



## Futures in Biotech, 37: Just a Touch of Green

### Leo Laporte

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[Music]

### Marc Pelletier

Welcome to Futures in Biotech. I am Marc Pelletier. This year, we're going to end on a high note, because today's guest is Dr. Marty Chalfie. Dr. Chalfie is a Professor and Chair of the Department of Biological Sciences at Columbia University and also shared the 2008 Nobel Prize in Chemistry with Osamu Shimomura and Roger Tsien for their discovery and development of the green fluorescent protein, GFP. I have invited Dr. Glen Ernstrom, who did his graduate work in Dr. Chalfie's lab, to help with the introduction.

And by the way, this is going to be a two-parter. In the first part, Dr. Chalfie describes his work exploring how organisms sense touch; and in the second part of course, Dr. Chalfie describes the work that led to him winning the Nobel Prize, that is, the development of GFP as one of the most important tools of modern molecular biology.

So, on to our introduction, and here is Dr. Glen Ernstrom.

### Dr. Glen Ernstrom

Yeah, so I was a graduate student with Marty from, let's see, '96 to about 2003. Actually I got out about 2002 but I got my degree in 2003, so.

### Marc Pelletier

'96 to 2002?

### Dr. Glen Ernstrom

Yes.

### Marc Pelletier

So, six years of pain?

### Dr. Glen Ernstrom

It was a roller-coaster, ups and downs. But it worked out well in the end.

### Marc Pelletier

So, yeah, tell me a little bit about what it was like to work for Marty Chalfie. And this is pre-Nobel era, right?

### Dr. Glen Ernstrom

Yes, well no, no. I actually – so I guess, well, yeah, pre-Nobel era, you would never have known that he was – had a key role in the GFP discovery. The thing that Marty – what's special about Marty is that – and his colleagues really recognize him for is his incredible focus on mechanosensation, or the study of the sense of touch. And that's what really got me interested in his lab, not so much – I mean, I didn't really know so much about what GFP was doing or I mean it was such a new technology when I was getting there. It was a couple of years I guess since the paper had been published and I just got out of undergraduate. And so I joined the lab because I was really interested in the ability to use genetics to study the nervous system. And how that could also tell us something about behavior.

And so he was – he studied this very simple system. And he – I mean for his career, he studied the six mechanosensory cells in this tiny, tiny nematode, it's as big as a grain of sugar. And within that tiny organism, he's just been so focused on trying to figure out how the six mechanoreceptor cells work. And I was really interested in being able to manipulate genes to understand how – it was a model system to study how behavior could work. And the particular behavior that you get when you touch the worms, they just, they run away from it. So if you touch them with a special tool, a special tool is an eyebrow hair glued to the end of a toothpick, and you touch them, and they run away.

And I thought well, we need to – in order to study complex – how molecules – the behavior, we have to start with something really, really simple. And I thought this was a good...

**Marc Pelletier**

A simple response, a worm...

**Dr. Glen Ernstrom**

Just a tiny little thing, nothing too complicated. And I think it's been really fruitful over the years. People who have gone through the lab and who contributed and another people in the field have really built on this system to make it a really super well-studied system on how a multicellular organism senses touch.

**Marc Pelletier**

[5:11] That's somewhat similar to the work using *Aplysia* right, that Kandel was doing?

**Dr. Glen Ernstrom**

Yes, Eric Kandel, yeah. So he had this – there's a – they have an aversive response, gill response and touching those things, they can...

**Marc Pelletier**

This is a sea slug, this is a giant...

**Dr. Glen Ernstrom**

Yeah, sea slug, yes, the *Aplysia* sea slug. And so Eric Kandel and his colleagues worked out the neural circuitry that led to that. The thing about *Aplysia* though that – is that it's not a genetically tractable organism. So it's hard to get on the molecular basis of what's going on there. Although through beautiful pharmacology and other biochemistry done on *Aplysia* they're able to get their handle on that.

**Marc Pelletier**

Right, they have giant, giant nerves, right...

**Dr. Glen Ernstrom**

Yeah, yeah. So they make them really accessible to do all sorts of neural recordings and study electrical properties.

**Marc Pelletier**

So these are the – sorry, sorry to interrupt. This is just – what’s shocking here is that there’s two very bizarre organisms...

**Dr. Glen Ernstrom**

Yeah.

**Marc Pelletier**

...that led to transformational science that changes our understanding of neuroscience, right?

**Dr. Glen Ernstrom**

Right.

**Marc Pelletier**

And one’s in a worm the size of a...

**Dr. Glen Ernstrom**

Sugar grain.

**Marc Pelletier**

...comma, and the other one’s this giant sea slug.

**Dr. Glen Ernstrom**

Yeah, yeah.

**Marc Pelletier**

And their simple, simple behaviors I suppose are what really make it interesting. Because then you can – you know, you don’t have a million things to try and understand, you’re just looking for a worm that runs away.

