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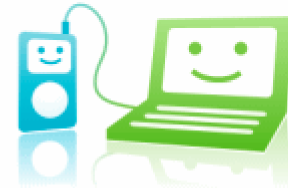

Futures in Biotech, 38: It Is Easy Being Green

Leo Laporte
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Marc Pelletier
This is Futures in Biotech, episode 38: It Is Easy Being Green. This is Part II of our interview with Dr. Martin Chalfie, 2008 Nobel Laureate in Chemistry for his work on the green fluorescent protein.

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[Music]

Marc Pelletier
What do I normally say? Welcome to Futures in Biotech. I am Marc Pelletier. Today is a Part II of a two-part series. It's an interview with Dr. Martin Chalfie who is the Professor of Biological Sciences and 2008 Nobel Laureate in Chemistry for his work on GFP. And the idea of interviewing Dr. Chalfie was actually that of Glen Ernstrom, who is a postdoctoral fellow in the Department of Biology and Howard Hughes Medical Institute at the University of Utah. So I have invited him again in this episode to help us with the introduction. I always want to get the introduction to our special guests right. And it's much simpler if you get somebody who's actually close to that guest. And so, you did your graduate studies in Dr. Chalfie's lab at Columbia University?

Dr. Glen Ernstrom
Yes. Hi, Marc. Thanks for having me. Yes, I was in the graduate program there, the neurobiology behavior program at Columbia from '96 to '02. And, yeah, I just have really happy memories there. It was just a great place to learn and do science in Marty's lab.

Marc Pelletier
So, tell me, you were saying that you had a reunion this weekend. Was it this weekend?

Dr. Glen Ernstrom
Yes, yes, it was really great. We had a big reunion. People came from all over the country, all over the world just to give their congratulations to Marty. There were scientific talks; people were invited to talk about some of their science that they're doing now. And after that, there was a session of joke telling and roasting Marty, and it really conjured up a lot of good memories and some funny things about Marty when we did that.

Marc Pelletier
So when you win the Nobel Prize, you sort of – you get put into a spotlight that scientists normally don't I guess want to be in, right?

Dr. Glen Ernstrom
Yes, yes, yes, it's kind of fun. I mean I guess when you're a professor, you don't realize the impact that you make on other people's lives so much, like little things that they say or do are really big events in other people's lives.

For instance, when I first met Marty, I was really wide-eyed and just so excited to be in his lab. I was with another graduate student at the time who was also starting her first day, Yun Zhang, who is now at Harvard. We were calling him Dr. Chalfie, Professor Chalfie, and he winced at this. He wasn't really – he

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didn't really like what we were calling him, like Professor Chalfie? What are you doing? And so he reassured us that while some people call him obscene names, I probably shouldn't say exactly what he said here – but that it was okay to call him Marty. Just call him Marty, and that's fine. But that was great, because what it did, it set the tone where he created this environment where it was fun and open to interact with him and the rest of the lab and other lab mates. It did get intense when grants were due, but for the most part, it was a great place to learn and do science.

Marc Pelletier
[5:02] Was he at the bench most of the time or did he – was he coordinating ten post-docs, because everybody has a different style of doing science?

Dr. Glen Ernstrom
Yeah. No, no, you'd occasionally see him once in a while at a dissecting scope looking at the worms. But for the most part, he was just pretty well established by the time I got there. He was more involved with thinking about the direction and the types of experiments he wanted to do and guiding post-docs and graduate students.

Marc Pelletier
So, when he would use an eyelash, a human eyelash to move those worms around, did he call over a graduate student and say, hey, you come here. Wink!

Dr. Glen Ernstrom
Yes, yes. Sacrifice your own eyelash, yes. It wasn't eyelash, that's the thing, I always forget. It's an eyebrow. So those are actually aren't too hard to get out. But eyelash, they're pretty painful...

Marc Pelletier
Graduate students, they're like a dime a dozen, like, hey you, graduate student, come here. Wink! So, one of the things that – when I was in discussions about with him talking about what we'd talk about, the talking about what we were going to talk about. Anyway, he said, "I don't want to talk about GFP, because I really don't know a lot about GFP." And he uses it as a protein. But he didn't feel comfortable talking about, I suppose, the biophysics and the biochemistry of the protein. So he said not to – we wouldn't be discussing it.

