

GFP: LIGHTING UP LIFE

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by

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“You can observe a lot by watching.”

Yogi Berra

“My companions and I then witnessed a curious spectacle... The Nautilus floated in the midst of... truly living light[,]... an infinite agglomeration of colored... globules of diaphanous jelly...”

Twenty Thousand Leagues Under the Sea – Jules Verne

“Now it is such a bizarrely improbable coincidence that anything so mind-bogglingly useful could have evolved purely by chance that some thinkers have chosen to see it as a final and clinching proof of the nonexistence of God.”

The Hitchhiker’s Guide to the Galaxy – Douglas Adams

I want to thank the Royal Swedish Academy of Sciences and the Nobel Foundation for this amazing and surprising honor. At first I wondered why I, a biologist and a person with less than enviable college grades in Chemistry, had been selected. Then I realized that this prize had actually been given to the GFP molecule, and I am one of its assistants. Thank you for letting me be part of the celebration of a wonderful tool for visualizing life.

Scientific inquiry starts with observation. The more one can see, the more one can investigate. Indeed, we often mark our progress in science by improvements in imaging. The first Nobel Prize, the Physics prize of 1901, was an imaging prize, given to Wilhelm Röntgen for his discovery of X-rays and their astonishing ability to allow the noninvasive viewing of the human skeleton. A few years later the Nobel Prize in Physiology or Medicine was awarded for the development of silver nitrate staining to visualize nerve cells by Camillo Golgi and its improvement and use by Santiago Ramón y Cajal to demonstrate the cellular nature of the nervous system. This research laid the groundwork of modern neurobiology.

Over the years several other imaging techniques and their developers have been honored with the Nobel Prizes including x-ray crystallography (William and Lawrence Bragg, Physics, 1915), the ultramicroscope (Richard Zsigmondy, Chemistry, 1925), nuclear magnetic resonance (Felix Bloch and E. M. Purcell, Physics, 1952), the phase contrast microscope (Frits Zernike,

Physics, 1953), large-array radio telescopes (Martin Ryle, Physics, 1974), the electron microscope (Ernst Ruska, Physics, 1986), the scanning tunneling microscope (Gerd Binnig and Heinrich Rohrer, Physics, 1986), computer-assisted tomography (Allan M. Cormack and Godfrey N. Hounsfield, Physiology or Medicine, 1979), and, most recently, magnetic resonance imaging (Paul C. Lauterbur and Sir Peter Mansfield, Physiology or Medicine, 2003).

My road to imaging was not direct. I had been interested in science from when I was very young, but after a disastrous summer lab experience in which every experiment I tried failed, I decided on graduating from college that I was not cut out to be a scientist. Instead I did a series of somewhat random jobs including teaching high school chemistry. During the summer break from teaching, I tried laboratory research one more time, working with José Zadunaisky at Yale Medical School (Figure 1). The successful experiments of that summer and his support gave me confidence to apply to graduate school, and I entered the Physiology Department at Harvard in 1972 where I did my thesis with Bob Perlman. Bob and I had a wonderful relationship, which continues to this day. He is one of the warmest, kindest, and smartest people I know, and a great person to talk over ideas with.



Figure 1. Influences on my career. The success I had working for José Zadunaisky convinced me that maybe I could be a scientist. Bob Perlman was an outstanding Ph.D. advisor who always had time to listen to my (often crazy) ideas. Working with Sydney Brenner, John Sulston, and Bob Horvitz during my postdoctoral years started me on my continued research with *C. elegans*. I am convinced that working with this transparent animal was a major reason why I was excited about the possibilities of GFP as a biological marker. Photo of José Zadunaisky from Bronner, F. (2006) Obituary: José A. Zadunaisky. *J. Exp. Zool.* **305A**: 103; reprinted with permission of John Wiley & Sons, Inc. Photo credits for the remaining photos are M. Chalfie for Bob Perlman, the Nobel Foundation for Sydney Brenner, John Sulston, and Bob Horvitz, and Adam Antebi for *C. elegans*.

My current studies, however, started when I was accepted as a postdoctoral fellow by Sydney Brenner at the MRC Laboratory of Molecular Biology and began working on the nematode *Caenorhabditis elegans*. In 2002 Sydney, Bob Horvitz, and John Sulston won the Nobel Prize in Physiology or Medicine for their work on *C. elegans*. All three shaped the direction of my research. Sydney gave me the opportunity to work with him and an amazingly gifted group of scientists, Bob, a friend since high school, gave me several crucial pieces of advice, collaborated on several projects, and served as an example of what one can achieve in science (I am still following in his footsteps), and John, with whom I collaborated the most and who taught me most about how to act honorably as a scientist, started me on the project that still occupies most of my time: the study of mechanosensation.

My colleagues and I often call their Nobel Prize the first worm prize. The second went in 2006 to Andy Fire and Craig Mello for their discovery of RNA interference. I consider this year's Prize to be the third worm prize, because if I had not worked on *C. elegans* and constantly told people that one of its advantages was that it was transparent, I am convinced I would have ignored GFP when I first heard of it. These three prizes speak to the genius of Sydney Brenner in choosing and developing a new organism for biological research.