**Dr. Glen Ernstrom**

Yeah, yeah, exactly, exactly. So yeah, I mean I think in order to wrap my brain around this – just how a molecule can affect a way an organism responds to something or responds to a stimulus, I don’t know, you’ve just got to start really simple, I guess, before you can – but I guess that tells us how much more work we have to do. I mean, we can – we’re barely starting to understand how worms run away from an eyebrow hair, trying to figure out how consciousness or – this is going to take a lot, a lot of work from a lot of people doing different things.

**Marc Pelletier**

Yes, great.

**Dr. Glen Ernstrom**

All right.

**Marc Pelletier**

So, thank you very much for coming on.

**Dr. Glen Ernstrom**

Thank you.

**Marc Pelletier**

I think I’d like to start off by asking you what are the main scientific questions that drive Marty Chalfie?

**Dr. Martin Chalfie**

[7:23] So I’ve been interested in several questions that relate to how we get different types of cells and what cells become the particular cells they do and how they work. In particular, I’m

interested in the nervous system, how we get different types of nerves cells and how the different nerve cells function. And in all this, I've really been interested in one group of six cells in a small worm, the nematode, *Caenorhabditis elegans* or *C. elegans*. And these six cells are the cells that sense touch.

And for years I've been studying these cells trying to answer two major questions. The first one is, for these cells, how do they become the specialized cells that they are? In other words, how do they differentiate or become different from all the other cells in the animal's body? And the other question is, because they're touch-sensing cells, how do they sense the mechanical stimulus that they're given? How do they sense touch?

And we can actually address both of these issues starting from the same point, and that is, we look for animals that are insensitive to touch. They can become insensitive to touch in basically two ways. First, they can become touch-insensitive simply because the cells don't develop appropriately, they don't become the mature cells at all, or they don't appear at all, or they die. So those – the mutants that are defective in those animals allow us to investigate the genes that are needed for the process of cell differentiation.

Others of the mutants are touch insensitive not because the cells have not developed appropriately, but because the cells don't work, and by understanding the genes that are defective in those animals, we can start to ask questions about the molecular mechanism underlying the sense.

Now, to back up just a little bit, why is that an important thing? Biologists have a good idea how – what the molecules are that allow us to sense light. We know that there's a molecule, rhodopsin in the retina, that really captures the energy from light and changes it, ultimately, into a chemical signal in the cell, it's in one of the way stations that allows that to happen. We also know what molecules are important for sensing chemical signals, so we understand the molecules needed for olfaction, we know how hormones and neurotransmitters act as signals within the body, because we know what the molecular receptors are for those chemicals.

But there are many senses that we have absolutely no idea how they work. How they capture the signal and change it into some signal that the cell can use. And the mechanical sensors are in this category. So we don't really understand the molecular basis of hearing, of our sense of balance, of stretch, of our detection of blood pressure, our sense of touch. All of these are senses that work because cells are moved or perturbed in some way, and that movement or mechanical disruption leads to an electrical or chemical signal in the cell.

But we haven't a clue as to how that actually takes place. The hope was that by looking for – at worms and looking at mutants that are defective in these animals, we can get some insight into the molecules needed for some of these senses. And we've been somewhat successful in finding these.

### **Marc Pelletier**

Just a quick question. And this is to sort of set up – so you're working with *C. elegans* which is a fantastic tool for genetics, sort of the fruit fly of the 21<sup>st</sup> Century. Could you tell us a little bit about it? We had a discussion with Cynthia Kenyon last week or last episode on ageing, and she described it a bit. But I think it'd be really great if you could give us a little sense of what this worm is, how it relates to humans, maybe in terms of scale of genomics and why is it a great animal to work with, especially for studying how a cell becomes a specific cell and maybe how a cell works? Why *C. elegans*?

### **Dr. Martin Chalfie**

[11:55] So the work on *C. elegans* was started in the early 1960s by Sydney Brenner in Cambridge, England. And what he was interested in at the time was to try to use a genetic way of trying to understand how the brain works, or any nervous system works. And his insight was to try

to use an organism that he could learn a vast amount of information and be able to describe as completely as possible.

And so he chose this small, it's only 1/25th of an inch, a millimeter long as an adult worm, because of many of its features. First, it is very small. It's very simple in structure. It's basically a tube. It is very quick in its growth; it grows from an egg to an egg laying adult in only three and a half days.

**Marc Pelletier**

Wow!

**Dr. Martin Chalfie**

At room temperature. It is – it has definable cells, in other words, doesn't look like a sponge, where you're not really sure what the cells making up the organism are. It has clear-cut nerve cells and muscle cells and skin and so on. And it has a couple of other properties, one of which is very important for the work relating to the Nobel Prize and that is that the animal is transparent.

And what all these features have meant is that from the early work that Sydney Brenner started, it was possible to prepare the animal for the electron microscope, and section the animal. The whole animal sectioning would take about 20,000 sections, and blow these sections up 10,000 times, and trace out what every cell looks like.