But he was very generous with his time, talking about, in the last episode, about the science that he did on neurosensory neurons, right? And the sense of touch, the basic molecular biology of touch, the molecular machines. But right before the end of the interview, I said, people are going to be shouting at their iPods, right? And yelling at me saying, "Marc, ask him about GFP."

Dr. Glen Ernstrom
Right, right.

Marc Pelletier
So I was hesitant. So I came out with a question, I think I'm actually going to edit it out because I fumbled all over the place because I was somewhat intimidated and I was – "Is it okay? Do you mind if I may, please, sir, ask you, sir, if we can talk a little about the GFP?" And then he was very charismatic and very open about talking about it. And so I just sat back and we had a great discussion about how this green fluorescent protein is used as a biological marker. That means you can track it within the cell as it's being made, where it's going, where it ends up. It's sort of like a molecular – almost atomic level or close to atomic level, marker of what's happening inside the cell. And that's what gave him the fame. It was his idea to actually use it to track biology inside the cell, look into the living cell.

So I got that right. I didn't screw up on the biology there? All right.

Dr. Glen Ernstrom
Yes, yes. I mean Marty is really remarkable for his focus on the particular area of biology. But he's always been creative and had this ambitious eye for using new techniques. And I think that combination of focus

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on a particular biological problem but a willingness to explore and try out new techniques that other people say, well, that's not how we do things, we do it this way. And I think that's what are ingredients that enabled him to get in the right place at the right time to do, to push this thing forward.

Marc Pelletier
So, I think I guess one of the take home messages here is, in part, one: it is important to be creative and ask good scientific questions. But also, don't fret about trying to tackle these questions with novel technologies. I think a lot of scientists tend to fear new technologies. They want to use standardized technologies and it's sort of like a classical musician sticking to classical music and using the same transcription instead of saying, well, I'm going to explore some kind of media interface and see if I can get a 150-piece band going in my studio and then doing…

Dr. Glen Ernstrom
Yeah, exactly. I think it helps to have that balance, absolutely.

Marc Pelletier
So let's get to the interview. And I really appreciate you coming on to help me. And as I said, I am going to edit that question out – maybe I'll post it on the website for fun for people to hear Pelletier here stumble as much as an interviewer ever will. So again, thank you for coming.

Dr. Glen Ernstrom
Thank you, thank you very much.

Marc Pelletier
On to the interview. One thing that I suppose I guess I should ask, right – and because everybody's probably shouting at their iPod right now – is the question of GFP. Right? Do you mind if I ask you…?

Dr. Martin Chalfie
No, no.

Marc Pelletier
[9:58] What I'd like to ask you is, how you used GFP to work out some of the big questions that you've – you were one of the, well the first person to really get an incredible depth of information from GFP. What – how does GFP relate to the biological studies that you're using? The biological questions that you're asking?

Dr. Martin Chalfie
Okay, so I'm going to answer it in two ways. So the first is, why would somebody studying touch suddenly publish a paper in which he showed for the first time that a molecule like GFP could be a biological marker? How in the world did I get involved in that sort of work in any way?

So, I have to take it back to where – when I first was hearing about GFP, which was 1989. I was listening to a seminar that had, as part of its introduction, a description of the jellyfish protein, and it was really the first time I had heard of GFP. I had known that jellyfish were bioluminescent and that jelly – the bioluminescence, the production of light, had been accomplished by a protein called aequorin and I also knew that aequorin – which is a fascinating molecule in and of itself – is a – is activated, that is, it produces light, when the calcium levels rise. And it had been used as a calcium indicator. Whenever calcium was present, then you'd get more light.