The year before I learned about GFP, my lab had begun looking at gene expression in the *C. elegans* nervous system. We were studying the differentiation and function of nerve cells needed for mechanosensation. Mechanosensors respond to physical perturbation; they underlie many of our senses, including touch, hearing, and balance. These senses are poorly understood; in particular the transduction molecules, the molecules that detect the mechanical signal, are virtually unknown. The genetic studies that I had done with John Sulston were directed, in part, at discovering such transduction molecules. We thought that by obtaining mutants that were defective in touch, which was sensed by six cells in the animal, we could identify genes that were needed both for the production and differentiation of these particular cells and for transduction. In the late 1980's my lab began cloning several touch sensitivity genes and testing whether they were expressed in the animal's touch receptor neurons. At this time three general methods were used to look at gene and protein expression. The first was the use of labeled antibodies, whose specificity created outstanding protein-specific markers. The second was the use of β -galactosidase from the *Escherichia coli lacZ* gene, which could be expressed as transcriptional and translation fusions and visualized by the cleavage and subsequent oxidation of X-gal to an insoluble blue product. The third was *in situ* hybridization to mRNA. We used all three methods to monitor gene expression (Figure 2).

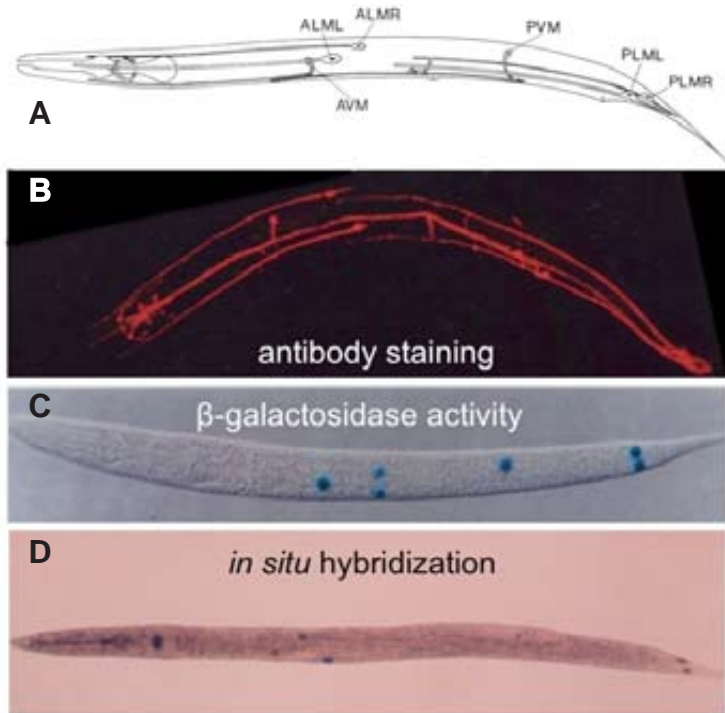


Figure 2. Gene expression methods used before (and after) GFP. A. Positions of the six touch receptor neurons in *C. elegans*. B. Antibody staining to the MEC-7 β -tubulin (taken from Savage *et al.*, 1994). C. β -galactosidase expression of a transcriptional fusion for *mec-9* (taken from Du *et al.*, 1996). D. In situ hybridization to *mec-7* mRNA (Shohei Mitani).

All three methods had considerable limitations; they required extensive and time-consuming tissue preparation. The animals had to be fixed and then permeabilized so either the antibody, the X-gal substrate, or the DNA probe could enter the tissue. This preparation, which needed to be done with each batch of animals, meant that we could only look at dead tissues, giving us a static picture of expression. If we wanted to understand changes during development, we had to compare images from many different individuals.

I first had the idea to put GFP into worms a little after noon on Tuesday, April 25, 1989. My department has a lunchtime seminar series on Tuesdays for those of us interested in neurobiology, and the speaker that day was Paul Brehm, who at the time was at Tufts University. He began his talk with a description of light production by jellyfish and similar animals, work that I subsequently learned had been begun by my co-Laureate Osamu Shimomura (Shimomura *et al.*, 1962; Johnston *et al.*, 1962) and then by Jim Morin and Woody Hastings (Morin and Hastings, 1971a, b). He first spoke about aequorin, which I had heard of as a calcium indicator. But then he talked about a protein new to me that fluoresced and allowed the jellyfish to produce green light instead of blue. Being primed from years of talking about the transparency of *C. elegans* and having just seen the work involved using antibodies and *lacZ* fusions, I immediately started to fantasize about how



Douglas Prasher



Ghia Euskirchen



Yuan Tu



Bill Ward

Figure 4. The GFP team. Douglas Prasher cloned the *gfp* cDNA, Ghia Euskirchen expressed the cDNA in *E. coli*, Yuan Tu expressed the cDNA in *C. elegans*, and Bill Ward compared the excitation and emission spectra of the native and recombinant GFP. (Photo credits: Douglas Prasher and Bill Ward for their own photos, M. Chalfie for the photos of Ghia and Yuan.)

During that day I learned that GFP had several features that made it a very attractive candidate for a biological marker. 1) It was a relatively small protein of only 238 amino acids. 2) It was active as a monomer. 3) It could be excited by ultraviolet or blue light. 4) It was a stable protein that had high quantum efficiency and did not photobleach easily. And 5) the active protein did not need a cofactor or other small molecule to fluoresce.

GFP had one feature, however, that might make it unsuitable for expression in organisms other than the jellyfish: the chromophore was formed by the cyclization of the peptide backbone between Ser₆₅ and Tyr₆₆ (Figure 5). No one knew how this cyclization occurred, but the prevailing hypothesis was that one or more converting enzymes were needed to change what was referred to as apoGFP to the fluorescent product (Cody *et al.*, 1993). If other proteins were needed, GFP would not be a very good marker.