And so this is the only animal that we know that it has precisely 302 nerve cells. And we know how every one of those 302 nerve cells are connected to every other cell in the animal.

**Marc Pelletier**

Wow!

**Dr. Martin Chalfie**

So it's the only animal we have what we call the wiring diagram. We know how it's pieced together, how its nervous system is pieced together. In fact we know what all the cells look like. That doesn't mean we know how all these connections work to make a functioning organism. We are still in the process of trying to understand that. But at least we have the basic blueprint on a cell-by-cell basis.

The other thing – and this is work that was done by John Sulston, who in 2002, with Sydney Brenner and also Bob Horvitz won the Nobel Prize for Medicine and Physiology for their work. John Sulston was able to look – because of the animal's transparency and the fact that it lived very – it developed very quickly, he was able to follow each cell division – from the fertilized egg all the way into the adult. And this meant he could map out every single cell division as it went from a fertilized egg to two and to four, eight cells, all the way to the final 959 cells of the adult.

**Marc Pelletier**

By the way...

**Dr. Martin Chalfie**

That's not including the germ line.

**Marc Pelletier**

I apologize for interrupting here, but what I think is really funny is I can imagine a postdoc or a graduate student sitting there for 72 hours tracking with little diagrams, every cell division, which is doable, that's a doable experiment.

**Dr. Martin Chalfie**

It's a doable experiment, and fortunately they can even get some sleep, one – John is a spectacular experimentalist, and he was able in – he developed various ways of mounting the

animal so he could watch it under the microscope, and found that basically he would take the animal and after he had viewed it for 10 or so hours he could then put it in the refrigerator. Its growth would slow down, and then he would be able to go to the bathroom or go have a meal or be able to come back the next day.

[16:03] But the really important thing and you are getting it, you're actually sort of hinting at something that's actually very important here. And that is, the development is the same from animal to animal, with very few differences. And so what it meant is he didn't have to follow one animal all the way through from the egg to the adult, he could follow the same pattern by looking at various individuals and making up the entire pattern together.

And so what this gave us is what we call the cell lineage of the animal. Other things that have been very useful, Sydney Brenner developed the genetics of this animal. It's very straightforward to be able to get mutant animals, so we can compare them to the wild type or normal situation or what we've designated as normal. And so we can have – we can make a comparison between the two types and study various types of cellular and developmental phenomena.

And then work principally led by John Sulston but also by Bob Waterston, excuse me, was that they were able to put together the worm genome project and so *C. elegans* was the first animal, the first multicellular organism, to have its complete genome, all of its DNA, sequenced. And this gave us again a great deal of information that we could then use for various studies and people study many aspects of basic cellular biology and developmental biology using the animal. In my case we use it to study nerve cell development and the sense of touch.

#### **Marc Pelletier**

One quick question, do you foresee a time – usually we go to the end of the interview at this, but this is the right time to ask it. Do you see a time when we'll have a complete cell lineage of the human body or are human's just absolutely too different to get a complete cellular map. I am hoping for cellular anatomy but I actually foresee perhaps a molecular anatomy, a human atomic anatomy eventually but do you see us being able to attain a complete cellular anatomy of the human body the same way we have in the worm?

#### **Dr. Martin Chalfie**

I think we'll be able to understand the different cell types that are in humans and other animals. I don't – but we are not going to be able to get the cell lineage in the sense of we – a cell always divides in a certain way, because we know that that's not how humans develop. In fact, that's not how the worm develops completely either. So, often in biology think about two different types of cells, the cells that make up the body of the individual and the cells that give rise to the gametes, so these are the germ cells that will give rise to the sperm or egg. And in this nematode the cell lineage for the somatic cells, that is skin, the gut, the nervous system, muscle and so on, that's reproducible from animal to animal. But that accounts as I said for 959 cells.

There is an additional approximately 2,000 cells that start off by – only a small set of cells, two in fact in the newly hatched larva and they develop – those cells are the ones that will eventually give rise to sperm and egg. And they don't have a defined cell lineage. One can't say, oh this cell will always divide produce two and then each of the daughters will produce two cells. There is no discernible pattern in that division and many tissues in mammals including humans are like this; that there is no defined pattern of cell division that gives rise to a set of cells.

So, we are never going to have a defined pattern of how all individual humans develop. It's just not the way the cells divide. But in this worm and maybe it has evolved to be able to be – to grow very quickly and to have more defined cell divisions to make precisely the right number of cells in the right places that it's needed that sort of very defined scheme. In other cases where you need lots of cells and you need them to interact before decisions are made about how the cells will develop along one line or another.

**Marc Pelletier**

How closely is our genome related to *C. elegans*?