But what I didn't know is the work of Osamu Shimomura, who was the person who had isolated aequorin, that he realized when he isolated that protein, that the light was produced by aequorin was blue, but the jellyfish were green. And they were green, he realized, and he actually has in his footnote for his paper in 1962, a small note saying, "Well, because of this color difference I went back and looked at my various samples and I found that there is another protein. And this protein is one that if you just take an ultraviolet light and shine it on the protein, it will produce a green light." So, it's a florescent molecule, and that's as now – he called it the green protein, it's now called the green florescent protein.

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And this was part of this introduction to this talk I heard in 1989. And if I had – I believe if I had been working on any other organism other than *C. elegans,* I might have heard that and perhaps ignored the talk. But because I had been working on *C. elegans* for the previous 12 years and giving seminars in which probably my third or fourth sentence for every one of those seminars was, "And the animal is transparent." And that we were studying these genes for touch and we were starting to look and ask the question: are these genes actually active in the touch-sensing cells? Where are these genes turned on?

Now, at the time, there were means of being able to ask where a gene was active or not. And most of them, many of them, involved procedures that you had to kill the cells or kill the organism in order to prepare it. So for example, one of the ways that people used and that we used in the lab was to have the gene's regulatory components turn on the production of the bacterial protein beta-galactosidase. And then there were nice chemical means, so that wherever that enzyme was turn on, you would get a blue color by the chemical reaction that it catalyzed.

But you had to fix the tissue, the animals were not alive and – but we were doing these experiments because we wanted to answer the question: where is the gene turned on. And when I heard this seminar and heard about the green florescent protein, I realized that we could use that instead of beta-galactosidase in the cells and answer our question. And we'd be able to look at a living animal and see this gene expression turned out.

So I got very excited about this and spent the next day at least calling up people and trying to find out who was studying this. And eventually found that a scientist in Massachusetts, Douglas Prasher, was in the process of cloning this gene. He had actually cloned the gene for aequorin and now was trying to clone the gene for green florescent protein.

We had a wonderful conversation and we set up a collaboration, that as soon as he was finished cloning the cDNA for GFP or green florescent protein, that he would send it to me and we would look at it in worms and this was going to be just wonderful.

That was in April of 1989. But I never heard from Douglas, and so I just assumed that he hadn't succeeded in getting the cDNA. Now he actually tried to get me, but I was on sabbatical. I had actually gotten married in the interim and I had decided I was going to spend my sabbatical with my wife and so I went to her university. And I think that he tried to call there and no one appropriately answered the phone. But in any case, he decided that I had probably dropped out of science, and I decided because he didn't get in touch with me that he had not cloned the gene.

[15:48] And so we were really ignorant of each other's situation for several years until 1992 in September when the new graduate students come to the department. And one of these graduate students, a woman named Ghia Euschirken came to do a rotation. Graduate students often try out labs and we call these try-out periods, rotation periods. So she came to my lab to rotate in the fall and Ghia had been at Columbia previously in the Engineering Department and gotten a Master's Degree in Chemical Engineering. And so – and her project was to work on florescence. And so I liked this idea, so I trotted out my old idea about how we could use GFP as a biological marker. And I said but this guy hasn't called me and I don't want to bother him, so I don't think I should call him. But the university has just put on our computers the MEDLINE Database, let's see if there's any other florescent proteins. And so we checked this new database online on the university computer system and found to my amazement that Douglas Prasher had just published, that February, his paper with the cDNA sequence for GFP.

We ran down to the library, got the journal out of the library, looked at it and made a copy of it and, wonderfully, his phone number was listed on the article. So I ran back upstairs, I called him up, we straightened out the fact that I still was in science and we still wanted to do the experiments, and he sent us the material and Ghia was able to put it in bacteria within one month.

Marc Pelletier
Wow.

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Dr. Martin Chalfie
And at that point, we knew that this was going to work. Now, there are some interesting things about this. The first is that, at the time that we started the experiments, people knew from the work of Osamu Shimomura that GFP did not need any other small molecule, any other addition to make it florescent. So, it was only the primary amino acid sequence that was needed. And that was a very important thing, because we didn't want to have to permeabilize the cells or tissue to add something to it.

