Exploring Biodiversity: The Search for New Medicines
Lecture 1 – From Venoms to Drugs
Baldomero M. Olivera, Ph.D.

1.Begin of Lecture 1 (0:18)

[ANNOUNCER:] From the Howard Hughes Medical Institute. The 2009 Holiday Lectures on Science. This year's lectures, "Exploring Biodiversity: The Search for New Medicines," will be given by Dr. Bonnie Bassler, Howard Hughes Medical Institute investigator at Princeton University, and Dr. Baldomero Olivera, Howard Hughes Medical Institute professor at the University of Utah. The first lecture is titled, "From Venoms to Drugs." And now, to introduce our program, the president of the Howard Hughes Medical Institute, Dr. Robert Tjian.

2. Welcome by HHMI President Dr. Robert Tjian (1:05)

[DR. TJIAN:] Welcome to the Howard Hughes Medical Institute and the 2009 Holiday Lectures on Science. As the new president of the Howard Hughes, this is my first opportunity to host this exciting event and to warmly welcome our in-house audience of high school students from the Washington area. I think these are going to be our future scientists. I'd like to also greet our webcast audience as well as DVD viewers.
I've had the good fortune to oversee the institute's many educational and research initiatives, of which these Holiday Lectures represent just one small but important part. To learn more about the institute, please visit our website, HHMI.org. The Holiday Lectures serve as a bridge between our research and education missions and gives HHMI an opportunity to share our science with a broader audience. You're in for a great treat with four presentations from two stellar scientists and especially gifted communicators. Bonnie Bassler is an HHMI investigator from Princeton and Toto Olivera is an HHMI professor from the University of Utah. Bonnie and Toto will be helping us to explore very different aspects of life on our planet. However, a common theme for their work is the essential connection between basic research and medicine and how nature through evolution gives us amazing drugs and medicines. And now, I'm pleased to introduce Toto Olivera to deliver our first lecture. As an HHMI professor, Toto is among a select group of scientists chosen and supported by HHMI to develop innovative programs for teaching science. Toto is a biochemist by training and as you will see, his work has taken him to interesting places and to forge partnerships with many different kinds of scientists, including neuroscientists and evolutionary biologists. In his first lecture, Toto will start us on a journey to understand how new medicines can come from surprising places. We will now have a brief video to introduce Toto before his lecture.

3. Profile of Dr. Baldomero Olivera (3:36)

[DR. OLIVERA:] I grew up in the Philippines and for one year, my dad, who's a newspaper man, got assigned to the San Francisco Philippine consulate. And so I went to school in the San Francisco school system. I knew I wanted to become a scientist because of experiments that I did when I was in second grade. Okay, so please sit down.

[CHILDREN:] Thank you, Mr. Olivera.

[DR. OLIVERA:] My outreach program really is based on what happened to me in the San Francisco public schools. So, I think it's an age when your mind is pretty open. I think the earlier one is able to do experiments the better because it's liberating in a way that you begin to realize that you can figure things out on your own; you don't necessarily have to believe what people tell you. So, I think one reason for outreach and for trying to broaden science education is, I think, in some ways, science is not just a

profession but an outlook on life, or at least an outlook on society, in which one wants to be able to make decisions that are rational and that are based on reality. And so I think for a society to have a good chunk of its citizenry believing in collecting the data and trying to make judgments based on real data is an important part of actually maintaining civility in the world.

4. Venomous animals (5:35)

It's a great pleasure to be here and I'm going to talk about a very special form of biodiversity. So, I think when you hear certain sounds... you may be reminded of certain types of animals that are venomous and the first thing that comes to mind when you talk about venomous animals is, of course, snakes. And there's been, of course, over human civilization, a lot of contact with venomous animals. And so some venomous animals are very familiar and have become cultural icons, and so what you see is a many-headed cobra that's protecting Buddha, which is an important part of Buddhist tradition, and more modern manifestations of the cultural interest in venomous animals, like scorpions, spiders, or wasps. What I'm going to do today, though, is talk about venom.

5. Venom defined (6:43)

And so, what is venom? Venom is a fluid made by an animal that has toxins, and the important attribute of venom is that it's generally injected. And so, in the snake, it uses its fangs to inject venom into the targeted animal and, of course, snake venom has very toxic components.