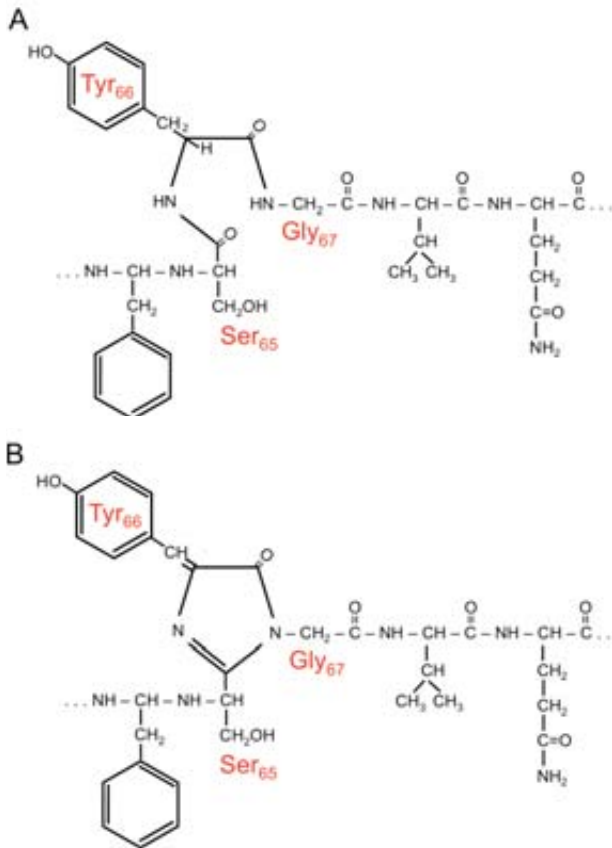


Figure 5. Formation of the GFP fluorophore. A. The primary amino acid sequence of GFP. B. Sequence after cyclization. Data from Cody *et al.* (1993).

The next important event in this story was my marriage in late 1989 to Tulle Hazelrigg, a scientist who lived and worked 2000 miles away from New York City at the University of Utah. Given the distance, I felt fortunate to be eligible for a sabbatical leave, which I took to work in her lab. Unfortunately, while I was away, Douglas finished cloning the *gfp* cDNA, tried to contact me, but failed to do so. He concluded that I had dropped out of science. For my part, having not heard from Douglas, I imagined that he had not found the cDNA.

We remained in mutual ignorance until September, 1992. At that time one of the new graduate students, Ghia Euskirchen (Figure 4), decided to do a rotation project with me. I was particularly happy about her joining the lab, because she had just finished a Masters degree in our Engineering School working on fluorescence. I told her about my idea of using a fluorescent protein to mark cells, and then bemoaned the fact that I had not heard from Douglas. But when we searched for “fluorescent protein” in the Medline database that the University had just installed on our computers, the first paper we saw was Douglas’ February, 1992 paper describing the isolation of the *gfp* cDNA (Prasher *et al.*, 1992). We ran down to the library, found the journal with the paper, discovered that the article included his phone number, and

rushed back to my office to call him. After we cleared up our misconceptions of each other's careers, Douglas and I renewed our collaboration.

Six days later Douglas sent the DNA to us. At this point, we had two choices as to how to do the experiment. Douglas had cloned the cDNA as an *EcoRI* fragment into a lambda vector. We could either obtain the fragment by cutting it out of the vector with the same restriction enzyme but this would give us additional non-coding jellyfish DNA, or we amplify only the coding sequence using the polymerase chain reaction (PCR), which was risky because it tended, at that time, to introduce base changes. I decided we should use the latter strategy, a decision that turned out to be fortunate, since we later learned that other labs using the restriction enzyme strategy failed to get fluorescence. Presumably, the extraneous jellyfish DNA interfered with the expression. Given the prevailing assumption that GFP needed one or more converting enzymes to fluoresce, the failure of bacteria to fluoresce could be interpreted as a need for other jellyfish components.

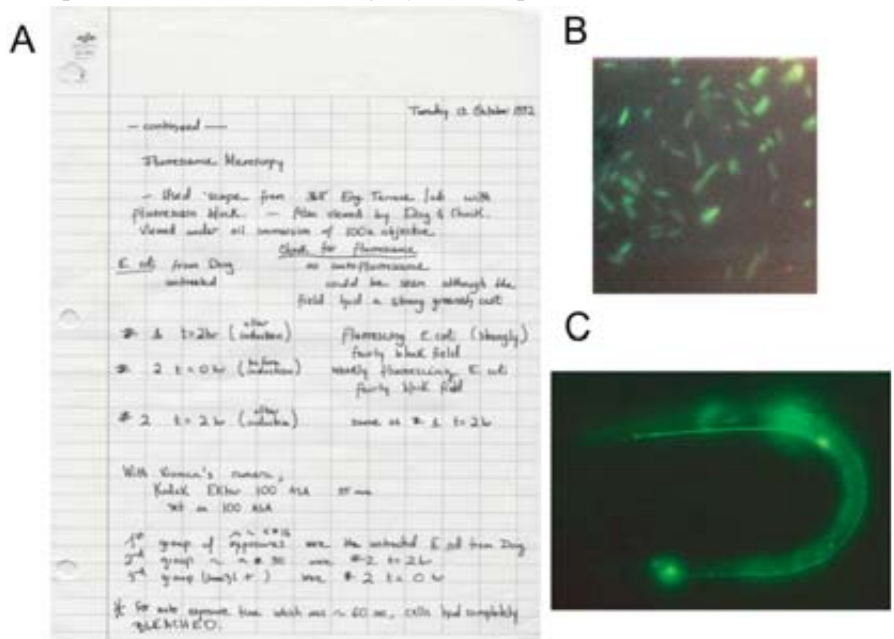


Figure 6. The first expression of GFP in heterologous organisms. A. The page in Ghia Euskirchen's laboratory notebook where she noted that *E. coli* expressing GFP fluoresced. The microscope she used was not in our laboratory. B. A picture of those first fluorescing bacteria taken by Ghia. C. GFP expressed in the *C. elegans* touch receptor neurons from Chalfie *et al.* (1994). Reprinted with permission from AAAS.