But there was a major problem. The major problem was proteins are a long string of amino acids in a polypeptide chain, and they're strung together by a chain of atoms that is a linear chain of atoms. But GFP is different because this backbone of atoms had formed a loop, a five-atom ring, that was part of the backbone. And no one knew how this rearrangement had taken place. And they hypothesized that maybe it would take one, two, three or maybe more enzymes to actually convert what was called apo-GFP into the functional fluorescent GFP.

And so when we did the experiment and put and expressed the protein in bacteria and showed that it was fluorescent, it meant that it did not need any other protein, it could be made completely on its own. Now if it had not worked, we might have said, it might be that it needs other components. So we were very fortunate that it worked really the first time out of the box for us.

Marc Pelletier
And had green bacteria.

Dr. Martin Chalfie
We had green glowing bacteria. Now of course, we didn't have green glowing bacteria in my laboratory, because it turned out that our microscope was no good to look at fluorescence. But fortunately, Ghia was able to take the bacteria to her old lab that had a much better fluorescence microscope and we were able to see the fluorescence. In fact, this was a bit of a problem in all the experiments because our microscope actually was so poor. It was designed for different things and frankly had too much glass to allow us to easily see the fluorescence. We could see it some of the time. That I was in desperate need of a microscope to do the subsequent experiments. And so I actually found a piece of paper describing all of this that I sent to NIH asking for a supplement to my grant soon after the paper was written. But at least what I wrote in that letter was that I had been inviting microscope representatives to bring in their microscopes so I could see them, in the guise that I was going to buy one – which I eventually did – and they thought they were demoing their microscopes to me, when what I was actually doing one of the experiments for the paper on that microscopes, which allowed me to do the work. But in the end, it all worked out quite nicely.

Marc Pelletier
I'd like to take a minute to thank audible.com for sponsoring Futures in Biotech. I think they've over 51,000 titles now of great audio content. And if you subscribe right now, you can get a free book, right. So you go to audible.com/biotech, and if you like it – like audio books, you get to stay and keep the subscription of course. But if you don't, you can actually cancel, but you get to still keep the free book. And I still have Glen on the line, so I thought it would be fun to ask Glen if he had read any books recently and if he wanted to give a recommendation, this week's recommendation, Futures in Biotech's audible pick of the week.

Dr. Glen Ernstrom
Yeah, hi, Marc, yeah. I've – a book I've recently picked up, I just saw that just came out is George Johnson's *The 10 Most Beautiful Experiments*. And I think this is a great book just for scientists and non-scientists alike, just great stories of individual scientists from Galileo [inaudible]. My favorite story, the Galvani and Volta being electrophysiologists, I thought this little story was great, talking about the controversies they had over deciding where does electricity in an animal come from. Is it artificial or is it actually generated and found in an animal? And they're doing experiments with frog legs. Just imagine, just walking around in, I don't know, 18th century Italy or wherever, and they're just electrocuting frog legs and they're trying to figure out where those electrical activities were coming from. So it's a great story.

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And the chapters and the stories they tell are really great because they're short little nuggets and in about as much time as it takes to watch a sitcom, you can get a nice cool scientific history lesson and some really interesting characters that really have changed science. So, I recommend that one.

Marc Pelletier
So, if you want to download *The 10 Most Beautiful Experiments*, you can go to audible.com/biotech. You will get the book for free. So without further ado, let's get back to our interview with Dr. Martin Chalfie.

Dr. Martin Chalfie
[23:09] Now, to answer your question about what do we use GFP for in my lab. I should mention that there were two really important series of experiments that were done, one important experiment and then a whole series of experiments that we've done subsequent to this. The next really important experiment was an experiment done by my wife, Tulle Hazelrigg, who was the first person to show that if you have GFP now fused to another protein, so the two would make a hybrid protein, then of course wherever that protein goes, you can see where the protein is, because you know that's where the fluorescence is. So you can actually follow proteins and ask where they go in cells.