6. Diversity of venomous animals (7:16)

So, when we look at different venomous animals, it appears that venom, the venomous lifestyle, has really arisen independently in evolution a large number of times and what you see are seven different lineages of venomous animals. So, snakes, bees, scorpions, spiders, they're all familiar to you; maybe a little less familiar are marine animals, such as the Portuguese man-of-war, which is, of course, venomous and has tentacles that can inject venom into their prey. You're, of course, very familiar with the duck-billed platypus, but many of you probably don't realize that platypuses make venom, but only the males and only during mating time when they compete with each other. So,

7. Uses of venom (8:15)

what I'm going to talk about, however, is the animal on the left hand side, which is a cone snail. And when you look at these different venoms, as you know, if you get stung by a bee or a wasp, it's painful, and if you get bitten by a cobra, you get paralyzed. So, different venoms have different effects, and the reason they have different effects is because they, in fact, have different biological purposes. So, as I mentioned, the platypus uses its venom for competition between males, the bees use their venom to defend the colony against any intruders, but the vast majority of venomous animals actually use their venom to capture their prey. And so, this seems to be the primary reason why venoms evolved; for predators to be able to capture their prey.

8. Predatory strategy spectrum (9:18)

So, if we look at predators, then you're very familiar, of course, with sharks and lions, and these are predators that are very well armed. They have great teeth, they run very fast, they have mechanical means for capturing their prey, but what we are going to talk about today are predators in the other end of the spectrum; predators that use a chemical strategy primarily. And the snails that I'm going to talk about are really at the very, very chemical end; they move slowly, they don't have anything to help them

capture their prey in the way of mechanical devices, and so they're really at one end of the predator spectrum.

9. Demonstration: Live cone snail (10:09)

So, what's a cone snail, which is the subject of my talk. And it turns out that we have some cone snails here, so what I'd like to do is show you what a living cone snail looks like. Here is a cone snail, and it is sticking its siphon out, and I'm going to dry it. I don't recommend that you hold a cone snail, but I know this guy is safe to hold because he's not sticking his proboscis out. But this snail, if you can see, doesn't move very fast, and you'd be surprised at what cone snails like this eat. So, this is a predator. It doesn't look like a lion, doesn't look like a shark, but it's just as effective a predator as great whites and lions in the jungle.

10. Video: *Conus catus* strikes a fish (11:12)

So, let's look at what these guys will eat. And if I could have the video, please. So, this snail has its proboscis stuck out, that's the translucent yellow tube, and you can see it stings a fish and it's pretty efficient at catching fish. So this snail eats nothing except fish; it's highly specialized for capturing fish and its venom really does the work to be able for this snail to succeed in evolution and eat nothing but fish. So, you see that the fish has very, very stiff fins. The snail will completely engulf the fish, and after about two or three hours, it will regurgitate the scales and the bones of the fish and it will regurgitate what it used to inject the fish, which is a single harpoon-like tooth.

11. Cone snail venom system (12:24)

And so, the snail injects its fish prey by extending its proboscis, and it locates where the fish is essentially by smelling it. These animals are largely nocturnal and they're able to find where the fish is by chemosensory mechanisms. If we look more carefully at the proboscis, at the end of the proboscis, a harpoon-like tooth comes out. And this tooth is hollow and the snail uses it only once. And so, part of the reason this group of animals has been so successful is because long ago, about 55 million years ago, they evolved the equivalent of disposable hypodermic needles. And so, this is really a drug delivery system and they use this tooth only once; they discard it. And if you look at the harpoons of fish hunting cone snails, what you find is the very tip looks like a harpoon. And so, this is both a disposable hypodermic needle and a harpoon for tethering the fish. And as you can see from the last video, the fish was not able to free itself once it had been tethered by the harpoon. So, this is how the snail injects its venom into targeted animals. And there is a diversity of different types of harpoons. Different types of cone snails have harpoons that have different morphologies, and what we find is that the harpoons are made in a sac and that sac is loaded with 50 to 100 harpoons in various stages of assembly and, as I said, the snail only uses it once and then discards it. The venom is made in a gland and is essentially moved from the gland through the proboscis to the harpoon where it's then injected into the prey or other targeted animal. And so this is very similar to what a snake does. In both cases, you have a specialized gland for making the venom, and you have evolved a way for injecting the venom into another animal. And in the case of the snake, of course, the injection apparatus is used over and over and over. In the case of the snails, the injection apparatus is only used once, so it's a disposable hypodermic needle type of strategy.