In any event, one month after receiving the DNA from Douglas, Ghia had *E. coli* that fluoresced green (Figure 6), although we had to use the microscope in her previous lab to see them. We were ecstatic. No other protein from the jellyfish was needed to convert the protein to the fluorescent form. Ghia took several pictures of the bacteria and I quietly started to show them to people. I couldn't contain my excitement. Soon afterward Ghia left my lab to do another lab rotation, and I asked Yuan Tu (Figure 4), my technician at the

time, to put GFP into *C. elegans*. Again the experiment succeeded, and for the first time we had GFP expressed in the touch receptor neurons of *C. elegans* (Figure 6). Bill Ward (Figure 4), a biochemist who had studied GFP for several years, then joined the project and showed that the protein produced in *E. coli* had the same optical properties as the native protein (Figure 7).

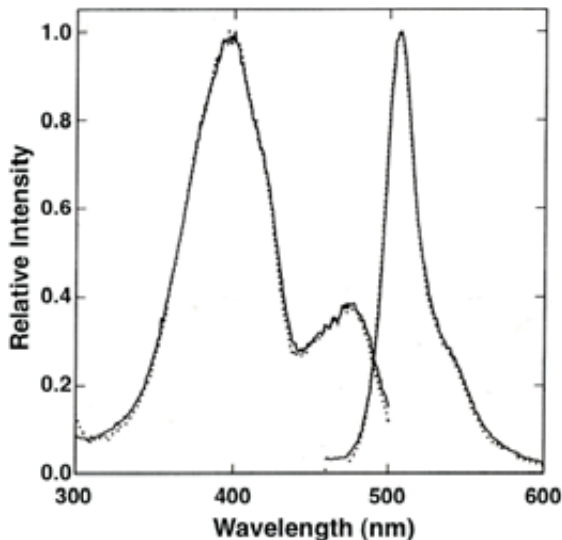


Figure 7. Excitation (left) and emission (right) spectra for native (dotted line) and recombinant (solid line) GFP (from Chalfie *et al.*, 1994). Reprinted with permission from AAAS.

We had one problem doing these experiments, as indicated by that note in Ghia's lab notebook that the bacteria were viewed in her previous laboratory: we did not have a working fluorescence microscope. This problem continued to plague us. I solved this problem by using the departmental confocal microscope and when I could not use that, by asking microscope sales representatives to bring demonstration microscopes to our lab, so that I could test them before purchasing them. In reality we were using the microscopes to do the experiments.

From the late 1970s through the 1990s *C. elegans* researchers had a tradition of notifying each other of research progress before publication, so the first written description of our work with GFP was in the October, 1993 issue of the *C. elegans* newsletter, *The Worm Breeders Gazette* (Chalfie *et al.*, 1993). This article started the flood of requests for the GFP vectors. Approximately 50 people asked for them before our official publication in *Science* in February, 1994 (Chalfie *et al.*, 1994).

Publication, however, was not without its difficulties. The first problem was the title. When we submitted the paper for the editors' consideration, the title proclaimed that we had found "A New Marker for Gene Expression." The editors told us that we had to change the title by removing that word "new" because every paper published in *Science* reported novel results. We were also asked to make the title more descriptive of the results. Partly in an-

noyance, I changed the title to a much longer one for the manuscript sent to the reviewers (“The *Aequorea victoria* Green Fluorescent Protein Needs No Exogenously-Added Component to Produce a Fluorescent Product in Prokaryotic and Eukaryotic Cells”). After the paper was accepted, however, the copy editor asked us to shorten the title. We submitted a new title that was almost identical to the original (“Green Fluorescent Protein as a Marker for Gene Expression”), and it was accepted.

We had a second problem, this time with the cover art. I had sent in a picture that I was quite proud of, since it showed a neuronal growth cone in a living animal. The art director, however, informed me that green was the color most difficult to reproduce on the cover, and asked if the color could be changed. Luckily, I convinced her that a color change in this instance would not be appropriate (Figure 8).



Figure 8. The cover of *Science* for February 11, 1994 showing GFP in *C. elegans* neurons. From *Science* volume 263, number 5148. Reprinted with permission from AAAS.

The third problem was that I had difficulty with one of the people who had already used GFP and whose unpublished data I had wanted to cite. Most people were happy to have their results discussed, but this person put special conditions on our use of her data (Figure 9). The fact that she was my wife may have had something to do with the added requirements. She is shown with our daughter Sarah, who is prize enough for anyone. Both have made me a better person and a very happy one.



Figure 9. Conditions set by my wife, Tulle Hazelrigg, if I wanted to cite her unpublished work on GFP in my paper. We still debate whether these conditions were actually met. She is shown with our daughter Sarah (Photo credit: Roger Tsien).

