Exploring Biodiversity: The Search for New Medicines
Lecture 2 – Shedding Light on an Invisible World
Bonnie L. Bassler, Ph.D.

1. Start of Lecture 2 (0:19)

[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 second lecture is titled, "Shedding Light on an Invisible World." And now, to introduce our program, the Vice President for Grants and Special Programs of the Howard Hughes Medical Institute, Dr. Peter Bruns.

2. Welcome by HHMI Vice President Dr. Peter Bruns (1:08)

[DR. BRUNS:] Thanks for joining us for the Holiday Lectures on Science this year, focusing on biodiversity and the search for new medicines. You know, we've already had one lecture full of all sorts of great stuff and we've got three more to go, but even then we won't be able to cover everything for sure. We have a way that you can get more information on this and other related topics, and that's on a website that we have. If you go to our own website, HHMI.org, and then on the bottom right, click on an area called Cool Science, you'll come to a page that has a number of options. In one spot you'll find BioInteractive. Click on that and you'll find a whole library of previous Holiday Lectures and, in fact, this Holiday Lecture, in the future, and other kinds of videos and animations and so on. Our next speaker is Bonnie Bassler. You will see Bonnie is an emphatic and energetic person, full of energy and ideas. She's always been curious and she is a tenacious problem solver. She started out trying to understand everything she could about chemical communication in an obscure group of bacteria. Deftly following that thread led Bonnie into ever-widening circles of interest concerning the evolution of multicellularity, the nature of communication, and the development of social interactions. In this lecture, Bonnie will introduce us to the fascinating cast of characters in the incredible realm of microbes. So, first a short video to introduce Bonnie.

3. Profile of Dr. Bonnie Bassler (2:52)

[DR. BASSLER:] So, at Princeton, we only do two things; we do research and we do teaching. And I love teaching for two reasons. One is a feeling of obligation. I can't believe my luck that I ended up a scientist, and that's because somebody, a few people along the way were great teachers. He or she was giving and I was getting it. So, I teach because somebody taught me. Right? And so, I think maybe I'm making the next me, or maybe I'm making the next better-than-me. That's my hope through teaching. And the second thing I should say about my teaching is I do not teach to scientists. I only teach to undergraduate humanists. And so, I've chosen here at Princeton to teach to people who will be writers, philosophers, scientists, politicians, because I think if they can bring science and scientific logical thinking to being a lawyer or being a politician or being a mom, they will be better. And then I also hope, you know, that maybe 20 years from now something they learned in molecular biology 101 will be useful to them as a lawyer or as a teacher or as a politician, and that would be neat. I'll never know about it, and that's okay, but it would still be neat. My hope for the Holiday Lectures is that you think quorum sensing's cool, is that if you decide to become a scientist and you pick some project that seems crazy to work on, glow-in-the-dark bacteria, that if you really in your heart believe that you are asking something fundamental, that that is a useful and a good pursuit. Right? So, if you just find something that you think you're curious about, and so for me it started as these glow-in-the dark bacteria and then it turned into how group behaviors happened on this Earth, right, go for it.

4. Hidden biodiversity: The microbes (5:00)

Well, hi. Good morning. I'm delighted to give part two of the first day of lectures and I want to take up where Toto left off, but I want to take it down a couple of notches. And so, what I want to try to do is to convince you that there's an amazing world out there that you've never seen. And so, to get into that, I want to sort of give the view of what we think of the world; maybe you go for a hike, or you do go outside, and this is sort of our idea of the world. We see objects, a tree, a nice bird in this case. And you know, if you get a little closer, like you stick your nose in the dirt or you walk up to that tree, you might see something smaller like an ant or a lichen or a fungus on that tree. But what you can't see is that on every one of these organisms out there are billions of other organisms that are too small to ever be seen by our eyes. And so, these are the microbes. And so, we're never going to see one of these in our life unless we look under a microscope. And so, I want to try to think about the biodiversity of these invisible creatures and talk about that with you this morning.

5. Family trees (6:02)

And so, the question is sort of how can we make sense of these weirdo invisible organisms. And what we can do is something that Toto sort of alluded to, is that we can make these family trees to think about how organisms are related. And so, on my simple family tree, I think that there's people that you recognize; these are the English royal family. And you guys have probably made these trees in your own family, you go a few generations or maybe its somebody that likes genealogy in your family, and they'll draw these trees like this. And, of course, these trees tell us something about how we're related to one another. So, you guys know that Queen Elizabeth is the mother of Prince Charles, and Prince Charles has two sons, William and Henry. And so, we draw these lines to show our relationships, but if you really wanted to think about these over more than a few generations, what those lines between the different organisms — in this case humans — are, is they show you how closely or how distantly each of these humans is related one to another. Right? So, the further a person is, the further they are related to the top. And so, those lines mean something about how distant our genes are, but they also mean something about time; so the longer the line, the more time that's gone by.

6. Evolutionary tress of related organisms (7:12)

And so, of course, we don't have to do this just for humans, or snails as Toto showed you. Because we have DNA sequences now, we can do this for any organism on Earth. And so, we can relate organisms that aren't the same species. And so, on this next simple chart, we're looking at a bunch of animals. And so, of course, a long time ago there had to be some ancestor, a long, long, long time ago, that gave rise over time and evolution to each of the animals that you see on this chart. And so, remember those lines mean time; so the longer the line, the more time that's gone by. And what we can do is by organizing these animals this way is we can tell how they're related to one another. And so, I don't know if this chart is a surprise to you. I don't know if you think it's surprising or not that hippos are more closely related to whales than they are to pigs. But we know this and we know it with certainty because we have DNA sequences.

