

Reflections On the GFP Nobel “Buzz”

By WILLIAM W. WARD, PhD

On October 8, 2008, the Nobel Prize for Chemistry was awarded to Osamu Shimomura, Martin Chalfie, and Roger Tsien ostensibly for the discovery, cloning, and applications of green-fluorescent protein (GFP). This protein is associated with bioluminescence in coelenterates, including jellyfish and sea pansies. This award is as much a celebration of basic research as it is an acknowledgment of the achievements of three accomplished researchers.

A Journey of Discovery

An exciting and mysterious phenomenon, bioluminescence has been studied by scientists and philosophers for centuries. In his classic 1952 treatise on bioluminescence,¹ E. N. Harvey quotes Pliny the Elder, Aristotle, Plato, and other ancient scholars who were captivated by bioluminescence and sought to learn its secrets. Even the word “luciferin” (the generic term for the substrate of a bioluminescent reaction) has ancient roots, coming from the Roman god, Lucifer, originally the bearer of light (and later the god of the underworld).

The GFP successes celebrated several months ago have their roots in hundreds of years of basic research—research for the sake of research. Contemporary scientists with long-term investments in the field of bioluminescence research began their studies because of the aesthetic appeal that bioluminescence

engenders. I feel that appeal every single day. I remember having taken the Kuder Preference Test in 9th grade, a test that measures the broad career areas that might appeal to a young student. My profile came out Scientific/Artistic. How prophetic that evaluation was, as I became a research scientist motivated by the artistic beauty of bioluminescence.

My first PhD mentor at the Johns Hopkins University, William D. McElroy, a world expert on the biochemistry of firefly bioluminescence and Biology Department chairman at the time, often counseled our entering class of graduate students. He said that we should all concentrate on doing excellent basic research and leave the applications to others who might follow. He assured us that applications would follow in time. So, with his advice, I began what has now become a 41-year-long quest to understand the basic biochemical secrets of light production in some of the most primitive multicellular animals on earth, ctenophores, jellyfish, and sea pansies.

Leading the Way

When, in my first year at JHU, Dr. McElroy left to become director of the National Science Foundation. His wife, Dr. Marlene DeLuca, directed his lab for several more months until she, too, moved into the DC area. Dr. DeLuca was an expertly trained protein biochemist who lacked a firm grounding in biology. One time, to the amusement of the biologists in the group, she asked us if a sea pansy is a type of marine flower. Years later at a national meeting, a graduate student, Keith Wood, who worked with Dr. DeLuca at the University of California, San Diego, showed a slide of the sea pansy, *Renilla*

reniformis, identifying the body of the animal as a rock—yet another source of levity.

My remaining years at JHU were spent purifying and characterizing the bioluminescent photoproteins from the ctenophores *Mnemiopsis* and *Beroe*^{2,3} in the laboratory of Dr. Howard Seliger, a physicist with training in the fields of optics and high energy radiation. My favorite JHU story is my presenting to Dr. Seliger, in the quiet of his office, the mathematical culmination of a year-long experiment I had designed and conducted. I had created a high resolution action spectrum for photoinactivation of the very light-sensitive photoprotein, mnemiopsin, and I had calculated the quantum efficiency of photoinactivation.⁴

I showed Dr. Seliger the numerical photoinactivation quantum yield I had calculated using a disjointed series of algebraic expressions and proportions. Instantly he barked, “*That won’t do!*” and he proceeded to derive equations on his office blackboard for the next three hours—not a chalk mark of which I was able to follow. When he finally finished, he triumphantly showed me the quantum yield he had systematically derived and calculated. “Here,” he said, “is the right number.” I glanced down at my pages of mathematical scribbles and meekly pointed out to Dr. Seliger that his answer was exactly the same as mine.

Soon, thereafter, as a brand new PhD, I joined the laboratory of Dr. Milton Cormier at the University of Georgia’s Department of Biochemistry. Within a few months, I was given the job of purifying and characterizing *Renilla reniformis* (the animal—not the marine flower nor the rock) green-fluorescent protein.⁵⁻⁷