And so we've used, in my work, we've used our original method, which was to ask where does the gene turn on, to ask what cells make a particular protein, and then we have used this protein fusion of GFP with the proteins we're interested in to ask where in the cell do the proteins go. So for example, we know that our channel complex proteins are not uniformly distributed all over the cell. They are there in a dotted pattern all along the cell process. And we've been studying why they are associated in the particular places they are and what other proteins are needed to localize them in the particular places. So having the protein fusions – fusion is a very important tool.

And the other great advances in GFP were done by Roger Tsien who improved the strength of the fluorescence and changed the color of it and made a whole series of molecular machines that have been proven in many labs to be extremely useful and the improvements he made really made GFP something that everybody could use.

To go back to what we do in our lab in addition to gene expression and looking where proteins are located within cells, we also use GFP in our genetic screens. Because after all, we can now label particular cells, in our case the touch-sensing cells, with GFP. So then when we look in the microscope and we have microscopes and we can have animals on plates growing around and developing and look at them, and what we see in them are just the touch-sensing cells that are lit up with the GFP fluorescence. And we can take these animals and we can mutate them and ask for animals in which the cells are abnormal.

So for example, these touch-sensing cells have a cell body – a long process that grows towards the head of the animal either from the tail midway or from midway up to the tip of the nose – and then near the end of the process, there's a small branch that's extended. We can mutate the animals and now look for animals that have abnormal forms of these cells. We can look for animals that don't have the cells at all, we don't see any fluorescence, or in which the process that grows out of the cell body has now grown abnormally, either grown too much or not enough or it's grown in the wrong direction. We can also ask about changes in the branching pattern. Do we see the branch at all? Does the branch occur in the right position? Does it occur more frequently in the cells? And we haven't got mutants that have all of these defects, but we've found several that are. So having GFP to illuminate the cells, if you will, allows us to look for variants that affect a great number of the processes that we're interested in, in terms of nerve cell development.

[26:58] In addition, we're sort of greedy people in the sense that we want to know not – we've identified several genes that are needed in the cells, but we really want to know, what are all the genes that are active in the cell? And so in order to understand that or to be able to try to answer that question, it would be very nice if we could find all the messenger RNAs that are produced by the touch-sensing cells. But that means we have to isolate those touch-sensing cells from all the other cells in the animal. And GFP allows us to do that.

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We can take animals that express GFP only in the touch-sensing cells. We actually take the embryos of these animals, we break the embryos apart into individual cells and we put them into culture. We usually wait about a day. And what we see is that only a few of the cells express GFP. And we know from other work that these are cells that also express all the other genes that we know in the touch cells.

And then there's a very nice machine called a fluorescence-activated cell sorter. What this does is it produces droplets. Now each droplet, if you work it out appropriately, will contain one cell. And as these drops are falling, they pass through a laser beam that will activate the fluorescence. So if they are cells that have GFP in it, you will get green light coming out from the activation by the laser, and that will be detected and those droplets will be directed towards one test tube. All the other droplets will go into another test tube. And eventually, you will sort the fluorescent cells from all the other cells in the cell culture.

And then we can take our purified cells, our selected cells and then study their messenger RNAs and try to find out what are the genes that have been activated in those cells. So GFP has been essential for us to be able to separate the cells one from the other – I also mentioned…

Marc Pelletier
I was wondering, you mentioned earlier on in the interview that you were taking specific cells out of the organism, and since *C. elegans* is the size of a comma, I was wondering how you were dissecting those. But that's one great way to do it.

Dr. Martin Chalfie
And that's exactly how we do it. And the other problem is these cells are very small. The cell diameter for these nerves cells is on the order of two microns, so two one thousandths of a millimeter, so extremely tiny. And so it's rather difficult to do the electrophysiological recordings that we need from these cells.

But I was very fortunate a couple of years ago that I had a very talented student come to do a post-doc with me. Her name was Miriam Goodman, she's now at Stanford. And I had the good sense to send her away from my lab and send her to Shawn Lockery's lab at the University of Oregon. And together – she did her first post-doc – and together, Miriam and Shawn developed a method in which one could record electrically from *C. elegans* nerve cells. And the way they did this utilized GFP because – I have to explain a little bit about this animal. It's an invertebrate and it has a very tough cuticle on its outside that's mainly collagen. And – but because it's an invertebrate, there's nothing else to it. But in order for it to move, it has to build up a very high internal pressure. So a standard way of doing electrical recordings from cells is to put a micropipette into a nerve cell and record from it. However, if you stick a micropipette into a worm, it explodes, which makes the electrophysiology extremely difficult to do. What they developed was a very nice method of relieving the pressure far away from the cell that you're interested in, and then making a very small cut near the cell they were interested in, and the nerves cells in that region will then come out gently out of the animal.