**Dr. Martin Chalfie**

[21:05] I don't know the exact number, so I'm going to be quite approximate, the *C. elegans* genome has approximately 20,000 genes in it, the human genome I think has between 30 and 40,000 genes and the numbers always change a bit. That's because as – first of all as we learn more about what's actually encoded by DNA and as we learn more about what the genome actually has within it, but – and there is not a one-to-one correspondence between the 20 that are in the worm and the about double that number that are in mammals or humans.

But a very large number of the genes are in fact in common. So, that I think something in the order of perhaps 70% of genes that have been implicated in inherited human disease have counterparts that are seen in *C. elegans*. So there is a great deal that we can learn from studying these genes in *C. elegans* or in *Drosophila* or other model organisms where we can do the genetic manipulations and do studies that we could never even contemplate in humans.

**Marc Pelletier**

And mutations I suppose, if a mutation is possible in a gene that's similar between the worm and the human you might be able to identify a human disease related by then going and studying the same mutation in the worm.

**Dr. Martin Chalfie**

Absolutely. So, for example we know that there are a series of genes, a series of diseases in humans including Huntington's disease in which one of the hallmarks is an extended series in the proteins of the amino acid glutamine, the so called polyglutamine diseases. And people have been studying in worms and as well as in *Drosophila* what happens if you have a gene that has a large number of glutamines, a string of glutamines, what does that do, what is the signal – does that signal to the cell? And it seems that many of the same defects that are seen in the human disease in terms of affecting cells, aggregates forming in the cells and so on, are actually mimicked in the worm or fly disease. And so people can then begin to study those and ask, for example, what other genes if they are defective or what other conditions will change the effects of having this expression of polyglutamine in the cell?

**Marc Pelletier**

I guess it'd also be great to produce a ton of material, right. If you have – you can grow a worm to maturity in three days, develop the disease, you could have thousands and thousands and thousands where you could triage them into wells and screen compounds to be...

**Dr. Martin Chalfie**

And people, people have actually done this where they have tried to use it as a way of screening for compounds that affect various processes including disease processes, yes.

**Marc Pelletier**

A very powerful little critter.

**Dr. Martin Chalfie**

It's been a lot of fun to work with for quite a number of people.

**Marc Pelletier**

Yes, and they don't fly away like *Drosophila*. Your lab is not swarming.

**Dr. Martin Chalfie**

Or complain, yes.

**Marc Pelletier**

Which is kind of neat. And they're – they're transparent which has helped sort of give you a good use for them.

Well, so let's move back to those two fundamental questions, and perhaps we could start – so the two questions being how do the six neurons – six cells that are neurons that you study, how do they become those neurons – or touch neurons and then how do they sense touch? And perhaps we should start with how do they sense touch? I, I – for some reason I'm drawn to that question. How does a cell or a neuron specific to touch sense touch? Are they little molecular machines or is it chemical? How does it ...

**Dr. Martin Chalfie**

[25:24] So as far as we can figure out, it appears to be a molecular machine and the question is what is the molecular machine? How does it work? So, one of the features that people over the years have found in terms of hearing, in terms of sensing in flies, the bristles on flies are very sensitive to touch and the touch systems in *C. elegans* have one interesting feature to them, and that is they respond with amazing quickness, so fast that one gets a response within, well, less than a millisecond, often much faster than a millisecond.

And the interesting thing about this, in comparison let me say to vision, when light is received by a photoreceptor that – the response comes in tens of milliseconds. Not sub-millisecond. And the difference appears to be that in vision the response has a chemical intermediate. The light is received, leads to a chemical intermediate that then affects the membrane of the cell so that channels are opened or closed, so that we change the electrical property of the cell.

But for hearing and insect bristle mechanosensation and for touch in *C. elegans* the response is so rapid that we don't know of any chemical reactions that can take place that can occur that quickly. So the thought...

**Marc Pelletier**

I have never heard of an enzymatic reaction going that fast ever at one millisecond. It's much...

**Dr. Martin Chalfie**

So we – we think that that's – that means that there cannot be a chemical intermediate between the actual sensing of the signal, we call it transduction, and the electrical response of the cell. So there must be some sort of physical detection, and that leads to a channel being open. That is at least what we and many others feel about this.

So when we studied the mutants affecting touch sensitivity in the animal, it was particularly nice to find that two of our genes that we found could be expressed just in the touch-sensing cells were in fact genes that encoded proteins that were components of channels.

We've subsequently found, in fact it's not two genes that do this, but there are probably five, at least five genes and maybe there will be more that contribute proteins to make a complex in the membrane that has as its central core a channel that seems to respond to the mechanical stimulus. And we think the channel itself is the sensor.

And our reason for thinking that the channel itself is the sensor is that we obtained of couple of very interesting mutant versions of the channel and those – let me explain that in some cases if you want to know is a protein needed for a process, you might say well what happens if we get rid of protein. You don't have it there then you shouldn't see the effect. And sure enough if we get rid of the channel proteins or any of the proteins from the complex, the animals don't respond to touch.

**Marc Pelletier**

How do they behave such – how do you determine whether a worm can feel – that's a...?