The work that Tulle finally allowed me to cite was actually the next really important advance in the use of GFP because she and her graduate student Shengxian Wang made the first protein fusion with GFP, showed that it could functionally replace the original protein, and demonstrated that it could be used to show where in the cell the normal protein resided (Wang and Hazelrigg, 1994). Typical of her modesty, Tulle didn't use the words "green fluorescent protein" or "GFP" in her title.

These papers demonstrated the usefulness of GFP as a biological marker for both gene expression and protein localization. And GFP had several distinct advantages over past markers. First, GFP, like β -galactosidase, was heritable. Because organisms could be transformed with DNA encoding GFP, strains could be established that could be studied at latter times. This property not only allowed repeated observations without extensive tissue preparation, but also the strains, with particular cells or proteins labeled could be used for a variety of studies. Second, visualizing GFP was essentially non-invasive; the protein could be detected by simply shining blue light on to the specimen. Third, GFP was a relative small and inert molecule that did not seem to interfere with the biological processes that were being studied. Moreover the active protein was a monomer, which allowed it to diffuse read-

ily throughout cells, particularly nerve cells, and outline their entire shape. In contrast, β -galactosidase monomers are four times larger than GFP monomers and enzymatic activity requires the formation of a tetramer, making diffusion difficult. Fourth, the fluorescence from GFP could be observed in living organisms, allowing a dynamic view of biological events. In addition, biological activities could be monitored for proteins outside as well as inside the cell (a property not shared by β -galactosidase).

Although native GFP was very useful for a variety of experiments, for it to be really established as a useful tool for biological studies, its properties needed to be improved. The person who started improving GFP and who continued to lead its development was my co-Laureate Roger Tsien. The first things he and his laboratory did were to devise ways to change the emission color and to greatly enhance the fluorescence output of the protein when irradiated by blue light. But these and the other inventive adaptations that his laboratory produced are the subjects of his talk, and I want to turn to how researchers reacted to our paper.

After the publication of our paper, people requested and we sent out approximately 1500 sets of the GFP vectors we had produced before turning over the distribution to others. Two aspects of the early requests for GFP struck me as interesting. First, more often than not an investigator asking for the GFP vectors would say that he or she had heard about GFP from one of their graduate students or postdocs, an indication that these people were the real drivers of innovation in the laboratories. Second, several people would start their requests by asking if I knew if GFP had been used in their favorite organism. I expected that they asked because they wanted to be first to use this method, so I was surprised that when I said “no,” that some would say they would wait until someone else had worked out the method. I am still somewhat dismayed by this reaction, although I suspect it does mean that we have fewer real competitors than I would have assumed.

In any event GFP was soon put into a staggering array of organisms from all three domains of life: archaea, bacteria, and eukarya (Figure 10). Moreover, a search of PubMed using the terms GFP or green fluorescent protein nets over 30,000 publications, a number that has been increasing exponentially since 1994 and is undoubtedly a lower bound. GFP has also entered the art world with Eduardo Kac’s GFP bunny Alba and even the movies as evident by the opening credits of the film *Hulk* by the director Ang Lee that was released in 2003. The implication is that the Hulk is the first human GFP transgenic.

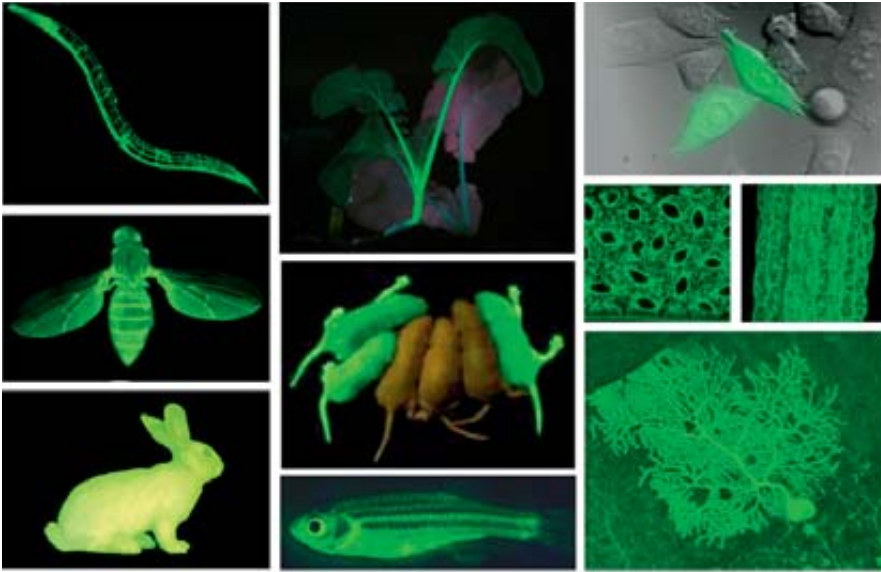


Figure 10. A gallery of GFP images. Photo credits by columns left to right: *C. elegans* (John Kratz), *Drosophila* (Ansgar Klebes, Freie Universitaet, Berlin), Alba the GFP bunny (Eduardo Kac), canola [M.D. Halfhill (St. Ambrose University) and H.A. Richards, R.J. Millwood, and C.N. Stewart, Jr. (University of Tennessee)], mice (Ralph Brinster, University of Pennsylvania), zebrafish (Brant Weinstein, NIH), cultured HeLa cells (Jerry Kaplan and Michael Vaughn, University of Utah), *Drosophila* embryonic cells (Jennifer Lippincott-Schwartz, NIH), *Arabidopsis thaliana* hypocotyl cells (David Ehrhardt, Carnegie Institution of Washington), mouse Purkinje cell (National Center for Microscopy and Imaging Research, University of California, San Diego).