Marc Pelletier
Oh wow.

Dr. Martin Chalfie
And now – the nerve cell bodies, the rest are intact in the animal. But then, one could take a micropipette and attach it to the cell that you're interested in. Well, how do you know what the cell is that you're interested in? GFP tells you that you have the correct cell.

Marc Pelletier
The green one.

Dr. Martin Chalfie
So you just look for the green cell, and you attach the pipette to it and you record that. Miriam, after doing this wonderful work with Shawn Lockery, came back to my lab and I had a graduate student in the lab,

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Bob O'Hagan, working with her and with me, developed the method of being able to record from the touch-sensing cells that we were interested in, and did these initial recordings of looking at the cells and how they responded in the mutants and the normal animals.

Marc Pelletier
[32:30] It seems like a whole series of very ingenious systems and put together are elucidating really the molecular mechanism of touch, which is – you've been during some really fun – and these are fun questions, this is incredible work on this.

Dr. Martin Chalfie
It's been very nice. But to just talk a bit about GFP, GFP has been very useful in all these experiments, I think because of three features. The first is that it's something that is inherited from organism to organism. It's not like staining with an antibody where you have to prepare the tissue and then use an antibody to identify components, which is something we and others do as well. But having something inherited means that every animal and its progeny has the marker in the way you want it to and that leads to a lot of these experiments being done.

The second thing is that we don't have to add any other components. This is a non-invasive way of looking at things. And that's really quite good in this, to be able to do this without tearing the tissue apart, trying to make it permeable.

And the third aspect of this which just sort of rolls things together is that we're looking at living organisms, we're looking at living tissue. And so we can not get just a snapshot in time of what's happening, but look at the dynamic changes that are taking place over time and actually do this in real time in an organism.

And I think those advantages, that it's non-invasive, that it can be done in a living animal and that it can be inherited, have really been what's made GFP very important. And what continues to, I think, support the work in which people have been modifying GFP in a number of different ways to look at a variety of questions. And to give you just two examples of things that we've done in my lab, a couple of years ago, I found out about some absolutely spectacular experiments that was done by a chemist at Yale, Lynne Regan.

Marc Pelletier
I know Lynne, I've worked with her.

Dr. Martin Chalfie
And she did a wonderful experiment around – and published it around the year 2000. And that experiment was, she took GFP and she cut it in half. Now not surprisingly, if you take a molecule and you cut it into two parts, it no longer works. So that's not the unusual aspect of the work. But the really wonderful thing about it is she found that if she took two – what we could call peptide zippers that could bring the two halves back together again, when these associating peptides came together, the halves of GFP came together, and it produced a fluorescent molecule.

Now a number of people have been astonished by this result including myself and many of the people have used it as a way of saying, could this be a way of studying how proteins interact with each other. Instead of having the molecules that she did, she used to bring them together, let's use our two favorite molecules, and each one will bring half of GFP together and if our favorite molecules will come together, then GFP will come together and this will tell us that the proteins interact. And there have been a number of people that have been studying that possibility.

My lab did something a little bit different from this. What we did is, we realized that this – the molecules that Lynne had produced would allow us an even greater refinement in what cells we could label with a fluorescent protein.

Let me explain it this way. I already talked about how development is a combinatorial process, that one gene may be expressed in cell A and cell B and cell C, another gene could be expressed in cell D and B

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and E. If we have the first gene expressing half of GFP and the second gene expressing the other half of GFP, the only cell that will get both halves is cell B. That will be the only cell that will light up green when we shine blue light on it. And so instead of having many cells expressing GFP, now we only have a very defined, restricted set of cells. And that allows us to do still other experiments, because we've been able to narrow down the labeling to only a very small set of cells. And so that turned out to be a very useful tool for us in experiments that we were doing.