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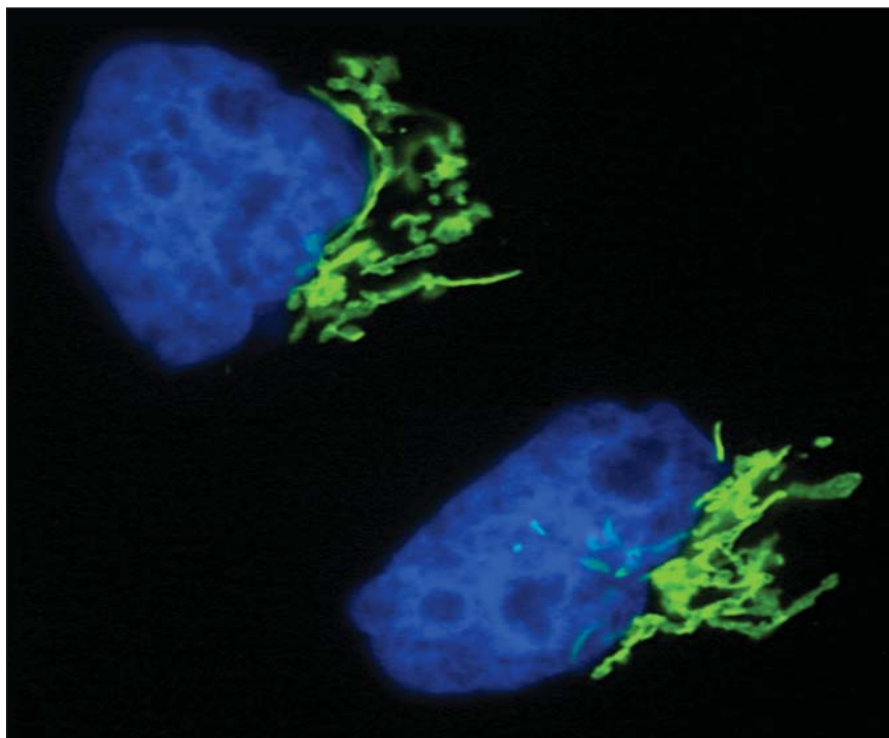
The most indelible memory of my years in Dr. Cormier's lab was witnessing the overnight demise of 40 milligrams of pure GFP. In the middle of the night there had been an equipment failure in a three-day-long preparative isoelectric focusing (IEF) run. The refrigeration unit had overshot, freezing solid the ethylene glycol/water circulating water bath and allowing the 7-watt power supply to boil away the entire sample while reducing the column to an amorphous lump of plastic.

The now "fried" GFP sample had taken a year to prepare from one hundred liters of sea pansy acetone powder extracted from many tons of sea pansies. Others before me had collected the animals over many seasons and I had spent an additional year getting that 40 mg sample 95% pure before subjecting it to preparative IEF.

Revised Methodology

Vowing never again to use preparative IEF, I needed an alternative process to achieve final purity in my next large-scale prep. All other methods having been exhausted, I needed to create a new protein purification technique to replace IEF. I experimented with alternate ways to elute GFP from a DEAE ion exchange column. I first eluted the column in a conventional way, with a linear salt gradient. Not yet having a pure sample, I applied the best fractions to another identical column, but instead of using a salt gradient, I eluted the column with a low ionic strength, descending the gradient of pH from pH 6.5 to pH 4.5. The GFP eluted precisely at its isoelectric point of 5.3, the point at which GFP carries no net charge.

I achieved final purity in a third round of DEAE chromatography—this time raising the pH from 9.5 to 11.5 in the elution buffer. Now I was titrating not the GFP, but the DEAE. These three sequential DEAE steps generated a cleaner sample with a higher percent recovery than had ever been achieved with *Renilla* GFP on IEF. This may have been the first time anyone had unknowingly performed chromatofocusing, a technique not yet named but later marketed by Pharmacia.



A Golgi stack containing the GFP-tagged glycosylating enzyme (GalNAc-T2-GFP).
(Photo courtesy of Markus Grabenbauer and *Nature Methods*)

Ironically, we never got their much more expensive chromatofocusing materials to work for us.

Focused Research

In 1977, I left the University of Georgia to accept an appointment as assistant professor in the Department of Biochemistry and Microbiology at Rutgers University in New Brunswick, NJ. In the 31 years that have followed, I have devoted nearly all my research to GFP, becoming the first person in the world to utilize GFP in a practical way — as a fabulous teaching tool for a hands-on continuing professional education course in protein purification. This course has been taken by more than 1500 industrial professionals from all corners of the globe (website: <www.rci.rutgers.edu/~crebb/protein.html>).

Monetary Realities

It is ironic that after decades of well-funded basic research on bioluminescence, Milt Cormier's and Douglas Prasher's research funding was abruptly turned down by granting agencies just as they were about to complete

the cloning of a GFP gene — a project with which Doug had worked for a concerted three years (and many years additionally). Rather than let the gene go to waste, Douglas gave it away to Marty Chalfie and Roger Tsien. And, as they say, the rest is history.

The capstone terminal cloning and ensuing applications of GFP that earned the Nobel Prize were built upon the work of Milt Cormier, John Wampler, Russ Hart, Doug Prasher, myself—and others who set a firm GFP foundation over four decades, block-by-block and brick-by-brick. The Nobel Prize take-home message to the world is not just that GFP (with some 20,000 publications and citations) has become an amazingly valuable tool in so many areas of life sciences. The underlying (and I would argue, the more important) take-home message is that these now celebrated applications of GFP rest upon decades of government-supported basic research carried out by dozens of scientists wanting just to solve basic research problems. Without decades of significant government support, there would have been no knowledge base

leading the Nobel Prize winners to their ground-breaking applications of GFP.