After publishing our GFP paper, I went back to the work on mechanosensation, thus turning from a developer to a user of GFP. For the next few paragraphs I want to describe how we use GFP in our research to provide examples of the many ways this protein can aid scientific discovery.

First, of course, we use GFP in transcriptional fusions to characterize the expression pattern of genes (Figure 6). Second, we used GFP translational fusions to examine both gene expression and protein localization (Figure 11). With the advent of different colored fluorescent proteins, we could also test for the co-expression of different genes. By studying mutants that are defective in touch, we have found that touch sensitivity in *C. elegans* requires a channel complex of at least four proteins and specialized extracellular matrix and microtubules. Using a MEC-4::GFP translational fusion, we showed that the channel complex is localized to discrete spots along the length of the neuronal process (Figure 11D).

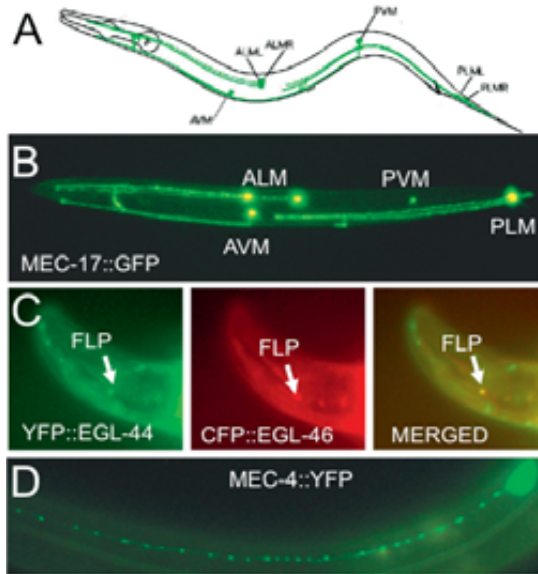


Figure 11. Uses of fluorescent protein translational fusions. A. Diagram of the six touch receptor neurons (green). B. The six touch receptor neurons are completely filled with a MEC-17::GFP fusion (taken from Zhang *et al.*, 2002). C. GFP protein fusions for the transcription factors EGL-44 and EGL-46 both localize to the nucleus of an FLP neuron (Ji Wu cited in Wu *et al.*, 2001). D. Localization of MEC-4::YFP to puncta along the length of a touch receptor neuron process (taken from Chelur *et al.*, 2002).

Once cells have been labeled with GFP they can be put to a wide variety of uses. Because of the strong genetics available in *C. elegans*, we have used such animals as the basis of screens for mutants defective in several aspects of cellular development. For example, mutating animals in which expression of GFP has labeled the entirety of the touch receptor neurons, we found mutants with more or fewer fluorescing cells (allowing us to study genes needed to control cell fate and number), mutants with cells in abnormal locations (allowing us to study genes that influence the positioning of cells), and mutants with cells (Figure 12A) with additional processes or abnormal branches (allowing us to study genes involved in neuronal outgrowth and morphology).

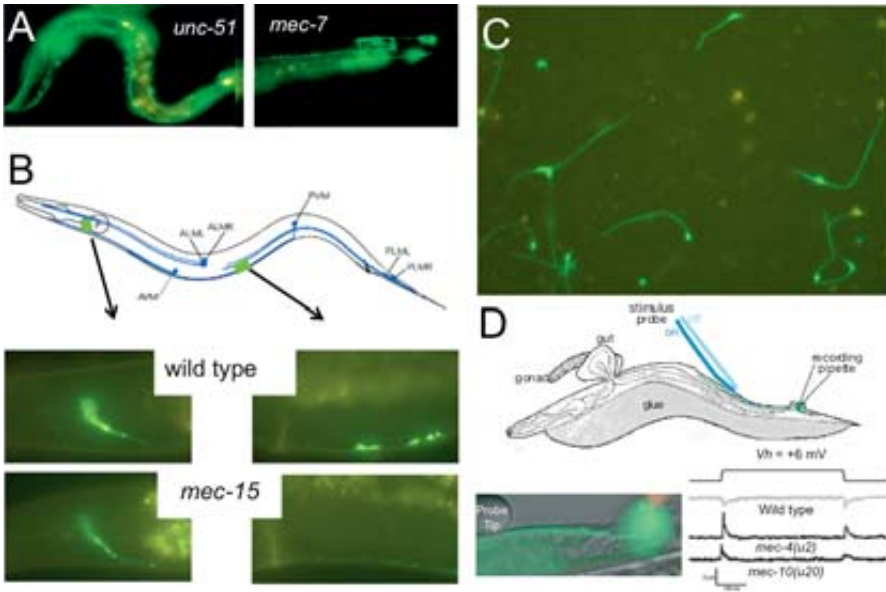


Figure 12. Using GFP for gene discovery and characterization. A. Mutations in the genes *unc-51* and *mec-7* affect process outgrowth of touch receptor neurons labeled with MEC-2::GFP (from Du and Chalfie, 2001). B. Mutations in *mec-15* reduce synapses, visualized by GFP::RAB-3, in touch receptor neurons (A. Bounoutas, M. Nonet, and M. Chalfie, unpublished data). C. Touch receptors neurons can be cultured from embryos, isolated by cell sorting, and used to identify cell-specific genes (e.g., Zhang *et al.*, 2002). D. Electrophysiology of touch neurons from wild type and mutants is possible because the cells can be identified by their fluorescence (taken, in part, from O'Hagan *et al.*, 2005).