**Dr. Martin Chalfie**

[29:12] Well, it doesn't complain, so, it's very hard to – so it's actually a very simple test. We take an eyebrow hair and the reason we use eyebrow hair is most of the time people don't cut their eyebrow hairs and so they are fine-tapered hairs. You glue that onto the end of a toothpick and under the microscope, for the animal is only a millimeter long, we basically stroke the animal on the tail or to the head and it will move away from that touch. An animal that is touch insensitive will not move away if you touch it.

Now we have one slight problem, a dead animal will also not move away. So, we actually touch the animals with this hair, if they don't move away with the hair, but they do move away if we hit them a little bit harder with a little platinum wire that we use to pick them up and move them from plate to plate, then we know that they are just not responding to gentle touch and that's what these cells that we study sense. And so if they don't respond to the tickling with the hair but they do respond to the little tapping with the wire then we know that the cells are not functioning correctly and they are not sensing touch.

Now we have more sophisticated ways of doing this and that is we can actually electrically record from the cells and when we touch the animals we can see that there is an inrush of sodium into the cells. And we can record that electrically, and monitor that and for these animals that are missing the channel, when we touch them we get no influx of sodium at all.

**Marc Pelletier**

How did you screen for these? Did you go around tickling worms until you found one?

**Dr. Martin Chalfie**

Exactly, exactly.

**Marc Pelletier**

You did?

**Dr. Martin Chalfie**

And I did and it's a great use of undergraduates at my lab.

**Marc Pelletier**

Worm ticklers.

**Dr. Martin Chalfie**

And, but it actually turns out that by appropriate ways of doing the experiments one can actually identify something on the order of 20 to 30 independent mutant animals, that is 20 or 30 different mutations affecting touch in a single day. So, it's a long day that you do this. But we've actually over the years collected over 450, now approaching 500 different mutant strains that are touch insensitive. We do that because we can try to get as many different mutant versions for each gene and we have actually many – for some genes we have as many as 70 or so mutant versions of the genes.

**Marc Pelletier**

Well, that seems to be really, really important because if someone comes across with a disease where they don't sense, you might be able to have the same allele, the same amino acid change.

I'd like to take a minute to thank audible.com for sponsoring Futures in Biotech. They are an incredible source of audio content on these "internets". I think they've over 51,000 titles now and this week's pick is The Snows of Kilimanjaro and Other Stories by Ernest Hemingway narrated by Stacy Keach. Here's a clip.

**Stacy Keach**

"For Christ's sake," he said, "That's been my trade."

He lay then and was quiet for a while and looked across the heat shimmer of the plain to the edge of the bush. There were a few Tommies that showed minute and white against the yellow and, far off, he saw a herd of zebra, white against the green of the bush. This was a pleasant camp under big trees against a hill, with good water, and close by, a nearly dry water hole where sand grouse flighted in the mornings.

"Wouldn't you like me to read?" she asked. She was sitting on a canvas chair beside his cot. "There's a breeze coming up."

"No thanks."

"Maybe the truck will come."

"I don't give a damn about the truck."

"I do."

"You give a damn about so many things that I don't."

"Not so many, Harry."

"What about a drink?"

"It's supposed to be bad for you. It said in Black's to avoid all alcohol. You shouldn't drink."

"Molo!" he shouted.

"Yes Bwana."

"Bring whiskey-soda."

"Yes Bwana."

"You shouldn't," she said. "That's what I mean by giving up. It says it's bad for you. I know it's bad for you."

"No," he said. "It's good for me."

So now it was all over, he thought. So now he would never have a chance to finish it. So this was the way it ended in a bickering over a drink. Since the gangrene started in his right leg he had no pain and with the pain the horror had gone and all he felt now was a great tiredness and anger that this was the end of it. For this, that now was coming, he had very little curiosity. For years it had obsessed him; but now it meant nothing in itself. It was strange how easy being tired enough made it.

Now he would never write the things that he had saved to write until he knew enough to write them well. Well, he would not have to fail at trying to write them either. Maybe you could never write them, and that was why you put them off and delayed the starting. Well he would never know, now.

### **Marc Pelletier**

If you'd like to download *The Snows of Kilimanjaro and Other Stories* by Ernest Hemingway, simply head over to [audible.com/biotech](http://audible.com/biotech). You can get the book for free if you are a new subscriber and of course if you like the service you stay on but if you don't you can actually

cancel the subscription and you still get to keep the free book. So, it's a win-win situation either way. So, back to the interview.

[34:52] So, one thing – so, you've got the worm ticklers identifying mutations; you have up to 70 mutations for specific – for some of the genes that you have and I was asking sort of have you correlated some of those mutations to some of the specific diseases, certain amino acid changes in this channel at the core of the complex?