Moving Ahead

But the times are changing. Federal funds are diminishing and important university research is suffering. So, to keep research funding at acceptable levels, universities need a much broader economic base than their near total reliance upon federal dollars. Other means of raising funds must be explored and, like it or not, universities need to cultivate and encourage the entrepreneurial skills of their faculty. In addition, key university administrators need to form more extensive alliances with the private corporate sector. Business people know how risky it is to base the financial health of a company upon just one major revenue source. Collapse of that revenue source means collapse of the entire company. By analogy, academic researchers and entire research programs at major universities are highly vulnerable if they depend almost exclusively upon federal revenue streams to support these research programs.

Historically, this dependency has been driven by university administrators who count on grant overhead to support research-related infrastructure and, as many of us suspect, to support nationally competitive intercollegiate athletics programs as well. The promotion system at major research universities places far more value on overhead grant support than on any other forms of support. Thus, diversification of the revenue base for university research is discouraged by university administrators. Sometimes diversification is thwarted, prevented, and even punished.

Creative Support Strategies

As federal funds diminish, university administrators must begin to encourage alternate ways for university professors to attract research dollars. One way is to reward successful faculty entrepreneurs through the promotion and/or merit systems. Currently, this is not the practice at most major research universities. My having raised about \$3 million via entrepreneurial activities has

been ignored by my university administration, and promotion denial narratives, instead, point out deficiencies in my acquisition of federal funds.

If we accept that financial diversification is essential for the survival of major research universities, then it is not sufficient for individual professors to “drum up business.” University administrators must begin raising research funds by dealing with large corporations, CEO-to-CEO, in order to broaden the base of support for university research.

- Why should taxpayers be the only ones to fund the research (via federal granting programs) that prepares graduate and post-doctoral students to leave a university and enter the commercial work force fully trained?

- Shouldn't the hiring corporations pay some of the training costs?

- Is this not the same situation we have with intercollegiate football and basketball in the United States?

- Does it make sense for taxpayers to foot the bill for “training” athletes, many of whom enter the professional ranks with their “training” having been fully subsidized by tax payers and tuition payers? Not to me.

The Issue of Technology Transfer

Tech transfer offices need to do more than push papers, process patents, and monitor conflict of interest cases. They must be given sufficient funds and authority by their central administrations to act as advocates for, facilitators of, and partners with entrepreneurial professors. Very little of this is happening now.

Commercially viable, early stage research results should be carefully evaluated by disinterested peers. Those projects that pass the “peer test” should be generously supported and encouraged at the local level with relatively long-term commitments contingent upon the researcher's achieving jointly agreed upon milestones. Tech transfer offices must be given the resources to facilitate the transfer of technology. “Corporate liaison” and “technology transfer” are words that must have real meaning. It is high time that the Offices of Corporate Liaison and Technology Transfer exercise their “consonants”

rather than just their one lone “vowel.” Who needs another “Office?”

In the meantime, we need to inform legislators and business leaders of the great economic values of basic research. I have been stimulated by the words of Pennsylvania Congresswoman Kathleen Buto (in a Chris Matthews-hosted panel discussion at the recent Biotech 2008 Symposium in Philadelphia), that all biotech scientists need to communicate more effectively with legislative leaders. (I would include business leaders as well). One way to excite leaders of government and business is to give them exposure to actual experimental science. I have found no better way to excite the lay public than to expose them to the wonders of GFP in an actual laboratory setting. This is especially true now that the Nobel Prize for GFP has shown us all the “fruits of basic research.”

In Conclusion

This country became the world leader in science and technology because our government has dared to fund imagination and innovation, not just practical goal-directed engineering. Now we need additional help from the private sector. We cannot afford to pull the plug on basic research funding now, especially as the rest of the world challenges us for science supremacy.

REFERENCES

1. Harvey EN. (1952) *Bioluminescence*. Academic Press, New York.
2. Ward WW and Seliger HH. (1974a) Extraction and purification of calcium-activated photoproteins from the ctenophores *Mnemiopsis* sp. and *Beroe ovata*. *Biochem* 13:1491-1499.
3. Ward WW and Seliger HH. (1974b) Properties of mnemiopsin and berovin, calcium-activated photoproteins from the ctenophores *Mnemiopsis* sp. and *Beroe ovata*. *Biochem* 13:1500-1510.
4. Ward WW and Seliger HH. (1976) Action spectrum and quantum yield determinations for photoinactivation of mnemiopsin, a bioluminescent photoprotein from the ctenophore *Mnemiopsis* sp. *Photochem Photobiol* 23:351-363.
5. Ward WW and Cormier MJ. (1976) In vitro energy transfer in *Renilla* bioluminescence, *J Phys Chem* 80:2289-2291.
6. Ward WW and Cormier MJ. (1978) Energy transfer via protein-protein interaction in *Renilla* bioluminescence, *Photochem Photobiol* 27:389-396.
7. Ward WW and Cormier MJ. (1979) An energy transfer protein in coelenterate bioluminescence: Characterization of the *Renilla* green-fluorescent protein (GFP). *J Biol Chem* 254:781-788.