12. Video: Philippines biodiversity (15:05)

So, I work in cone snails and people always ask me, well, how did you end up working on cone snails, that seems very esoteric, and the answer is that I grew up in the Philippines and I collected shells as a hobby, and so, cone snails were among my favorite shells, they have very pretty patterns, and the

Philippines is a place where there are lots of shells, in fact, a lot of marine biodiversity. In the Philippines, what you have is an archipelago with lots of islands, over 7,000 islands, and around these islands there are coral reefs and all kinds of animals, some very familiar to you, and many of these are venomous or poisonous. So this is a blue ringed octopus, which is fairly deadly, and here's a cone snail crawling around on the sand near a coral reef. So, the Philippines really has a very rich biodiversity and as a result of that, many people in the Philippines get their food from the oceans. Fisherman, villages go out during low tide to gather all of the shells that they can find, and in small islands of the Philippines, essentially all snails, marine snails are eaten. And what you see is a market where these sea snails are being sold, and they are cooked in a variety of ways. And a byproduct, of course, of eating snails like this is that their shells are... used to be just thrown away, but a shell trade has grown up in the Philippines and they're now big business to sell these shells all over the world. So, if you go to Florida or you go to the Mediterranean and you buy a sea shell, it's more likely to have come from the Philippines than to be a local product.

13. Geological reasons for the Philippines biodiversity (17:13)

So, the marine biodiversity has yielded lots of shells, and I became interested in these as a kid, but the other reason why the marine biodiversity in the Philippines is so rich is because of its geological history. So, as I said, the Philippines is an archipelago, and what we've done here is colored different islands in the Philippines with different colors, and the reason is that, close to 50 million years ago when, in fact, cone snails were evolving for the first time, the first adaptive radiation of cone snails, the different parts of the Philippines were really in very different places. So, you had the orange bits far north, the purple parts that were still underwater way south of the equator and very far east, and then about 35 million years ago or so, when cone snails really had their big second radiation, you can see that the three parts of the Philippines are still widely separated, and therefore evolving really quite different types of marine fauna. And towards the middle of the Miocene, these groups grew closer and today they are the Philippine archipelago. And so, the geological history means that all of these separated islands met in what is the Philippine archipelago today, and so you have this very rich biodiversity as a result of having essentially separated islands where you got island type evolution of species that then combined to give you the present day marine biodiversity of the Philippines. So, a lot of people like to come to the Philippines to do biodiversity research. And the slide shows a Russian scientist who is collaborating with people in the Philippines to do molluscan taxonomy and my own work there, and so we have been able to get lots of cone snails, and that's pretty easy to do in the Philippines.

14. Phylogenetic tree of cone snails (19:32)

So, if you look at cone snails, you can easily tell from their shells that there are a number of different species. So, let's look at seven different cone snails. And what you have here are a diverse set of cone snails, and what you can do these days is extract the DNA from each of these species and you can sequence particular genes in each cone snail and you can compare how closely related these genes are. And so, if you take *Conus aulicus* and *Conus textile*, what you find is that the genes are very close in sequence. There are very few differences in the nucleotide sequence of the same genes. On the other hand, if you compare *Conus textile* and *Conus monachus*, then you find many more differences, and so the DNA sequences are much more divergent. And so, based on these sequences alone, you can construct a tree and the greater the differences, you can make the species further away, and the closer the sequences are, you can put them together, and what you end up with is a tree. And so, this tree is based purely on DNA sequences. And when you do that, what you find is that in fact, the cone species that have somewhat more similar biology are closer together on the tree. And so the orange branches are the cone snails that hunt fish, the green branches are the cone snails that hunt other snails as their main prey, and the yellow branches show you the cone snails that hunt worms. So, this is purely based on DNA sequences and yet it groups together cone snails with similar biology.

15. Video:*Conus textile* strikes a snail (21:42)

So, cone snails not only hunt fish, but as the next video will show, they can also hunt other snails, and so you're going to see *Conus textile*, the cloth of gold cone, which is a snail hunter, begin to attack its prey, and as you can see, the prey has behaved in a very dramatic way. It's essentially a snail undergoing a seizure because its nervous system has become hyperexcited and that's a snail hunter. Typically snail hunters inject their prey multiple times, not just once.