Another thing about GFP that people have learned over the years is that it's an extremely stable molecule. Once you make GFP, its sticks around for a nice, long time. And that's a wonderful thing. And we've recently, however, needed to use a GFP that was not so stable, that was made and becomes fluorescent, but then is gotten rid of by the cells. And there have been other people that have made constructs to do this. We in the last year or so have made our own different construct that allows GFP to be made, and – but then it is rapidly destroyed by the cell's mechanisms that get rid of proteins.

Marc Pelletier
You put a whole bunch of lysines on it or…?

Dr. Martin Chalfie
[38:32] Well, we actually put a domain that allows it to be targeted for protein destruction.

Marc Pelletier
All right.

Dr. Martin Chalfie
And this has proven to be useful an experiment that we found in which we found that – well, I'll describe one experiment we had. We know that for certain cells, transcription factors need to be turned on. It uses something that many people study, what transcription factors are needed to be activated. But we also found that by studying this rapidly degrading GFP, that there is at least one transcription factor and we think there are undoubtedly going to be many others, but the one that we were studying, that not only had to be turned on, but it was also turned off. Now what this meant is that the cells that were turned on during the time it was turned on, those cells were green, but when – because it was rapidly degrading when it is no longer being generated, eventually the GFP is cleared from the cells and they are no longer green.

So we wanted to ask the question, what turns it off? Not what turns it on, but what gets rid of it or what makes it so that it is no longer made. And so we simply took our animals that had the rapidly degrading GFP and if we look in adults, there are no green cells. But then we looked for mutants in which the adults did have green cells. That's leading us to try to understand the genes that are needed to turn off the transcription factors. So that's leading in a completely new direction that the lab is going to be going.

Marc Pelletier
I remember back a few years ago, maybe around 2000 or year 2001 or 2002, when people were burning – taking a laser and burning the GFP, because there was, because you could identify the cells expressing GFP during the laser and then you cook it. And sort of then watching that photobleaching get restored by more new fluorescence. But I think if you have a GFP that can turn off quickly, then…

Dr. Martin Chalfie
Well we can't turn it back on. And so having something that is going to be made. So there's still a very good use for – there's been versions of GFP that are photoactivatable and also things that can be inactivated, as you said, by laser light. So there's a number of different variants that have been produced by people over the years that have proven to be very useful.

Marc Pelletier
I would like to take a minute to thank GoToMeeting for sponsoring Futures in Biotech. GoToMeeting is a service provided by the makers of GoToMyPC. And what it allows you to do is hold your meetings by sharing your desktop over the internet. Works both with a PC and a Mac, and it's secure. Where I find this

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is interesting, at least as a scientist, is that you can share your data without having to travel. So it saves you money, it saves you time. And by doing so, you can share your data at an earlier stage. It's really, really important to design experiments that allow you to address your question, to do it in a way that allows you to have an answer with as much certainty as possible, and by sharing and communicating this data, the preliminary data with your colleagues and by doing it with a desktop, which makes it so much easier, you can save a lot of time in terms of science as well. They are currently offering a 30-day free trial. For more information, visit gotomeeting.com/biotech. Now, back to the interview.

[42:15] It's amazing, right, the – I never saw how useful GFP was I suppose until the Nobel Prize was awarded. And then at that moment when I first read it in The New York Times, I was, of course, this protein really has led to – in terms of development and in terms of proteomics, it's probably the most important tool for the human molecular anatomy – human atomic anatomy, as I call it. Because it has allowed us to see where the proteins are in the cell, where they're moving to, where they're going, how long they will be there, in a non-destructive way as you mentioned in a live organism.

So then it became absolutely clear, yeah, the Noble Committee made the right choice and I would like to say congratulations. I always feel a little awkward in this situation, but I'm humbled by the – and I – I really appreciate you coming on, because what I'm really trying to do with this show is really get a first-hand experience or account of some of the really great science and it's absolutely clear that you found some tools and made such great use of them that the rest of the world watched and then followed your footsteps and…

Dr. Martin Chalfie
Well, let me change that just slightly.