7. Three main branches of life (8:07)

And so, of course, we can do this, as I just said, for any organism on the Earth. And so, if we go down to looking at all of the biodiversity on Earth, what we find is that we can relate all the organisms that we know about on the Earth into three big branches of life. And these are the Archaea, the green branch, and so those are the oldest organisms. They are microbes, and they live in very inhospitable environments. And so, that's why we think they're the oldest because they like hot and cold, like when the Earth was very hot or very cold. And the Archaea gave rise to the other two branches of the family which are the

bacteria, what we're going to focus on today, and also the eukaryotes. Eukaryote, that word means having a nucleus. So, all higher organisms on Earth, including us, are eukaryotes.

8. Bacteria predominant on Earth (8:54)

So, we can break it down into these three big domains, but in fact, if you're me, this is a very skewed view of the Earth, because this is what it really looks like if we actually start to put how many organisms there are in each of these families. The truth is, is the Earth is microbial and it's mostly bacterial. So, right, the blue represents bacterial species, and so that's most of the whole pie of life on Earth. The green, you remember the Archaea, they're also microbes, and what you might not know is that most eukaryotes are microbes, too. So, every animal, every insect, every human, everything you've ever seen fits into that little slice of the pie. Right? So, those are all animals. So, everything else on that pie is invisible to us, and what you can see is that is most of all of the life that's on this Earth. And it doesn't get very much press because we don't see it, and so we don't think of it as part of our day-to-day life. But I want to try to give you a glimpse of what this world is and how important it is both to your health and both to the, in the environment.

9. Bacterial cell structure (10:01)

So, I'm going to focus my talk on bacteria, the blue guys, because that's what my lab works on. And so first, what are these critters? This slide shows a cartoon of a sort of a generic bacteria. If you look at the bottom part of the slide, bacteria mostly look like that guy; they kind of look like sausages. And then most all have a tail that you see. We call that a flagellum. What that is is a rotor. It works like a boat propeller and it propels the bacterium from here to there. And so, that gives the bacterium motion and allows it to move around in the environment. But then if you look at the bigger cartoon, that's sort of a breakdown of the components of a bacterium, they're very simple. They're all covered by a membrane, which we have in red, and you can think of that as their skin. It keeps the outside out and the inside in, exactly the same as our skin does for us. Inside is a liquid environment that we call the cytoplasm. And in there, sloshing around, are all the biomolecules, the proteins, the enzymes, the lipids, that keep the organism alive. And then what is special about bacteria is that they all only have one piece of DNA. So, they have only one chromosome and only a few thousand genes. So they don't have very much genetic information to allow them to do all of the things that they do in the world.

10. Bacteria traits appear simple (11:20)

So, because they're so simple-looking, single cells, one piece of DNA, they've always been considered to be very primitive and kind of boring. So, if you look at a bacterium, like sort of what it does for a living, what bacteria do is that they consume nutrients from the environment, they grow to twice their size, they cut themselves down in the middle, and one cell becomes two and so on and so on. And so, because at sort of first blush this life looks so simple, just growing and dividing and making more of themselves, they weren't thought to have very sophisticated traits, like the traits that we know about in higher organisms. And so, what I hope to get to today is to try to show you that they're not quite so dull and that they have all kinds of traits that are very similar to ones that we have.

11. Bacteria inhabit extreme environments (12:04)

Okay, so those are the bacteria. So, where are these guys? Well, it turns out, as I've already alluded to, they're everywhere on Earth. And so, what scientists can do is go to the bottom of the ocean, where the magma is roiling up from the center of the Earth like a volcano. It's 300 degrees, all of this metal smoke is coming out of these vents, and what do we find there? Bacteria, living and thriving. Likewise, we can go to the coldest places on the Earth. So, scientists have looked under layers of ice that have not thawed in

tens of thousands of years and we find microbes. So, these are environments that we couldn't exist in, and yet these bacteria manage to occupy these niches. And so, probably you and I don't get to go to those places very much, but we do get to be around here.

12. Humans are covered in bacteria (12:51)

So, now I want to move into what these bacteria mean to you. And so, this is just a picture of a person's arm and what we've done is to take a micrograph of the elbow. So look under a microscope at the skin on a person, random human being's elbow, and what I hope you can see is that there are dozens of species of bacteria there. So, this is how you are as well. And so, each of those different shapes and color is a different species of bacteria. So, on your skin, just in this one little patch, you have dozens of bacterial species. A few more on your teeth, so you guys, I hope, brush your teeth every morning, right?
You clean your teeth because you have those little sweaters on them. Those sweaters are called biofilms. They are made up of bacteria. There are 600 species of bacteria on your teeth every morning. And so, the final picture shows what your teeth look like before you brush them, and these bacteria are not willy-nilly. They are in an architected community where one guy sits next to the correct neighbor and so on and so on.They are an amazing community that is working together and flourishing. The middle picture is to inspire you to change your toothbrush kind of frequently. That's just a bristle of a toothbrush, because you're brushing these bacteria off. So, you have bacteria all over you all of your life. It's this invisible world that covers your body and they are your body armor that keeps you safe from environmental insults. So, they cover you and they protect you from bad and harmful things in the environment.