16. Video: *Conus imperialis* strikes a worm (22:25)

And the next video will give you an example of a cone snail that hunts worms. This particular cone snail hunts a very unusual type of worm. I don't know how many of you have been scuba diving, but if you go diving in the tropics, you're told never to touch this worm. This is called a fire worm and its bristles are painful, they sting, and yet, that's what this snail likes the most. It's specialized in eating fire worms.

17. Hunting specialization and phylogenetic tree (22:57)

And so, there's all kinds of specializations, and if we construct a larger phylogenetic tree, as I said, there are about 700 cone snail species and this is a small fraction, you can get a phylogenetic tree that shows branches with different types of cone snails. And it turns out that some of these branches have species that hunt fish, there's one branch that has all of the species that hunt other snails, and the majority of branches are the cone snails that hunt worms. And we generalize and call all of these worm hunters, but in fact, the worms that they eat are very diverse, everything from hemichordates to polychaete worms, which are related to earth worms. So, each of these branches, we call a clade, and that shows you the clade of cone snail species that like to eat fire worms. And so, we can now begin to group cone snails based on this phylogenetic tree into their different specializations. And so, this is really an overview of the biodiversity of cone snails.

18. Demonstration: *Conus geographus* can kill you (24:20)

Now, of course, in the slides that I've been showing you, the cone snails are shown pretty much all the same size, but they're not. So, here's a little cone snail that hunts fish, and here's a much bigger cone snail that also hunts fish. And indeed, size matters, because if this little cone snail stings you, you might feel pain and a little numbness, but it can't kill you. But this snail, which is large, has enough venom so if it should sting you, your life is in danger. And in fact, in the absence of medical intervention, when people get stung by this particular species, the geography cone or *Conus geographus*, 70% of the people die. So, the rate of lethality is very, very high with the stings of *Conus geographus*, and it is, in fact, for that reason that we decided to study *Conus geographus*, because we knew that the venom of *Conus geographus* could kill you. And so, what I'm going to do is explain to you why it might be interesting to study the venom of a snail that's capable of killing people.

19. Biodiversity of venomous snails (25:54)

So, as you can see, the biodiversity of venomous snails has led to some very unexpected, unpredictable evolutionary paths. You might not have predicted *a priori* that snails would be able to specialize in eating nothing but fish, after all, fish can swim away. So, why are there 100 species of cone snails that eat nothing but fish? And you might not have predicted *a priori* that a snail would be able to evolve a venom that would be dangerous to people in which 70% of all people being stung, if you don't get them to a hospital on time, die. And so, I will explain in the next little while, why this is interesting from a

scientific point of view. But now we should take a break for some questions, and I'd be happy to answer any questions that you have so far. Yes?

20. Q&A: Does *Conus geographus* make deadlier venom or just more? (26:58)

[STUDENT:] You said that the larger snail, that size matters. Is this the quantity of the venom or does the snail have, like a different strength, or concentration, or chemical makeup of its venom that makes it so much more powerful?

[DR. OLIVERA:] It's actually both. So, a snail this small can only inject maybe a few microliters of venom, and no matter how potent that venom is, it's probably not going to kill you. However, the snail in the tank grows just as large as this snail, particularly in Hawaii, it grows even larger than this snail, and yet that snail has never killed anyone. People have been stung, but no one has died. And the reason is that it turns out that the toxins in this venom are just as potent on people as they are on fish. That has toxins that are equally potent on fish, but the effect of each individual component on a person is a parameter that's not selected for and so varies randomly. And it so happens that although that snail can inject just as much venom as this snail, that snail has never killed anybody. Question there?

21. Q&A: How many people are stung by cone snails? (28:25)

[STUDENT:] I was wondering how often people were stung on a regular day basis, especially since getting cone snails is part of trade now, as well as when you go on recreational trips?