By using animals making GFP fused to proteins that localize to particular regions of the cells, one can obtain mutants with even more specific defects. For example, Michael Nonet at Washington University in St. Louis is interested in genes needed for the formation of the chemical synapses that connect nerve cells. He has created fusions of GFP with proteins found at synapses that label these connections (Figure 12B). These fusion proteins label the two regions in touch receptor neurons that contain chemical synapses. We are collaborating with him to study a gene needed for touch neuron synapses.

Mutageneses are not the only uses for animals with labeled cells. The animals can also be used to isolate and study the labeled cells. For example, we have disrupted embryos and grown the embryonic cells in culture. The touch receptor neurons can be detected among all the other cells because of their fluorescence (Figure 12C). Remarkably, these cells have the same morphology they do within the animal; other labeled nerve cells look entirely different. The cells can be isolated using a fluorescence-activated cell sorter and used to identify messenger RNAs that are specific to the cells. Using these methods, we have identified 200 additional genes that are overexpressed in these cells.

Labeling the cells has also allowed us to study the electric properties of the touch receptor neurons, and to my particular joy identify the first transduction molecules for a eukaryotic mechanical sense. *C. elegans* is a wonderful

organism for a wide range of studies, but one of its disadvantages is that its nerve cells are tiny. Fortunately, Miriam Goodman working with Shawn Lockery at the University of Oregon developed a GFP-based method to make electrophysiological recording from *C. elegans* (Goodman *et al.*, 1998), which she then adapted to the touch receptor neurons as a postdoctoral fellow in my lab (Figure 12D). Using this method Bob O'Hagan, a graduate student in my lab, Miriam, now at Stanford, and I found that specific mutations affecting the channel subunits in the touch neurons change the normal inward current elicited by touch to an outward current, a demonstration that these channel proteins are directly required for transduction.

None of the results that I have mentioned would have been possible without the use of GFP in living animals. Despite my enthusiasm, however, GFP does have some limitations that have led us to devise modified proteins. One problem is that GFP expression is dependent on gene regulatory elements. We have been fortunate in studying the touch neurons because we know of genes that are expressed only in these cells and we can use their regulatory sequences. But such cell-specific expression is not common in *C. elegans* or other organisms, so specific labeling with GFP can be difficult. We have taken advantage, however, of a remarkable finding by Lynne Regan of Yale University to overcome this difficulty.

Lynne's lab found that GFP split into two pieces could be reconstituted if the two parts were linked by interacting peptides (Ghosh *et al.*, 2000; Figure 12A). Her lab and others have used this property to study protein interactions. Two members of my lab (Shifang Zhang and Chuck Ma) realized that this two component GFP, which we named recGFP, could help solve the specificity problem, if the two parts were expressed from different promoters. This two-component system should enable the labeling of 80% rather than 20% of the existing neuronal cell types in *C. elegans* (Figure 13).

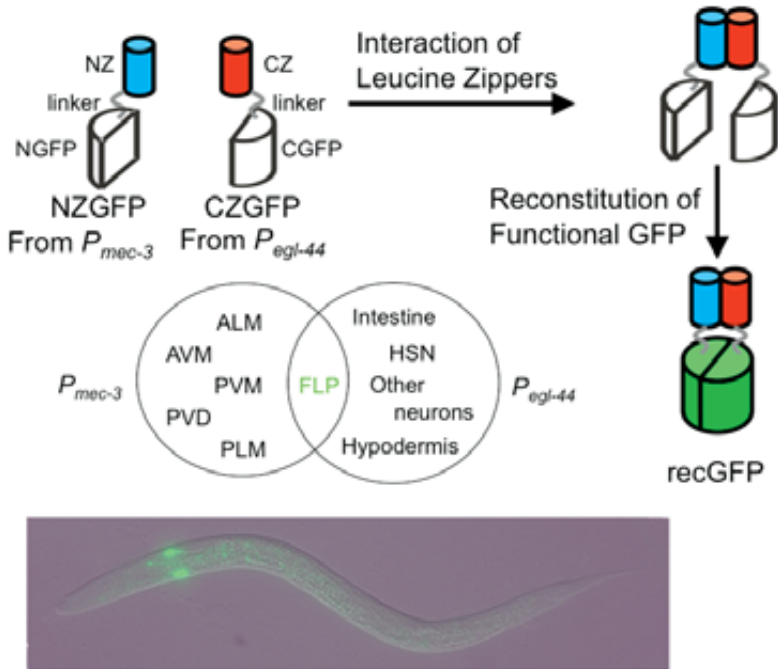


Figure 13. Use of recGFP to label the *C. elegans* FLP neurons (modified from Zhang *et al.*, 2004).

Another feature of GFP that can be both an advantage and a disadvantage is that the protein is very stable. This feature is wonderful when accumulation of GFP can give a brighter signal, but we wanted to study situations when gene expression was turned off rather than on, and stable GFP is a hindrance in these instances. We have found that fusions of GFP with the RING finger domains of certain E3 ubiquitin ligases creates an unstable GFP. We have used unstable GFP to learn how disruption of microtubules in the touch receptor neurons causes a generalized reduction in protein levels in the cells. Untreated animals fluoresce as adults, whereas animals treated with the anti-microtubule drug colchicine, which selectively causes touch insensitivity do not (Figure 14). We have identified several genes needed for this down regulation by mutating these animals and then looking for adults with visible fluorescence. These genes include members of the p38 mitogen-activated protein kinase (MAPK) signaling pathway and one putative transcription factor. I find these experiments exciting for two reasons. First, axonal outgrowth, neuronal branching, and synapse formation are dependent on the state of nearby microtubules. The connection of microtubule state and MAPK kinase activity may help explain the role of microtubules in these events. Second, we now have a means of investigating the down-regulation of gene activity. We have identified other instances where gene expression is reduced in post-mitotic cells, and we are using the unstable GFP as in this example to identify genes that regulate that reduction.

