chinese ballistic missile program. In speaking of his research on molecular engineering Tsien joked, "I'm doomed by heredity to do this kind of work" [50].

Tsien grew up in Livingston, New Jersey, where he attended elementary and high school. At the age of 16, he won first prize in the Westinghouse National Talent Search with a project investigating how metals bind to thiocyanate [50]. He attended Harvard College on a National Merit Scholarship and graduated *summa cum laude* with a B.S. degree in chemistry and physics in 1972 at the age of 20. He then received a Marshall Scholarship to attend the Physiological Laboratory at the University of Cambridge, UK, where he received his Ph.D. degree in physiology from Churchill College in 1977. He remained in Cambridge as a three-year postdoctoral fellow at Gonville and Caius College. From 1982 to 1989 he was a faculty member of the University of California, Berkeley. Since 1989 he has been Professor of Pharmacology at the University of California, San Diego School of Medicine and Professor of Chemistry and Biochemistry at the University of California, San Diego in La Jolla. Tsien is also an investigator of the Howard Hughes Medical Institute [47].

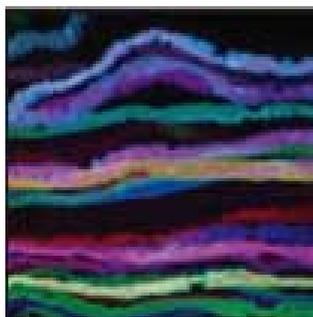


Figure 18. Nerve Cells in a Mouse's Brain Fluorescing in all the Colors of the Rainbow. (Courtesy, Nobel Foundation).

Tsien's primary contribution to GFP was the elaboration of varieties with many new colors that glowed longer and with higher intensity (Fig. 17). He exchanged various amino acids among the 238 that constitute GFP, a procedure that allowed the protein to both absorb and emit light with different wavelengths in other parts of the molecule. These variants of GFP shine more strongly and in colors such as cyan, blue, and yellow. Researchers could now mark different proteins in different colors to visualize their interactions.

Tsien was first unable to produce a variety of GFP with a red color. Red light penetrates biological tissue more readily and would be useful in studying cells and organs inside the body. Russian scientist Sergey A. Lukyanov (b. 1963) found GFP-like proteins in fluorescent corals, including a red one, "Dsred," which consisted of four amino acid chains instead of one for GFP and which was less useful as a fluorescent tag [9, 51]. Tsien's group succeeded in reshaping Dsred, which can now be easily connected to other proteins. It joined other proteins with names according to the color that they glow: mPlum, mCherry, mStrawberry, mOrange, and mCitrine. The "Tsien palette" was completed by proteins from other researchers and companies, and it includes all colors of the rainbow. Three of these proteins were used in genetically modified mice to produce varying amounts of the colors yellow, cyan, and red within the nerve cells of their brains. The result was a mouse brain that glowed in the colors of the "brainbow" (Figure 18).

Tsien received numerous honors and awards, including the Young Scientist Award from the Passano Foundation (1951), the Columbia University Alden Spencer Award in Neurobiology (1991), the Belgian Artois-Baillet-Latour Health Prize (1995), the Canadian Foundation International Award (1995), the Basic Research Prize of the American Heart Association (1995), the Pearse Prize of the Royal Microscopical Society (2000), the American Chemical Society Award for Creative Invention (2002), the Heineken Prize for Biochemistry and Biophysics of the Royal Netherlands Academy of Sciences (2002), the Christian B. Anfinsen Award of the Protein Society, and Israel's Wolf Prize in Medicine (2004) "for his seminal contribution to the design and biological application of novel fluorescent and photolabile molecules to analyze and perturb cell signal transduction." He received the Herbert Sober Lectureship from the American Society for Biochemistry and Molecular Biology (2000).

Tsien was elected to the Institute of Medicine (1995), the American Academy of Arts and Sciences (1998), the National Academy of Sciences (1998), and the UK's Royal Society as a Foreign Fellow (2006).

The Nobel Ceremony

In Stockholm on December 10, 2008, the 112th anniversary of the death of Alfred Bernhard Nobel (1833–1896), each of the three laureates received the Nobel Certificate and Gold Medal from the hands of Swedish King Carl XVI Gustav and shared equally the 10 million Swedish kronor (\$1,241,570) prize money [52]. In his presentation speech to the laureates, Måns Ehrenberg, member of the Royal Swedish Academy of Sciences and the Nobel Committee for Chemistry and Professor of Molecular Biology at Uppsala University, addressed the laureates:

You are rewarded for the discovery and characterisation of the green fluorescent protein, for first expressing GFP in fluorescent form in important model organisms and for the development of GFP and its homologues to a universal set of genetic tags for protein localisation, protein movement and protein interactions in the cells of all types of organisms [53].

Two days earlier the laureates had presented their Nobel lectures at the Aula Magna of Stockholm University [54–56]. Four days earlier Tsien participated in a Nobel Symposium, "Beyond Genes," at Karolinska Institutet, Stockholm, where he gave a lecture titled "Imaging Protein Sociology and Cell Signaling" [57].

On December 10, 2008 in his banquet speech, Tsien magnanimously conceded:

We [three laureates] never directly collaborated with each other, nor was GFP the prime focus of any of our research careers, yet our contributions fortunately synergized with those of many other people, for example Douglas Prasher, to revolutionize the way that scientists image molecular events inside living cells and organisms. So we also thank the many collaborators and colleagues who really made this revolution happen....On the personal side, we owe great debts to our spouses Akemi Shimomura, Tulle Hazelrigg, and Wendy Tsien, who have patiently (well, *almost* always patiently) endured and even abetted our maniacal obsessions with our research....We hope this prize reinforces recognition of the importance of basic science as the foundation for practical benefits to our health and economies [58].