[DR. OLIVERA:] Yes, so, the answer is that the probability of you being stung by a cone snail is rather small. First of all, they're nocturnal, and so most cone snails are very hard to find alive if you are out in the middle of the day. But even if you should be scuba diving at night and you pick one up, most cone snails are not at all aggressive. Their first line of defense is to simply go into their shell. Okay? Most of the people who've been stung have done something to the cone snail that makes the cone snail think that it's a predator. Thanks so much for the questions. We should proceed with the second part of the lecture.

22. Venoms as research tools (29:26)

And so, what I'm going to do is to explain why we decided to study *Conus geographus*, the geography cone. And the reason was when we got started in this research at the time, just a few years earlier, someone in Taiwan, Chen-Yuan Lee to be precise, had studied the multibanded krait and isolated a very important molecule from the venom from that snake called alpha-bungarotoxin. And this turned out to be a key moment in neuroscience because neuroscience up to that point was dominated by electrophysiology, there was very little molecular work, and having this snake venom component available allowed scientists to pull out from the nervous system the first purified signal molecule in the nervous system, the receptor target of this snake venom component, alpha-bungarotoxin. And so, it became a way to begin to examine at the molecular level the important molecules in the nervous system. And so, our hope was, alpha-bungarotoxin, of course, is a paralytic molecule that can kill you; you don't want to be bitten by the multibanded krait. And so we thought, okay, this snail can also kill you; 70% of people who get stung die. And so, we decided that we would purify the venom components of the snail and try to figure out which were the venom components that were responsible for killing people.

23. Chromatography helps identify venom components (31:17)

And in order to do that, what you have to do is separate out all of the different venom components, and it turns out that these venoms are very complex and they have lots of components. And so, what we did was we dissected the venom duct of the snail, collected a lot of venom, and today the best way to

separate out these different venom components is a technique called high performance liquid chromatography, or HPLC. And so, what you do is very similar to experiments that you may have carried out called paper chromatography. So, you can put a mixture of dyes on paper, spot the paper, and then run a solvent, and the dyes will move with the solvent at different velocities and so you can separate them. And so, of course, you can see the results of this as shown on the paper chromatogram that's on the slide. And the problem with the venom is that the venom components aren't colored and so you have to have a way of finding them. And so, you have to use UV light because the venom components do absorb UV light, and when a molecule is in front of UV light, it absorbs light. And so, if you have a detector, you can tell whenever one of these molecules is moving in front of the UV light, and what you end up with is you pass the crude venom through an HPLC column and you pass the solvent and the different venom components stick to the column with different abilities and as they pass through the column, you can detect them using a UV light detector, and you can collect the fractions afterward, and you end up with what is called a chromatogram that shows you all of the different components that are separated out.

24. Conus geographus has over 200 venom components (33:18)

So, here's a chromatogram of *Conus geographus* venom and so, the materials on the left came out earlier than the materials on the right. This turns out to be a function of both how big that component is and how organic it is. And then, what you see is that some of these components absorb more light, which usually means there's more of it in the venom, and some of the components are just small peaks. But what this reveals is that there are a lot of different venom components in *Conus geographus*. And, in fact, if you do this very carefully, you can count more than 200 different components in the venom of the geography cone, *Conus geographus*.

25. Venom components that paralyze mice (34:08)

And so, what we did as our initial investigation was to ask which ones will be paralytic to mice, as a mammalian model system, and we were able to purify and characterize a number of the components that were paralytic. And so when people die, they get paralyzed and apparently they die mainly because their diaphragm muscle gets paralyzed and they can't breathe, and so typically people will die a few hours after they're stung by *Conus geographus*. And so, we isolated the first component of *Conus geographus* venom, and we were surprised to find that it was somewhat different compared to the snake venom components. So, I told you alpha-bungarotoxin was a small protein, proteins are made out of amino acids, and so this toxin from the snake is a string of 74 amino acids, but when we isolated the paralytic component, the first paralytic component from the snail, it was much, much smaller; only 13 amino acids. And so we call those peptides, and the peptide could readily be synthesized. So, because it was so much smaller, we could use chemical synthesis to study this peptide. So, that's how our work began, but, in fact, we thought it was going to be a relatively short term project.