Marc Pelletier
All right.

Dr. Martin Chalfie
Because I don't think that gives it – you have the sort of timing a little bit off. I mean we developed this tool, and I imagined that it was going to be useful in many respects. There were certain things that I thought it could be used. But the really wonderful thing about – I think also – about the prize and about what's happened with GFP, the interesting thing about the Nobel Prize is that it wasn't given to one person for this. It was given to three people. The person who discovered the protein, the person that showed that it could be used as biological marker and a person who improved it.

It was really given for a process, not so much a single discovery. And the amazing thing that I find in this, is that this process is continuing. I would never have imagined all the various uses that people have put GFP to and the modifications they've made to this and the improvements that they've made and alterations. It really is the combined brain power of so many people that have developed this into the useful tool that it is. So it's really more of a community and an interaction of a number of different people that have added their own value to this work. So, it – the thing that's been astonishing to me is all the wonderful ideas that people have put into it that, as I say, I would never have guessed.

Marc Pelletier
Well, you know, it took someone to say "And let there be light." You know it sort of – or did it? I don't know. I'll let the audience decide. But I guess that's what makes it so incredible. There is now light inside the cell. We can see what's going on in a living organism. And that's so much fun. And I don't think there's a molecular biologist out there that would rather use immunofluorescence and do immunocytochemistry over using GFP. I mean just having – doing one genetic construct, and then, as you said, have generations of organisms. And in popular science, it's been taken too to all kinds of levels where the green cat recently in the news, right? The fluorescent cat. So…

Dr. Martin Chalfie
So there have been that – actually, my favorite use of GFP in – not real use of GFP, but it turns out that there was a student apparently on the set of the movie The Hulk that Ang Lee brought out several years

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ago, and told him about GFP. He was interested in jellyfish among other reasons. And if you look at the opening credits to The Hulk, Ang Lee's The Hulk, what you see is a jellyfish and a hypodermic cell going into the jellyfish and seeming to pull out a green fluorescent solution from the jelly fish. If it could only have been that easy to do the experiment. And a notebook that then says, in 1965, green bioluminescence. And the implication is that The Hulk is green because he's the first GFP transgenic human. And I thought it was a wonderful thing that to see in a movie.

Marc Pelletier
[47:30] That's amazing. That's a great – a great connect. By the way, it's my wife's favorite protein, absolutely by far. She became – she started working with adenovirus or adenovirus and it was using GFP markers and they were using it to characterize all kinds of cells. And she used the FACS, the fluorescence-assisted cell sorter, that was her favorite machine. So I was always expecting her – because adenovirus can give people colds, that she'd come back with fluorescent, a fluorescent nose or skin that is a little bit off color. But she never turned into the Hulk, so. Well, I'll let you go and I really, really appreciate you coming on the show.

Dr. Martin Chalfie
It's been fun.

Marc Pelletier
This is fun, and this is really why I do this show. Because the whole process of science and discovery is – it takes an enthusiasm and your enthusiasm was infectious. So we can all go out there and get back to the bench and do some more – do some more work. So thanks again for coming on the show.

Dr. Martin Chalfie
Great, nice talking to you.

Marc Pelletier
I really would like to thank Dr. Martin Chalfie for being a guest on this show and for sharing his science with us today. He is the William R. Kenan, Jr. Professor and Chair of the Department of Biological Sciences at Columbia University. I'd also like to thank Dr. Glen Ernstrom who helped to coordinate this interview or these interviews, Part I and Part II, and thank him for his contributions as well in the introductions. And also the audio pick this week. I'd also like to thank Phil Pelletier and Will Hall for the opening and closing themes.

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And lastly, I'd like to close the show with a song that's near and dear to my heart. But I don't think it's true anymore, it's pretty straightforward to do. So, for Futures in Biotech, I'm Marc Pelletier.

[Music – It's Not Easy Bein' Green by Kermit the Frog]

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