Quite appropriately Tsien warned against the disastrous human aggression against the environment. He deplored the decline in the population of jellyfish in their Pacific Northwest habitat by more than a thousand-fold over the last ten years and noted that "fortunately, the crucial research on GFP was done before the population collapse" [58]. However, he asked, "What other potential scientific breakthroughs may never happen because of man-made pollution and global warming?" [58].

He concluded his speech by singing the praises of the lowly jellyfish and by warning about our deleterious actions on the oceans:

While environmentally friendly or so-called "green" chemistry has become the rage in the chemical community, no human chemist can yet match what a single jellyfish gene directs: 238 ordered condensations + 1 cyclization + 1 oxidation, all done in a few minutes in aerated water with no protected groups, only one slightly toxic byproduct, and essentially 100% yield of an extremely useful product that literally glows green. Corals produce yellow and red fluorescent proteins with the same chemistry plus one extra oxidation. Yet coral reefs are also



Figure 19. Green Fluorescent Protein, Created by Julian Voss-Andreae in 2004. (Courtesy, Julian Voss-Andreae).



Figure 20. Steel Jellyfish, Created by Julian Voss-Andreae in 2006. (Courtesy, Julian Voss-Andreae).



Figure 21. Eduardo Kac and Alba, the Fluorescent Bunny. (Courtesy, Eduardo Kac).



Figure 22. Kac's white bunny labeled with a gene from jellyfish and illuminated with blue light glows a bright green light. (Courtesy, Eduardo Kac).



Figure 23. Glofish Fluorescent Fish Photos. (Courtesy <http://www.glofish.com>).

under world-wide jeopardy, due to acidification and warming of the oceans. So my final thanks are to both the jellyfish and corals: long may they have intact habitats in which to shine! [58].

Exotic Uses for GFP

In addition to its use in scientific research GFP has been applied in various fields including the fine arts, such as sculptures based on the structure of GFP, creation of a green fluorescent rabbit, and the breeding of fluorescent fish and pigs. Julian Voss-Andreae (b. 1970), a German-born physicist turned artist who, after thinking about using some larger biomolecules as new candidates to demonstrate matter's wave property as he had done with C-60 buckyballs, began to specialize in "protein sculptures" [59, 60]. He has created sculptures based on the structure of GFP, including the 5'6" (1.70 m.) tall "Green Fluorescent Protein" (2004) (Figure 19) and the stainless steel 4'7" (1.40 m.) tall "Steel Jellyfish" (2006) [61] (Figure 20). The latter is currently on display at the site of Shimomura's discovery of GFP, the University of Washington's Friday Harbor Laboratories on San Juan Island.

Eduardo Kac (b. 1962) [62] (Figure 21), a contemporary Brazilian-born American artist who lives and works in Chicago considers himself a "transgenic artist" or "bio-artist." He uses biotechnology and genetics to create provocative works that explore scientific techniques and critique them. Internationally known for his interactive net installations and his "bio-art," he commissioned a French laboratory to create a genetically modified rabbit impregnated with a GFP gene from a jellyfish. This glowing rabbit, dubbed "Alba," fluoresces green under a specific blue light [63, 64] (Figure 22).

The U.S. company Yorktown Technologies markets green fluorescent *zebrafish* (*Danio rerio*) (GloFish)TM available in three striking colors—Starfire RedTM, Electric GreenTM, and Sunburst OrangeTM to aquarium shops [65, 66] (Figure 23). It was the first genetically modified animal to become publicly available as a pet. In 2006 green fluorescent pigs were bred by a group of researchers led by Wu Shinn-Chih at the Department of Animal Science and Technology at National Taiwan University [67].

Microorganisms can be made to glow in the presence of heavy metals, explosives such as TNT (trinitrotoluene), and other chemical substances, allowing them to be used as sensors to find materials in the environment. GFP has also been proposed for detecting arsenic in water wells.

Conclusion

Chemical educators will find useful classroom and laboratory material available in books [68–70] and

experiments [71]. In viewing the events described in our article, Tsien stated, “None of this would have happened without the jellyfish” [8]. But one mystery remains: Why does *Aequora victoria* shine? No one knows what has caused *Aequora Victoria* to evolve aequorin and GFP.

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 44. A minor controversy has arisen concerning the exact American-Yiddish word uttered by Chalfie. According to Leo Rosten, the ultimate authority on such words, a shnook is “a timid shliemiel; a meek patsy; a passive, unassertive, ineffectual type”...A shnook is pathetic but likeable; if not likeable, he would probably be called a shmuck.” He defines the latter, which many report was the word used by Chalfie [for example, 35, 45, 46] and is actually a vulgar expression referring to a protuberant portion of the male anatomy, as “a dope, a jerk, a boob; a clumsy, bumbling fellow” or “a detestable fellow; a son of a bitch” (Rosten, L. *The Joys of Yiddish*; Pocket Books: New York, NY, 1970; pp 363–364 and 360–363, respectively). A Google search of Chalfie’s name and schnook scored 158 hits, while a search of his name and schmuck scored 598 hits, but since this is hardly a valid way of resolving the controversy, on February 12, 2009 GBK emailed Chalfie, who responded 34 minutes later via email reply: “It is schnook.” He directed GBK to the following website, where the word can be clearly heard: Smith, A. Phone Interview with M. Chalfie, 2008 Nobel Prize, Chemistry. http://www.youtube.com/watch?v=SG_5yc8dwqQ (accessed February 2009).
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