26. Undergraduate pioneers new assay (35:47)

So, why am I working on cone snails today many, many years after we started this project? And the answer is that there was an 18-year old kid, an undergraduate named Craig Clark, who came to our lab and he said, you know, I want to do the assay differently. I don't like the assay that you guys are doing. You're just injecting the venom either intramuscularly or into the body cavity of a mouse. I want to inject the venom directly into the central nervous system of a mouse because there's a blood brain barrier, and so the venom components can't reach the central nervous system. And I remember I wasn't so convinced he should do this and I tried to dissuade him, but the reason why I think the most creative work is done at universities rather than anywhere else is because the students do what they want, not what their professor tells them. And so, Craig proceeded to inject venom components directly into the

central nervous system; this is called an intracranial injection. And he convinced me that he could do this with innocuous proteins, or saline, and that the mice are perfectly normal, so he knew how to do these injections, and what he found is shown here.

27. Behavioral spectrum from intracranial injection (37:13)

So, I told you about the paralytic components of the venom, but then Craig found, by intracranial injection, a peak that made mice jump and twist as they're jumping. The next peak made mice completely uncoordinated. The next peak, which is a major peak in this venom, put mice to sleep, and then they'd wake up 12 to 24 hours later and they'd be perfectly fine. But this was only true if the mice were under three weeks of age. If they were over three weeks of age, injecting mice with the same component made them climb the sides of their cages constantly and run from corner to corner. And so, that was really interesting because the effect on mice depended on the developmental stage of the animals injected. This component made the mice drag their back legs. This component made the mice run around in circles; we called it the circular peptide. This component caused head swinging, another caused trembling, another made them depressed, another made them scratch, another caused convulsions, and so what you can see is that what Craig Clark's experiment revealed was that the venom of a cone snail is not a mixture of a few paralytic toxins, but is this incredibly complex pharmacologically diverse mixture of peptides that vary in size from somewhere between 10 and 30 amino acids. So, the lesson from this early work was pharmacological complexity and small peptides that were between 10 and 30 amino acids.

28. Undergraduate discovery leads to new pain medication (38:54)

And so, after Craig Clark developed this assay, we were in the very lucky position of having a whole troop of undergraduates come into the lab, and they could choose any cone snail that they wanted, and they could follow any activity they wanted, and the job of all of this undergraduate work was to isolate the pure peptide that caused a particular effect on the nervous system and figure out its structure, its amino acid sequence, so we could then synthesize it, and determine further what it did. And so, this young man, Michael McIntosh who was 18 years old, and at the time, right out of high school came to our lab. And he decided to work on the venom of *Conus magus*, the Magician's cone, and sure enough, using Craig Clark's assay, when he assayed various factions, he got a whole bunch of different types of effects on the mice. So, this was a direct injection into the central nervous system, and he decided to purify a component that made mice shake, which he called the shaker peptide. So, why am I telling you this? The reason is that today, this peptide that Mike characterized and purified for the first time is a drug, an approved drug for intractable pain, called Prialt. And so, what I'd like to do is to tell you what Prialt does. So, in order to tell you what Prialt does, we have to look at what happens whenever you contract a muscle. So anytime you move or anytime a fish moves, then an electrical impulse moves along the nerve towards the muscle and it reaches the junction between the nerve and the muscle which we call a synapse and that is the target of Prialt, the synapse between nerve and muscle. That's the target of Prialt and that's the reason the snail has evolved this particular venom component. So, the next animation will show you this and we'll just review what happens as electrical signals are transmitted through the synapse from nerve to muscle.

29. Animation: Prialt blocks motor synapse in fish (41:25)

So, here is a fish and electrical signals are coming down the nerve, and this is how a fish swims. And at the junction between nerves and muscles, the electrical signals have to get through the synapse before you get electrical signals on the muscle, which are striated. And so, you see this in close-up. And now we're going to look at what happens at a synapse. And what you see is an electrical signal coming down the nerve, reaching the nerve end, and calcium channels open as a result of the electrical signal, and

calcium enters, and the entry of calcium causes vesicles to fuse, release their contents, which is the neurotransmitter acetylcholine, which is then free to bind to the receptors on the muscle side. Those receptors are ion channels that open up. They let sodium in, and the entry of sodium then triggers the electrical signal on the muscle. So, this is what happens at the synapse and this is how the electrical signal from the nerve gets transmitted to the muscle. So, when you have Prialt present, what happens in a fish is that you plug up the calcium channels, and so even though an electrical signal arrives at the nerve ending, no calcium enters through the calcium channels because they've been plugged up by Prialt. And as a result, none of the other events that normally occur, the release of neurotransmitter, the opening of the receptors in the muscle end, none of those occur. And so, what happens is that there is no electrical signal that's elicited in the muscle, and that's why the snail makes this; because it's part of its strategy for paralyzing fish. When you can't get the electrical signal from the nerve to the muscle, the fish is paralyzed. And so, if you inject Prialt into a fish, it becomes paralyzed.