13. You are mostly bacterial (14:20)

And so, you are covered with them, but I want to tell you about relative numbers of you versus bacteria. So, what we've done here, this is my idea of a human being and the cells that make up a human being. So, we know how many cells are in a human being; there's about a trillion cells that we each need to make us alive and do all the things that we do. And so, what we've done is to draw those little circles.
Each of those circles represents a fraction of the human cells in a typical body making a live person, so one trillion human cells. Well, it turns out at any time of your life, you have ten times more bacterial cells than human cells, so you have ten trillion bacterial cells in you or on you every moment of your life. So, the truth is you're only 10% human, you're 90% bacterial. But you guys are all crack science students and so you know it's not really your cells, it's your DNA, right? So, it's your genetic material that gives you your oomph and your personality. So, what we've done is we've redrawn our man in the As, Ts, Gs, and Cs, right, the nucleotides that make up every genome. And so, of course, we have the sequence of humans, and there's 30,000 genes. We know how many genes there are in the human body.
So, it takes 30,000 genes to make a person. Well, if we count how many bacterial genes are in you or on you, there are 100 times more bacterial genes in you and on you, and those genes encode proteins that do things, that have functions, just like your genes do. So, I don't know which of these metrics you like, but at best you are 10% human, most likely you are really only one percent human; 99% of you is bacterial. It's just that we don't realize it's there because it's just a part of our day-to-day invisible life.

14. Most bacteria are beneficial or neutral (16:06)

So, I hope you're convinced that you have all these bacteria all over, in and on you. And so, the question is, what do they do? Well, most of these bacteria are good for us. They're living in a mutualistic symbiotic association and because they have that vast repository of genes that I told you about, they encode all kinds of functions that we need but we don't have the genes for. And so, the one that you hear about the most is that we have these bacteria in our gut that help us to digest our food, right? They help us do things. So, for example you have *Escherichia coli* in your gut and it helps you digest your food, but it also makes vitamin K. So, you need vitamin K to be alive, you need it for certain enzymes, and it turns out that you

can't make it. But E. coli has the gene to make that and it provides it to you. So, you don't actually have to be petrified that you're covered with all these bacteria. It turns out you're supposed to be, and they provide us all of these beneficial and essential functions that we have not evolved ourselves.

15. Pathogenic bacteria (17:05)

But, of course, you have heard about bacteria, actually you even heard about them this morning, because sometimes bacteria get in or on you and they don't belong there. And so, this happens infrequently in our lives, and when you get invaded by a bacterium that isn't one of these commensals, we call that pathogenic. And so, there are many pathogenic bacteria that don't belong in or on you, and when they get in or on you, they unleash some of these factors that they have for surviving in nature, but those factors make us sick. And so, I don't want to be Pollyanna about this. Most bacteria are good, but indeed we have a terrible global problem with pathogenic bacteria and infectious diseases. And so, the one on the right, that's a picture of tuberculosis, the one that Dr. Tjian was telling you about this morning, so, a terrible pathogen.

16. How can tiny bacteria affect humans? (17:56)

So, the question that I want to pose today and that my lab is curious about, is whether you think about these bacteria because they're doing good things or you think about these bacteria because they're doing bad things, the question is how can they do anything at all? They're incredibly tiny, so how can they get any bang for their buck since they're such small organisms. And so, we want to try to understand what they're doing, what the host is doing, and how these organisms live in a community between a eukaryote and this microbial world. And so, what I want to try to talk about now is how can we possibly understand this complicated association?

17. A model system to study host-bacterial interaction (18:32)

And so, here's a slide you've already seen. So, you have these thousands of species of bacteria in or on you, and even though the ultimate goal is to understand what all of their jobs are and why we need those bacteria, it's hard to study that. And so, what scientists try to do is we try to get our hands on model systems, like systems that approximate this complexity, but they're much simpler. And then what we hope is that if we learn about more simpler associations step by step by step, we can learn about these more complicated situations that are sort of hard to study all at once. And so, what we do is to look for what we call a model system. So, what would be ideal in my mind to study these bacteria-host relationship is if we had a one-to-one relationship. So, if we had a eukaryotic host, so my simple man, the stick figure, so a simple host that lived in a one to one association with a particular bacterium, then we could study the host and we could study the bacteria, and hopefully get insight by studying that one partnership into partnerships that are much more complicated, like the microflora on our bodies.

18. The bobtail squid and bioluminescent bacteria (19:40)

And so, what's wonderful about, you know, hunting down these model systems is that nature provides these for us. So, in fact such a one-to-one relationship exists, and it's a crazy one. It's in the ocean and it's between this animal, which is called the Hawaiian bobtail squid, and a bacterium named *Vibrio fischeri*. So, *Vibrio fischeri* is a symbiont of this squid, so one animal, one species of bacteria, and what's especially good about this system is something that the bacterium does. So, this bacterium is bioluminescent. It makes light like fireflies make light, and remember bacteria are invisible, so the things that they do are even more invisible than the bacteria. And so, what's fantastic about bioluminescence is that it makes the invisible world visible. We can just see light with our eyes. We can measure it and we can use it as a reporter that the bacteria are there and the things that they are doing. So, now I want to tell

you about this partnership, and what I'm going to do is first tell you about it from the squid's point of view and then tell you about it from the bacterial point of view. So, first this squid— if we could have this movie, please—

19. Video: Bobtail squid swimming and burrowing (20:49)

so, this squid is a nocturnal animal. And so, what we've done is we've put this poor guy in his water during the day and he doesn't like it. So, he looks kind of uncoordinated, he looks a little nervous, because he's not supposed to be out during the day. So, what he likes to do is this; he likes to bury himself in the sand and sleep all day. This also keeps him away from those nasty snails of Toto's.
So, he buries himself and he's thorough. He wants to be buried, he wants to be hidden during the day, and he sleeps. And so, he's not quite done, even though he already has camouflage, right? So, he already has camouflage, but even that's not good enough. Those snails hunt those things down. So, anyway, he has to totally cover himself and then he's going to retract those tentacles and that should happen in a minute. And if you didn't know where that squid was, you wouldn't know where it was, right? You know where it is because we're zooming the camera out. So, this is the life of the squid in the day. It just sleeps away, hidden, invisible during the day to escape from predators.