**Dr. Martin Chalfie**

So, there're no actual diseases pertaining to these particular proteins in humans but having the many mutations is very useful. So, let me back up a bit and say that in trying to understand how these things work, we find that if we get – have mutations that in fact get rid of the channel proteins, of course, the animals are touch insensitive, we have no electrical response but that only tells us that these proteins are needed for the process, but they don't tell us where these proteins are needed for the process because they get rid of something.

This is like trying to figure out how a car can move and saying what's essential for a car to move; oh! I have this key that lets me into the car, it's the door key, and if I lose the door key, I can't get in, the car won't move. So, the door key is very important but it's not what really makes the car move, it's just something that's needed.

And so getting rid of these proteins and saying, well, the animals now can't sense touch, we don't know if it's a developmental problem or if this component is actually needed for the sensing. And this is where the other mutations come in as being important because if we take these – we look at these other mutations, sometimes we don't simply get rid of the component, we change the component. So for example, we found that normally these channels let sodium into the cell and that's the electrical signal that turns the cells on and starts the whole chain of command that eventually signals other cells so that the animal moves away from the touch, but we found some of our mutations affecting the channels change the channels so that they didn't let sodium into the cell but instead when the animal is stimulated they sent potassium out of the cells.

Now the cells – that signal of potassium coming out is not going to be conveyed, so the animals are touch insensitive. But what that told us was that touching the animal directly led to this ion movement, sodium in or potassium out. It's very hard to come up with a scheme in which that channel cannot be the sensor and have those two different effects. So, it's much better instead of getting rid of something to change its properties and we were able to change its properties and that's what told us that this channel, and by extension the complex proteins that are at the membrane, is actually the transducing molecule, the sensor of touch in the animals.

**Marc Pelletier**

And I would like to sort of try and get a visual idea of what this protein complex that senses touch looks like. Has the structural biology been elucidated or can you describe what this little machine looks like?

**Dr. Martin Chalfie**

[38:29] I wish I could but most membrane proteins have not had their structures determined. And although we would dearly love to have this information, we don't have it now. But it's interesting as to what we have found for these proteins because what we found is that in addition to the proteins that actually make up the channel itself – the sodium – that allows for sodium to come into the cell, we found several other proteins that are essential for the efficiency of this channel.

And one we have studied, or two of them, we've actually studied to a little more extent than the others and that is that – and one in particular, this is a protein that has three properties; one, it binds and associates with the channel protein – we knew that, that's why it's part of the complex – but it also binds to itself, so there is many copies if it associated with the channel. And the third property is that it binds the molecule cholesterol. Now, cholesterol is a normal component of cell

membranes, but we feel that it's the property of this protein that binding cholesterol, it changes the local environment of the channel so that it actually works properly.

So it looks like this complex needs to have not only the channel but other proteins that change the associated membrane around that channel, so that it's in a particular state so the channel can actually sense touch. So we are quite intrigued by these associated proteins, some of the others also seem to have at the moment a rather indirect effect on cholesterol as well. We think that's important in how the channel functions. And we think that this may be a general property for some membrane proteins that they have associated proteins with them that change their local environment and by that change their properties.

### **Marc Pelletier**

It would seem that such a molecular machine, there's got to be some membrane bending in there where the, you know, the shape of the membrane is contorted changing the orientation of those complex proteins – the complex of proteins around the channel.

### **Dr. Martin Chalfie**

So this is getting at the basic problem of how does touch lead to a channel opening a protein. That's a channel that will allow ions to flow across a membrane. How does it change its properties? How does touch actually change the properties of the channel to open and close? We don't have any answers to this.

Actually the best answers that people have been able to find have been in work on channels that really don't look like our channels but sense osmotic changes in bacteria and those channels are fascinating, the bacterial channels, because when a cell swells up because, for example the bacteria in the wild might find itself in a puddle of rainwater, for example, as it's swelling up as that water keeps rushing into the cell, eventually that cell is going to explode, we say that it lyses. Now, there has to be some way of compensating for that increased osmotic pressure as the thing blows up like a balloon.

And what bacteria have evolved are specific channels that as the cell enlarges and the forces on the membrane change, the forces cause a change, or the bending of the membrane, causes a change in these channels so they open up. And in fact for some of them they open up just before the breaking point, just before the explosion is going to take place. And what they do is they let out salts and small molecules, sort of jettisoning all of the things that are needed in the cell that are bringing water in, and as these things go, water goes with them and the cell can survive for a short time.

Then, as the concentration – concentrations change and the cell starts to shrink then of course it can replenish its small molecules. But this is sort of an escape valve if you will for the cells as they start to swell up. But it's in the study of these proteins that people have found that they don't need any other associated proteins, these channels in the bacteria, they don't need any associated proteins. What changes is the forces in the membrane and it's a little bit as if that when the forces change, the molecule shifts its shape, it moves a little bit like an iris in a camera, if you will, and that allows for the opening of the channel and then when the forces – when the cell starts to shrink and the forces change, it folds back into the closed state of the channel.