30. Animation: Prialt blocks pain signaling in mice (43:54)

It turns out, however, that there are many types of calcium channels, and this shows you a phylogenetic tree of calcium channels. And it turns out that the type that's sensitive to Prialt is shown in green, and you find that type of calcium channel in the synapse between nerve and muscle of fish and frogs. But in mice, it turns out that there's a different type of calcium channel, and that calcium channel is resistant to Prialt. So, in mice what happens is when the electrical signal gets to the end of the nerve, those calcium channels also open up, but they're Prialt resistant. And so, as a result in a mouse you get a different effect of Prialt. And so, now notice we have the red calcium channels opening up, letting calcium in, and even though Prialt comes, because these are Prialt resistant calcium channels, they don't affect the synapse at all. So you can have Prialt at very high concentrations, calcium enters normally in response to an electrical signal, the neurotransmitter is released, it binds the receptor in the muscle end, and so as a result, you get the electrical signal elicited by the muscle. So Prialt does not paralyze us, and we can take a very high dose of Prialt and our nerve muscle junctions work very well. However, in a mouse, as well as in us, the same channels that are present, the green ones, are in the pain circuitry, and they're found instead at the ends of special nerves called pain fibers. And there is a synapse between the pain fiber and a nerve cell in the spinal cord. And now, if you introduce Prialt, what happens is the electrical signal reaches the end of the pain fiber, it does not get transmitted across the synapse to the nerve cell in the spinal cord, and it's that nerve cell that takes the pain signal to your brain. So, even though the pain fiber is firing very frequently, and normally you would feel intense pain as a result of the firing of this pain fiber, when you interrupt the transmission of the signal to the nerve cell in the spinal cord, then what happens is that you completely alleviate the pain; you don't perceive it because it doesn't get to your brain. And that's why Prialt is an analgesic for humans, although it's a deadly toxin for fish.

31. Review of synapse function and Prialt action (46:53)

So, that's a lot of detail, and what you've seen is that to get through synapses, there's a whole series of events that have to sequentially occur; an electrical signal arrives, calcium channels open, calcium enters, vesicles fuse, neurotransmitters released, the signal is seen at the receiving end, and when the receptor is activated, you start an electrical signal on the other end of the synapse. And where Prialt is able to inhibit is at this step, and so what happens is that you don't get all of the other steps that follow because no calcium enters the nerve ending and so you don't get vesicle fusion, you don't get neurotransmitter release, and you don't get activation on the muscle side.

32. Prialt-sensitive and Prialt-insensitive channels (47:53)

So, this works in the pain circuitry, and the reason why it works as a drug is because those types of calcium channel in the spinal cord are only found in the pain circuitry. And so, what you see on the slide

are two labels of the spinal cord; one that only labels the Prialt-sensitive calcium channels, and one that labels two types of calcium channels, both the Prialt-sensitive and the Prialt-insensitive. And so, if this venom component were not absolutely specific, you'd get all kinds of side effects because you'd also be inhibiting all of the other synapses in your spinal cord, okay? But the fact that this venom component is very sensitive for only one type of calcium channel means that now it can be used as an analgesic drug. So, when we inhibit this particular type of calcium channel in fish, we get one physiological effect; the fish gets paralyzed. But when we introduce Prialt to our nerve muscle junctions, we don't get paralyzed because we have a different type of calcium channel there. Instead, when we introduce it to the pain circuitry in the spinal cord, we're able to alleviate pain.