20. Bioluminescent bacteria for camouflage (21:51)

The problem is you have to come out to eat, so he comes out at night to hunt. And this squid is not invisible at night so, this squid lives in just sort of shallow, knee-deep water. And so, the light that the moon or the stars make, since this water is so shallow, can penetrate the depth of the water that the squid lives in. So, if you're a predator under this squid and you looked up, that's what you'd see; it's a giveaway. Right? And likewise, if you were just swimming, minding your own business under this squid and the squid happened to swim over you, it would cast a shadow that would also alert the predator that the squid is there. So, this is where the bacterium comes in. What this squid has evolved is a specialized light organ that houses this *Vibrio fischeri* bacteria at something like 10 to the 12th cells per ml. So, on the left-hand side of the picture, that's the squid sort of like you saw it, on the right-hand side we've turned the squid over. That's the eye on the bottom and then up on top, that big black blob, that's a specialized home for this *Vibrio fischeri* bacterium. So, the bacterium lives in that light organ at incredibly high cell numbers. So, the selection evolutionarily for the bacterium is the squid feeds it and lets it grow to numbers it could never achieve in the ocean free-living. So, the bacterium gets to make more sons and daughters. The squid is interested in this partnership because it wants the light from that bacterium. So, the way that this symbiosis works, so now there's two pictures of this squid, the one if the bacteria wasn't there, but what the squid does is it uses that bioluminescence that the bacteria has and it shines that bioluminescence out from its lower half. So, if a predator looked up, because it's countershading itself to make it look like the water, it's much harder to find. And likewise, because it's got this beam of light coming down, what it does is it countershades its shadow, so it has two ways to keep itself invisible from the predator; can't see it when it's up and you can't see it when it's down. So, it's an amazing anti-predation strategy. So, the squid feeds the bacterium, and the bacteria like that, and then, in exchange, the squid gets bioluminescence that it doesn't have the genes to make.

21. Daily cycle of bacterial growth in squid (24:02)

And so, now I want to go through the day-night cycle that this squid uses because there's an interesting part about the bacterium here. So, on the left, the blue line, that's the number of bacteria in this squid-like organ. So, we're starting in the morning, right? The squid has buried itself in the sand, and there's very few bacteria in there. So, it wouldn't matter, the squid doesn't need light, you know, so it doesn't matter; there's only a few bacteria. The squid is feeding them so the bacteria grow and grow and grow during the day. Then at night when the squid has to come out, there's lots of bacteria in this, 10 to the 12th per ml

bacteria, in this light organ. They can make a lot of light, enough light to counter-illuminate that squid. But the squid can't keep that going forever because it's too hard to feed all of them. So, what happens is when the sun comes up the next morning and the squid's going to bury itself in the sand, it has a pump that's attached to its circadian rhythm. And so, when the sun comes up, it pumps out 95% of the bacteria; it sort of looks like toothpaste coming out of the side of the squid. So, now these bacteria are dilute but, of course, the squid isn't really using them. It's asleep in the sand. It's growing, growing, growing them so that at night, they've grown up to high cell density, there's a lot of light there, and the squid uses it. And this happens over and over and over during the life of the squid and it's how it keeps this bacterial culture fresh and growing and glowing when it needs it. So, that's the squid's part of this symbiosis. And what I want to do is to stop for a minute and ask if people have questions before we get to the bacterium's point of view. Yes, sir?

22. Q&A: What makes bacteria so resilient? (25:36)

[STUDENT:] What evolutionary aspects do you think contributes to bacteria's resiliency?

[DR. BASSLER:] I think that the reason bacteria are so resilient is that they have had so much more time to evolve and perfect these strategies than we do. So, bacteria have been on this Earth for billions of years; human beings, maybe a couple of hundred thousand. So, what they have had time to do, and plus they do evolution on a fast scale, right? Half hour, right? And so, they have time to explore the chemical diversity of the Earth, the environment, right? And they make some more of themselves so somebody gets a fortuitous mutation that helps it adapt to a particular environment, and then you have sort of your next species. So it's time which has given them all of that incredible diversity that we don't have in their genes. Good question. Yes, ma'am?

23. Q&A: Can the bacteria live outside the squid? (26:28)

[STUDENT:] Once that bacteria is expelled from the squid, can it survive, or does it immediately die?

[DR. BASSLER:] Right, and so the *Vibrio* — that's a good question, too. You were supposed to ask that. Anyway, thank you. Anyway, so *Vibrio fischeri* that gets squirted out, they are alive, they are making no light out in the free living state, they only make light in the squid, and they're alive and fine, but that's the poor man's life. So, they have to fend for themselves, get these nutrients, and they divide very slowly because the ocean is sort of a nutrient desert. So, they're out looking for another squid. So, being in this squid light organ is the rich, happy, fat life. Right? But, indeed they're alive, they're fine. Yes?