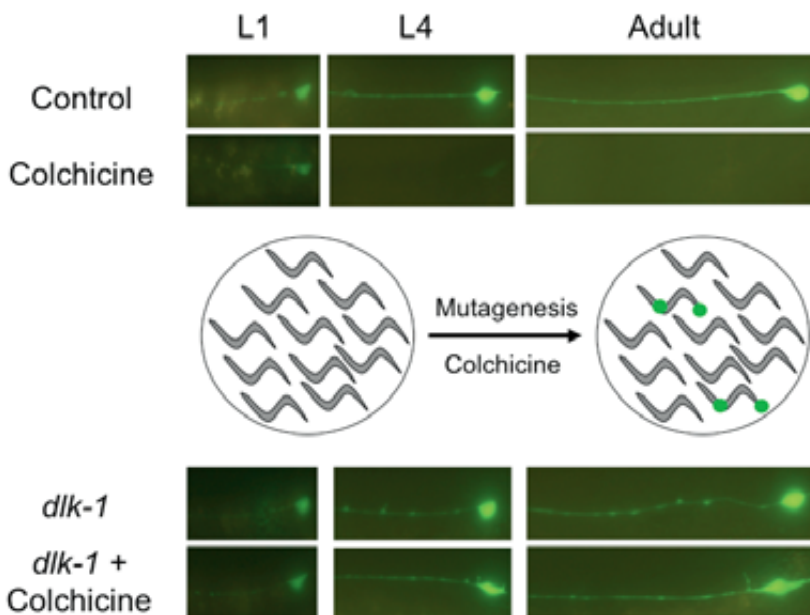


Figure 14. Gene discovery using a rapidly degrading GFP (praja::GFP). GFP fluorescence is absent in adult touch receptor neurons grown in the presence of colchicine. Mutation of wild-type animals yields some animals with adult fluorescence. One gene identified by this type of genetic screen is *dlk-1*, which encodes a mitogen-activated protein kinase kinase (A. Bounoutas, C. Ma, L. Emtage, and M. Chalfie, unpublished data).

In closing I would like to mention two reasons I am appreciative of this Prize, in addition to the obvious reasons for feeling so honored. First, this award is a Prize for basic science. GFP was discovered by Osamu Shimomura in a quest to answer a fascinating question: how can some organisms produce light? And the tools that Roger Tsien and I have developed enable additional investigations in cell biology, developmental biology, neurobiology, in fact throughout the life sciences. I would also add that the study of *C. elegans*, a nematode, has also taught us so much about life, as evident, as I mentioned, by this its third Nobel. These days one hears much talk about how science should be directed more toward translational research, research that applies understanding gained in the laboratory to problems in human health. Although the application of knowledge to human health and well-being is obviously important, I feel that many statements urging a switch from basic to translational research are based on two false premises. The first is that scientists are either uncaring or ignorant of the implications of their research for human disease. I find this attitude ironic and false, since virtually every scientist I know thinks deeply about the meaning and implications of his or her research. Second, I feel that some people who promote translational research act as if we have already learned all that we need to know to cure human diseases. A look at any sequenced genome, whether worm, fly, mouse, or human puts a lie to this assumption, since most of the predicted genes encode products described as “proteins of unknown function.” We have so

much more to learn. Not only what these proteins do, but also how they interact together to sustain life and development. As many people have said, one needs to have material to translate to have translational research. And when we consider how few organisms scientists have studied, the mystery that surrounds us becomes even greater. What will be the *A. victoria* and *C. elegans* of the future? Indeed, what will be the GFP of the future?

Second, by awarding this Prize to the three of us, the Royal Swedish Academy of Sciences and the Nobel Foundation have recognized that scientific progress is not a solitary event, but a process. Each of us has been a step in the very large development of GFP, and we have not been alone. Hundreds of others have adapted and varied fluorescent proteins forming a wide array of rather spectacular research tools. I have been astonished by the ingenuity and creativity of my colleagues from around the world. In a real sense, GFP is a wonderful metaphor for our work as scientists. Just as GFP and other fluorescent molecules absorb light of one wavelength and convert it to light of a different wavelength, we, too, take in what others have learned about the world, add our observations and insights, and produce additional gains in human knowledge.

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I dedicate this lecture to three people with whom I would truly have loved to share this honor, but who are no longer alive: my maternal grandmother, Madeline Friedlen, a kind, intelligent, and remarkable woman who started her own business in the Depression and who, when I was ten or so, wanted me to become a chemist or, more particularly, a metallurgist; my mother, Vivian Chalfie, who always claimed that intelligence had skipped a generation in our family but was wrong, and my father, Eli Chalfie, from whom I inherited my sense of humor and my love of the guitar.

Finally, I want to thank all the past and present members of my laboratory on whom I have relied for many years for their intelligence, hard work, and companionship. You've kept me on my toes. Thank you.

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- Portrait photo of Martin Chalfie by photographer Ulla Montan.