33. Calcium channel diversity (49:22)

So, there are two things about the nervous system that allow Prialt to become a drug, okay? One is that the molecules in the nervous system are extraordinarily conserved. And so, that green calcium channel that's Prialt-sensitive is essentially identical in structure in fish and in our own bodies. And, indeed, one of the big surprises in molecular neuroscience in the last decades is that all nervous systems use the same basic components, and all those basic components are conserved. And so, you can look at calcium channels in a worm, *Caenorhabditis elegans* or in *Drosophila*, and it will look like the calcium channels that I just described. However, as you've seen, the expression pattern of those conserved elements varies from nervous system to nervous system. And so, in fish we find this Prialt-sensitive calcium channel at the synapse between nerve and muscle. But in our own bodies we find a different calcium channel there. Instead, that Prialt-sensitive calcium channel is found in the pain circuitry and that's why Prialt can become a drug. So,

34. 22 years from discovery to drug (50:51)

you can see that that's how this component discovered by Michael McIntosh became a drug, and it took a long time. That shows what Michael looked like when he discovered the drug, and it took 22 years before it was approved and he, by that time, was a professor of psychiatry at the University of Utah, and is my close colleague in collaborating on what these *Conus* venom components do. So, this is Prialt. This is the structure that Michael determined and which is chemically synthesized. And so, the snail uses it to paralyze its prey. We use it, so it's now chemically synthesized as a commercial drug, to alleviate pain when morphine especially no longer works. So the name Prialt is meant to imply primary alternative to morphine.

35. Prialt is one of thousands of candidate drugs (51:50)

So, I want to emphasize that Prialt is only one component of the venom of one snail and each snail has perhaps 200 different components. Furthermore, there are 700 cone snails and the remarkable thing, and I'll rationalize why this appears to be true in the third lecture, is that the venom components for one *Conus* species do not overlap at all with the venom components of another *Conus* species. And so, we don't find the same peptides in one cone species versus another. And what this means is that there are probably 140,000 different peptides in the venoms of living cone snails, and so these, of course, will lead to potential drug development of which Prialt is only, we hope, the very first example. And so, I think I'll stop there. Thank you very much for your attention. Let's have some questions.

36. Q&A: Would Prialt work with all human calcium channels? (53:10)

[STUDENT:] I was just wondering because we have these calcium channels which are somewhat specific to Prialt and some are not, how do we know that it works for every mammal, if not really every

human. Because of divergent evolution, there are some... well, a lot of proteins have acquired mutations through divergent evolution, so how do we know it works for everyone or would work well?

[DR. OLIVERA:] That's a good question. And the answer is that in general for every species, one has pretty much the same type of calcium channel in the same place. There are, of course, mutations in calcium channels, and some of them cause very severe pathologies, but what Prialt sees is only a small part of the total sequence of any one calcium channel and the probability that you will find a lot of variation in that in people is probably fairly small. And so far, that hasn't been the main problem in using Prialt as a clinical drug because it appears that it does always inhibit that particular type of calcium channel, and that at least in people, that calcium channel is always present in the pain circuitry. Other questions? Yes?

37. Q&A: How many components have been tested and approved as drugs? (54:46)

[STUDENT:] You said that there are thousands of potential drugs that can come from these 140,000 peptides. How many of the peptides have actually been tested and approved or proved beneficial to humans?

[DR. OLIVERA:] Yes, so at this point there are six that have reached various forms of human clinical trials, so six compounds from cone snail venoms. There are a larger number that are being looked at in preclinical investigation for possible application. And so, as I'll talk about in the third lecture, in fact, the pool of components from marine snail venoms is much larger than 140,000. We have found that by looking at very different branches of the phylogenetic tree, different clades, you very often will find novel pharmacology that you can't find by just looking at closely-related snails. So, a lot of our work was originally focused on fish hunting cone snails because we reasoned that fish are closer to humans than are worms, for example, which are the prey of most cone snails. But it turns out that there are nervous system components that those snails have evolved for their own purposes that affect worms that are found in esoteric parts of our nervous system and may be able to address some human pathological conditions. And I think we have to stop there.

38. Closing remarks by HHMI President Dr. Robert Tjian (56:45)

[DR. TJIAN:] Toto, thanks for a fantastic lecture, as always. Also, I want to thank the students in the audience with their excellent questions; I'm just sorry that we couldn't take more. I think Toto made a very strong case; first of all, not to go pick up any cone snails, and the second that this collection of cone snails is anything but a frivolous hobby since the consequences to the world of medicine are obvious. In the next lecture, Bonnie Bassler will introduce us to another unappreciated realm of biodiversity, the hidden world of microbes.