24. Q&A: How do the bacteria get into the squid? (27:07)

[STUDENT:] Are these bacteria, they're born without the bacteria in them, right?

[DR. BASSLER:] Right, so how does the squid get inoculated? So, the squid, the female squid, they lay these clutches of eggs and they're in an egg sac, and what we think they do, so the eggs are sterile, and what we think that they do is the mother loads that egg sac with *Vibrio fischeri*, so these would be like the free-living ones, so it's covered. So, as the hatchlings emerge, they can't help but get inoculated. And what's so amazing, if I can answer your question, is they have these little crypts on their side that the bacteria crawl into and then this entire light organ develops like you saw in that slide, and we can grow these squid; we can hatch them and grow them in the presence of antibiotics in the lab, right? So, with no bacteria they're not going to get eaten; there's no predators. And it turns out the squid will grow up completely, but that little light organ doesn't develop, so there's some amazing conversation that that bacterium is giving to make the entire development of that squid happen, and we do not understand that. But, presumably we don't see that in the ocean because they get eaten by the snails. Yeah. Yes?

25. Q&A: How big is the squid's light organ? (28:19)

[STUDENT:] Does the squid light organ cover the entirety of its belly or just...

[DR. BASSLER:] No, it's a little organ, but it actually, it's small. It's like a small... it's sort of like this on me. But the squid has reflectors. So first of all, it can open and close how much light comes out by using its ink sac, so it actually matches the amount of light coming out of the bottom to how much moonlight, so on a starlight, on a cloudy night, not so much comes out, and it can actually reflect it based on, you know, the angle of the moon and stuff, so it's, actually the bacteria are simply providing the light and the squid is maneuvering that to counter illuminate itself as best it can. Yes?

26. Q&A: Is the use of antibacterial soap detrimental? (28:57)

[STUDENT:] You said that bacteria on the surface of the human body serve as environmental protection, so I was curious as to the detrimental effects of using an antibacterial soap, and if there would actually be any positive effects from using pro-bacterial?

[DR. BASSLER:] Yes, so that's a good question, right? Because we are obsessed with this keeping ourselves clean when you're never clean, right? You're always covered with that. And, so, I am a proponent of washing your hands. Soap and anti-bacterial soap, to me, it's the same things. Soap is a greasy molecule and it pops bacterial membranes, so it works the same as these antibacterial products, so just washing your hands gets rid of them. And that is a good idea because this is how we share all these viruses, right? You know, you get colds and stuff from touching other people's hands. So, washing your hands is a good idea. I don't think we actually right now need probiotic soaps, right? Because you get this bacterial microflora from your mother. You have it your whole life. It is incredibly hardy and incredibly hard to perturb. So, the truth is in this country, we hardly ever get really sick from infectious diseases because this consortium — you have yours and I have mine, right? — is protecting us, right? And so, you're not doing so much by washing it off or you don't need, if you're a healthy person, to make it better necessarily. Yes?

27. Q&A: How do bacteria survive in extreme environments (30:15)

[STUDENT:] When you talked about bacteria being able to live in a wide variety of environments, I was just wondering how the bacterias can keep themselves from freezing...

[DR. BASSLER:] Yeah, so, right, right, so how do they, right. So, absolutely they have to have, the ones that are living in the ice versus the ones living in the vent, have different sets of genes — and I don't want to pretend that we understand this all — that protect their, you know, yours and my DNA would melt or freeze, right? But in fact, they have special ways of rolling up their DNA that people are trying to understand, like what are the genes and the features that allow them to actually thrive in these incredibly inhospitable niches. But if you looked on that, you know, chart, right, the guys over here have very different genes, like, very different genes, than guys that are living in other niches, and it goes back to this first question, right? You get fortuitous mutations that allow you to inhabit ever-wider environments.

[STUDENT:] So, basically the genes allow the bacteria to keep...

[DR. BASSLER:] So, absolutely. So, somehow the, like the ones that live in the hot places have genes that allow their membranes not to be melted, right, by the heat. But if you took me or a bacterium that lives just in the regular terrestrial environment and plunked it down there, right, it would be dead. Absolutely. So, that's evolution to occupy those niches.

28. *Vibrio fischeri* only makes light at high cell density (31:39)

So now, okay, so now you guys have this idea that we've got this model system, we've got our hands on that, right? This one to one relationship, and now I want to move to the part that is my actual favorite part, which is what are these bacteria doing. So, it turns out that the bacteria are pretty smart about this relationship as well. So, the answer to the question is what is also amazing about the bacteria is that they don't make light all the time. So, the observation is when the bacteria are alone, so when they're dilute, they make no light, but when they grow to a certain cell number, high cell density, all the bacteria turn on light together. And so, this makes sense in the terms of that squid, remember they're only at high number at night when the squid is using the light. So, that makes sense, again, from the squid's perspective. But

29. Quorum sensing and autoinducers (32:26)

the question for me and my lab is how can it be a bacterium that is supposed to be so primitive, how can it know times when it's alone from times when it's in a community, and then do something different under those two conditions. And so, what we have learned about it is that the trick they use is that they make and release small molecules that we call autoinducers, and those are depicted by these red circles on the slides. You can think of these like hormones. So, they make and release these molecules, and when the bacteria are alone, when they're in low cell number, the molecules just float away, so the bacteria don't perceive them. So, they don't make light. But when the bacteria grow to higher numbers, since all the members of the community are participating in making and releasing these molecules, the molecules increase in concentration externally in proportion to cell number. And when the molecules hit a certain amount, the bacteria detect that they're there, and they all turn on light together. So, the way that the bacteria know when they're alone from when they're in a group is by whether or not those molecules are there. They're using those molecules as a proxy for cell number. They actually have no idea how many other cells are there. They believe, if you will, that because these molecules are there, it means they have neighbors. And so, we have a name for this. We call this quorum sensing. The bacteria vote with these molecules, these chemical words, they talk to each other, they vote, they put their vote out there, it gets counted, and then everybody responds to the vote in a unified group, a quorum.