No one really knows what the forces are or how they change for the proteins that we are studying in the touch system in *C. elegans*. But we've hypothesized maybe a somewhat similar situation is taking place, not that the cell is swelling but maybe the association of our channel complex with other proteins is actually pulling or pushing it. Well, that would change the forces on the molecule on the complex and so that – as those forces change in the membrane then again it would open and close, in analogy to what's – what we know is happening in the bacteria. But we haven't been able to prove this yet, so it's at the realm of a hypothesis right now.

### **Marc Pelletier**

Sounds like a lot of – there'll be a lot of potential great work if someone were to join your lab. A lot of great work in your lab.

**Dr. Martin Chalfie**

Oh, there's always lots of work in the lab, there's no question about that.

**Marc Pelletier**

[44:32] It's so fundamental, right? And such an important biological question, how do we interface with our world outside of us, not through – I mean, eyes – sight is important, hearing is important, but touch is important as any – it's critical. And so...

**Dr. Martin Chalfie**

Very much.

**Marc Pelletier**

A fun scientific question to be following up on. And it looks like you have this molecular machine with still so many questions to answer. How does it work? You know it's the machine that's working but then I suppose even just getting a picture of what it looks like, have you guys reconstituted the complex in lipids and gone on to the electron microscope for potential particle analysis and imaging and what – ?

**Dr. Martin Chalfie**

We haven't been able to do that. In fact, one of the things about mechanical sensing that makes it so difficult to study is that the context of the sensor is extremely important. So, for example, in hearing, we know that the hairs and the hair cell in the inner ear are the cells that are sensing the signals for hearing. These various appendages that are coming off of these cells seem to be attached one to the other by structures which are called tip links. So it's not just a sensor in the membrane, it seems to be associated with something outside of it. Well, this seems to be the case for many mechanical systems.

So that also seems to be the case in our cells too that it's not just the cell on its own that can respond in the sense, it's because the cell is associating with other cells. So, when we take these cells out of the animal, put them into tissue culture, they don't work anymore. So now one of the experiments we hope to be able to do in the near future is actually take these cells out and see what other components we need to add to other cells to reconstitute the system so that it will work. So, it's not just an isolated cell that is the sensor, it's the cell and the associated material outside the cell, sometimes contributed by accompanying cells that's really important for its function. So, it's a very complex problem and maybe one of the reasons people have been – it's been very hard for people to actually identify these molecules.

**Marc Pelletier**

In the long run, I mean, understanding this is really – is the beauty of nature I suppose, I guess that's what drives scientists is the natural beauty of these machines. Do you see that potentially translating into some nanotechnology? Because the one-millisecond sensor for a tickle, I mean, would be – or are there machines out there already that are equivalent to these sensors?

**Dr. Martin Chalfie**

I can't really tell you about that. That's not in the area that I am really knowledgeable about, so I think I'm going to have to let that one pass.

**Marc Pelletier**

All right, all right. Well, maybe I'll get some engineers and have them compare their machines to yours.

**Dr. Martin Chalfie**

Fine.

**Marc Pelletier**

Yeah, so, maybe we could move a little bit to – so you said there are six touch neurons or you are studying six cells in particular in the – out of the 302...

**Dr. Martin Chalfie**

That's right.

**Marc Pelletier**

...neurons that exist in the worm? And maybe you could tell us a little bit about how you study their development into those touch nerves? How – what would you call these? The mechanosensitive nerves?

**Dr. Martin Chalfie**

So, we call the cells the touch receptor neurons.

**Marc Pelletier**

Touch receptor neurons.

**Dr. Martin Chalfie**

[48:27] Touch receptor neurons. The – so, as I said there are six of these cells, there is two cells – when the animal hatches there is only four, so two of the cells have their cell bodies in the tail of the animal and send processes that go half the length of the animal to the middle of the animal and then there is two cells, each of these are one on each side of the animal, that have their cell bodies near the middle of the animal and have processes that go up to the head, so the second half of the animal is taken care of as well. And then there's a pair of cells that arise as the larval animal develops. And – but, we can really talk about the two pairs of cells, the one in the tail and the one midway along the length of the animal, because those are the cells – ones that are in the tail, when you touch the animal in the back half of it, it goes forward. And other cells in the head or midway in the animal and send their processes into the head, if you touch the animal in the front half then the animal moves backwards. So, it's really two separate sensing systems, one in the back to make it go forward, one in the head to make it go backwards.

**Marc Pelletier**

Pretty elegant.

**Dr. Martin Chalfie**

And, so the question is, how do – why do we have just in this animal, why do we have six cells, because many of the cell types in this nervous system with only 302 nerve cells there is not a lot of cells that look like a lot of other cells. Most of them come in bilaterally symmetric pairs. Why do we have six of them? How is it that the animal has developed six of them and how did the cells develop as particular touch-sensing cells? And, as I said, our mutants have helped us try to answer these questions.