30. *Vibrio harveyi* is not symbiotic (34:00)

And so, now my guys, my lab, would love to study that. We think it is amazing that bacteria have the genetic wherewithal to be able to talk to each other with a chemical language and do things in synchrony, do things as a group. And so, now we want to actually have even simpler model system. We don't want to study *Vibrio fischeri* that only makes light in the squid. We want to study this quorum sensing on its own. And so, in order to do that, we have to go back to these evolutionary trees, this business we were talking about before. And so, what we've done is we've just taken a little branch of the big bacterial tree and we have just some of the *Vibrios*. So, there's lots of different *Vibrios* species. And so, what's interesting is that all of these *Vibrios*, and in fact probably all bacteria, have quorum sensing, but what's important for now is that all of these *Vibrios* have quorum sensing. They talk to each other.
But they have evolved to use quorum sensing for the different niches that they occupy. So, *Vibrio fischeri* on the top uses quorum sensing to make light inside of that squid. On the bottom, *Vibrio cholerae*, that's a pathogen you've probably heard about that causes the disease cholera, it uses quorum sensing to control pathogenicity. But in the middle is a bacterium that we like a lot named *Vibrio harveyi*. It uses quorum sensing to control bioluminescence, but it does it free-living in the ocean. And so, now we have a bacterium that makes light, that makes itself visible to us, that uses quorum sensing, and that does it free living. And so, we want to use that bacterium now as the model to think about this in the molecular terms from the bacterium's point of view.

31. Demonstration: Glowing bacteria in a flask (35:38)

And so, we grew up some *Vibrio harveyi* for you this morning. And all the lights are going to go out in the room, so don't worry, and I'm going to show you this bioluminescence, I hope, that these bacteria make. So, if the lights can go out. Okay. There they are. Alright. Can you guys see that? Right? So those bacteria are in those flasks. They're quite dense. Those autoinducers have been released. The bacteria in there recognize that those autoinducers are there, and in response to that, they're turning on bioluminescence because they realize they're in this crowd of other bacteria, all of which is dictated by whether or not that little chemical, the autoinducer, is there. So those are the glowing bacteria, and that's because of those autoinducers.

32. Quorum sensing activates a large network of genes (36:31)

Okay, so now we have this wonderful model system where we've extracted the bacteria from this host association and we can just study quorum sensing. And so, quorum sensing controls bioluminescence, I think you believe that now, but what is interesting is that when a bacterium makes the switch from "I'm going to go alone" to "I'm going to act as part of a community," it takes a lot of genes to change. It's a big deal to change from the program of gene expression that allows you to thrive when you're alone to that that lets you thrive when you're there in a community. So, even though light, bioluminescence, is what we can see as the readout of quorum sensing, we've gone in and studied these more invisible traits.
And it turns out in *Vibrio harveyi* alone, over 70 genes are controlled by quorum sensing. So, the genes that encode luciferase, the enzyme that makes that blue light that's quorum sensing controlled, but also the ability of bacteria to take iron up from the environment. Bacteria have to put a molecule outside of them and get iron and bring it back in. They like to change their DNA, exchange DNA; this helps them evolve faster. And so, when they give their DNA away, and then likewise they have to secrete lots of different exoproducts that help them consume nutrients from the environment. All of those genes are controlled by quorum sensing in *Vibrio harveyi*.

33. Pathogens also use quorum sensing (37:50)

What we're also learning is that this is not some weird anomaly that has to do with bioluminescent bacteria. We are now learning that quorum sensing is universal in the bacterial world. So, both beneficial bacteria use quorum sensing, but so do pathogens. And so we just put one pathogen up there, *Pseudomonas aeruginosa*. This is the pathogen that kills people who have the disease cystic fibrosis. So what *Pseudomonas* does with quorum sensing is it makes a biofilm — remember we talked about those — a biofilm on the person's lungs, so it sits down on the surface of their lungs. It secretes this goop, it's called alginate, that covers the biofilm and keeps it so antibiotics can't get there. And it also secretes 50 or 100 proteins that are virulence factors that chew up the person's lung tissues. It's just trying to get nutrients, but the problem is is that those toxins damage the host's lungs.

34. Quorum sensing allows bacteria to act collectively (38:43)

So, this is a terrible disease, but if you think about this from the bacterium's point of view, it is a fabulous strategy, both whether it's beneficial or pathogenic, for bacteria to be able to have an influence on their environment. All of the genes that are controlled by quorum sensing are ones that encode proteins that the bacteria have to put out there. And so, you're never going to get your own back. If I give you some protein for feeding, I'm not going to get it back. But if you participate and you participate and we all work together, what quorum sensing lets bacteria do is to be multicellular and to carry out tasks as a collective and accomplish feats that they could never accomplish if they simply acted alone as individuals. So, quorum sensing is all about these kinds of traits that you need lots of cells together or the trait is completely unsuccessful. And so, that's for good or for bad. And so, we are interested in understanding

that. Like, how did collective multicellular behaviors evolve on this Earth. Again, we like the idea that we can study this in bacteria because they're so much simpler than real multicellular organisms. So, my lab is interested in thinking about the molecular biology under that. So, the question is how do these bacteria, just like us, how do we process information from the environment? How do cells get information? And it turns out that signals come in from the environment. So, things change in our environment and then, of course, also in the bacterial environment and then we have mechanisms for interpreting that sensory information and behaving correctly.

35. Bacteria receive signals by membrane receptors (40:19)

So, in the case of bacteria, typically if something happens in the environment, something changes. In today's example, we're talking about quorum sensing, so that would mean whether or not that autoinducer is there. But this is true for many different sensory stimuli, but just think about the autoinducer. So whether or not that molecule is there, that gets detected by a membrane bound protein that's called a receptor, and the receptor actually goes through the bacterial membrane. So, it has a portion of the protein outside and a portion of the protein in the cytoplasm. So, transmembrane receptors connect the outside world of the bacteria to the inside world. And so, they receive the information and they send information to the DNA to turn on and off genes which encodes functions which allow bacteria to respond appropriately to whatever happens in the environment. And I just want to say I'm going to tell you the mechanism for this signaling in a minute. It is exactly the same biochemistry that your cells use to send signals to tell your cells what to do and when. And so, even though this evolved in bacteria, we can learn about higher organisms just by studying the biochemistry in bacteria. So, now we're going to take this step-by-step. So, the first thing is that there's this membrane-bound receptor. It's sort of the nose. It's sticking out there waiting for something to happen. And membrane receptors have two jobs; one is to sense a signal, a stimulus, and the other is to send that information to the DNA.

36. Receptors as kinases and phosphatases (41:46)

And so, the way that they do that is by changing their internal activity. So, the receptors can be kinases or phosphatases. Kinase means put a phosphate on someone. Phosphatase means steal a phosphate from someone. So, if you look at the left side, when the receptor is a kinase it's going to take phosphate, which we have as that yellow circle, and put it on a protein, in this case that's bound to the DNA, and then that protein can go turn on or off genes that the bacteria needs. Phosphatase, on the other side, is when the receptor takes the phosphate off of that downstream protein, and now the protein can't act. So, kinase means "put phosphate on," phosphatase means "take it in the reverse direction."

37. Molecular basis for quorum sensing pathway (42:36)

Okay? So, that's how receptors work. And so, now let's put this into our quorum sensing circuit. So, at low cell density— so remember this is when that chemical word, that autoinducer isn't there— when the molecule is not there, the receptor is a kinase. It puts a phosphate, the yellow circle, on itself. But then it has to get that information into the cell. So, what happens is it sends the phosphate to a connector protein called LuxU. LuxU's job is to take the phosphate from the protein that's stuck in the membrane and bring it down to a protein called LuxO, which sits on the DNA. So, this is how the bacterium has connected the outside world to gene expression. It's through a kinase, a phosphorylation cascade, that ultimately impinges on this protein LuxO. And when LuxO is phosphorylated, so when it has that phosphate on, its job is to shut off quorum sensing. So, it shuts off light and it shuts off all the other quorum sensing genes. So, at low cell density, phosphate flows to LuxO, quorum sensing is shut off, there's no light. At high cell density, so that's when these autoinducers accumulate — so remember that's the red molecule — they bind to the receptor, and that flips a switch. It tells the receptor "stop being a kinase, be a phosphatase." And so, now phosphate flow reverses. The phosphate goes from LuxO to that connector protein, LuxU,

and then LuxU puts it back on the receptor. So, now LuxO, the protein sitting on the DNA, is not phosphorylated, so it can't inhibit quorum sensing. So, all the quorum sensing genes turn on, including the luciferase genes, which make light. Okay? So, whether or not the cells are going to do quorum sensing is whether or not those molecules are there and it just matters which way phosphate is flowing in that circuit.

38. Quorum sensing turns on or off a gene regulator (44:25)

And so, now I want to take you one more step and tell you what is that protein LuxO do? What does it mean to inhibit quorum sensing? So, the first part, the first line, you've already seen this. At low cell density, phosphate flows to LuxO, and its job is to inhibit quorum sensing. What LuxO does is to inhibit the production of a special protein called LuxR. LuxR is the master regulator of the entire quorum sensing response. So, LuxR is a protein that sits on DNA and tells all these 70 genes to turn on or off. So, at low cell density, LuxO stops LuxR from being made, so there's no quorum sensing. In our little example of bioluminescence, that means that the LUX, that's luciferase, the LUX operon, so the piece of DNA that encodes the enzymes that produce light, don't get transcribed. There's no Lux AB protein, those are the enzymes that make light, and so there's no light. Right? So, low cell density, there's no LuxR, there's no quorum sensing, there's no light. At high cell density, remember phosphate is going to go backwards through the circuit. When LuxO is dephosphorylated, it can't inhibit. So, now what happens is that now LuxR gets made, and remember that's the master factor. So, LuxR then tells RNA polymerase come to all these genes, transcribe them, translate them into proteins, and we change into high cell density mode. In our case the important one is the Lux, or luciferase operon, so LuxR gets that transcribed and translated into luciferase, which is called Lux A and B, and the bacteria make light.