So, what we have found, for example, is we know because of the work that I described by John Sulston looking at all the cell divisions that take place in the animal, we know how these cells develop and one of the genes that we identified – mutants that are defective in this gene result in animals that never make the touch-sensing cells, touch receptor neurons. And when we looked at the animal to ask why they didn't make these cells, we found that the pattern of cell divisions, the cell lineages needed to make these particular cells did something different. It repeated an earlier pattern of cell division and as a result of repeating something over again, it never got around to making the cell division that would lead to the touch-sensing cells. So, the cells were never made. And all six cells were missing. So that told us something about an instruction that was needed to produce the right cell.

Another one of our mutants, the cell lineage was perfectly normal, but the cell that should have become the touch-sensing cell never did so. It never made this particular – these particular channel proteins and associated proteins that we know now are needed to sense touch. And, so this gene seemed to control the differentiation, development of this particular type of cell. So, it was very interesting for us to try to understand what the nature of that gene was and we found that that gene encoded a transcription factor, that is a protein that's needed for the activation of other genes. In fact, it is needed for the activation of the channel protein, the genes that encode the channel proteins and several other genes as well.

So, these genes that I've described so far tell us something about the instructions that are needed to make these cells and they are more and more restricted. But we had a problem when we did this, because we found that this last gene, one that if it's missing, the cell is made but doesn't develop appropriately, well, we found it was needed for all of the six touch cells but it was also needed for two other pairs of cells that didn't express the genes that the touch receptor neurons did. So, it was needed for the cells that we were interested in but it was also needed for a few other cells as well. And this immediately said that development could not be one gene saying, okay, this is – you are going to become this particular cell. There must have been some combination of genes that were needed to give the signal that a cell should develop as touch-sensing cells. And, so we went off to seek what these other genes were. And we have been able to find a number of these other genes.

[53:25] And, so what we have is a big combinatorial network. You can imagine it is a large Venn diagram. You need a gene A and you need gene B, but you don't need gene – or you can't have gene C, if you have that you can have something different as well. And so, we have been able to actually show that for the six touch-sensing cells, we need to have the original gene we needed plus one other gene in return. What about the other two pairs of cells? Well, for one of those pairs of cells it only has the touch cell gene. It doesn't have the second gene that is needed and so, those cells don't become touch cells. For the other pair of cells, it has both of those genes, but in addition it has two other genes that then prevent it from becoming a touch-sensing cell in the same way as the other six.

So, it is a combination of both activating and inhibiting factors that are needed to give the instructions on how to make a particular type of cell. We're still trying to understand the question of how is it that the cells in the head are different from the cells in the tail, and we are still trying to understand that, and those are experiments that we actually hope to do in the next couple of years or so, because there are some fascinating things that go on. For example, the cells in the head and the cells in the tail really look virtually identical to each other, not entirely identical but virtually identical. And they make many of the same connections but the cells in the head make a particular type of connection to one set of neurons and a different type to a second set of neurons, whereas the cells in the tail make exactly the opposite set of connections to those same cells, but they both recognize the cells but they do so in completely different ways.

And, we don't know if the cells are actually identical but they are in different environments, that is, they are up in the head where there may be different interacting – interactions that they encounter or if the cells are intrinsically different and there are genes that are saying, we are going to fool the scientists that are looking, and we're actually two different cells here. But we are in the process of trying to study that problem.

#### **Marc Pelletier**

Wow, that seems like a really fun question to try and figure out because there is obviously a crosswire going on and were having one protein is inhibiting something and the other protein is activating – it could be the same protein doing two jobs or it could be two different proteins, I suppose.

#### **Dr. Martin Chalfie**

Yes, all of the – all of those possibilities.

**Marc Pelletier**

Well, I guess you are going to put the potential possibilities on a board and pin some dollar bills and get the postdocs to pin some dollar bills and make some bets. It'd be fun to be...

**Dr. Martin Chalfie**

We actually do bets for chocolate cake around here.

**Marc Pelletier**

Oh!

**Dr. Martin Chalfie**

Instead of money.

**Marc Pelletier**

I'd really like to thank to Dr. Chalfie for being so generous with his time today. We also would like to mention that this is indeed a two-parter and in the next episode, we are going to talk about Dr. Chalfie's work on the development of the green fluorescent protein, and one of the most important tools of modern molecular biology.

Transcripts are available at [futuresinbiotech.com](http://futuresinbiotech.com). They're pretty easy to find, you just have to scroll down a little bit. They are kindly provided by the team at Pods in Print. If you need transcripts done and you are in a specialized field, they can handle it. They can handle biotech; they can handle pretty much anything.

I would also like to thank Will Hall and Phil Pelletier for the opening and closing themes. I would also like to thank Leo Laporte and Dane Golden for co-producing the show. If you have any questions or comments, feel free to contact me at [marc@twit.tv](mailto:marc@twit.tv).

For Futures in Biotech, I'm Mark Pelletier.