39. Animation: The LUX operon controls light production (46:05)

So, what you're seeing first, this is just DNA in the bacterial cell, and we've color-coded the genes. So, that's the Lux operon. There's five genes in that operon, and somewhere else on the DNA is encoded the gene for that master protein, LuxR, in purple. So, the first thing that's going to happen is that RNA polymerase, that's RNA polymerase, the green protein, is going to come to the DNA encoding LuxR. It gets transcribed into a messenger RNA, that's the molecule you'll see now, and then ribosomes come onto that messenger RNA, and they translate the message into protein, right? So, now the pink protein, that's LuxR, the master transcription factor getting made in the cell. When that protein gets made, which is only at high cell density, it's going to go and tell all these genes to get turned on so the bacteria can switch into quorum sensing mode. We're showing you it getting on to the Lux operon. Its job is to tell RNA polymerase "come here, come here, come here. This is a gene that we want to make the protein now." So, it's sitting on the Lux operon, here comes RNA polymerase, and then it's going to transcribe this long operon. There's five genes on this messenger RNA, so they're in colors for you to see this, and then the ribosomes get on at each gene, and they translate the message into proteins. We're just showing you two important proteins that are called Lux A and Lux B. Those are two proteins that fit like locks and keys, and when they get made at high cell density and come together, they give off photons of light. So, they lock in together, and light gets produced, which is what you saw in the demonstration. But of course, these bacteria are machines. It's not just one, you know, transcript getting made. There are thousands of copies of LuxR on thousands, you know, turning on thousands of messages to get made so that the cell can make thousands of copies of luciferase so that there's enough of those Lux A and B proteins coming together that the entire cell actually makes perceivable light. And so, that's what we're showing you there. And then if we zoom out of the cell...remember this is high cell density, so the outside wall of the cell, those autoinducers are there, the bacteria know they're at high cell density, they want to switch into quorum sensing mode, enough luciferase gets made that the cells turn on light and you can see it.

40. Summary (48:31)

Okay? So, that's how it works. And what I hope you've learned today in part one of my talk is that it's neat to work on model systems because they let us work on things that would be hopeless to take apart in a single step. So, we often try to find these simple systems and then relate what we learned from them to how more complicated systems work. In the case of what my lab is interested in, quorum sensing, besides letting us understand how bacteria talk to each other, what we think is because bacteria have been on this Earth for billions of years, by understanding how these collective behaviors, how do you get synchrony in a bacterium, we're going to be able to say something about how collective behaviors work in higher organisms, how the different organs of your body work. We think we're going to learn the principles because these bacteria evolved it billions of years ago, and often, just like you heard in Toto's talks, these themes are universal from organism to organism. What we're going to talk about tomorrow is that quorum sensing, as I've already said twice, is not just about *Vibrio harveyi* and *Vibrio fischeri*. What we think now is that all bacteria must have a way to know when they're alone and to know when they're in groups so they can do different things, and then we're going to think about that in terms of pathogenicity tomorrow. So, because both pathogens and symbionts use it, both beneficial and harmful behaviors are controlled by quorum sensing. It's just what the particular bacterium is doing in its niche, how it learned to survive in that niche, which is going to define what genes are under quorum sensing control in different species of bacteria. So, sometimes pathogenesis genes because it takes lots of bacteria to make a human sick, sometimes beneficial genes because it takes lots of bacteria to make a human healthy, right? And so, then, of course, what I really want to get to in sort of the opposite way of what Toto did, is to tell you how we're going to exploit what we've learned about how bacteria communicate with these languages to try to prevent them. So, if we could make bacteria that can't talk or can't hear, could we make new kinds of therapeutics to fight these global infectious diseases that you heard, for example, Dr. Tjian talk about this morning. So, that's where we're going to is can we actually do something practical from starting with just this crazy glow-in-the dark bacterium. And I will take questions. Yes sir?

41. Q&A: Can you add autoinducer and make the bacteria glow? (50:58)

[STUDENT:] If we take a low density bacterium and put them in a closed environment, and I don't know what the makeup of autoinducers are, but if you could create...

[DR. BASSLER:] Tomorrow.

[STUDENT:] ...create autoinducers and inject them in their environment to artificially...

[DR. BASSLER:] Absolutely.

[STUDENT:] ...would we be able to find out if just autoinducers are the things that stimulate receptors, or are there other signals...

[DR. BASSLER:] Exactly. Absolutely. So, that's a great question. So, we can do that. So, you're going to find out tomorrow. You're going to learn all the chemistry of the autoinducers. So, we can make these molecules. And so, I can take diluted cells and squirt that autoinducer on, and they turn on light and all the other quorum sensing behaviors. So, that actually was the proof that it's simply the autoinducer that is running that show. Right? And so, tomorrow I'm going to show you exactly that. That's a great question. Yes, up there. I know the rest of that audience is here. Yes, ma'am?

42. Q&A: Can *Vibrio fischeri* make light outside of the squid? (51:48)

[STUDENT:] You said that when the *fischeri* are not in the squid and when they're pumped out they don't produce light, but if they were in high density outside the squid, would they still not be able to produce light?

[DR. BASSLER:] Right, so that's a great question. So, there's two things happening -- you understood everything — so one is when they're outside the squid, they're dilute, so they should be in the individual mode. So, why would you do quorum sensing?But what is amazing about *Vibrio fischeri*, and this gets back to your question, is that even if you put them in high cell density like this, they don't make light. So, in that case, the squid must be providing some other signal that says you're inside. So, they're using the autoinducer and some host signal that we don't know what it is to say "this is when you make light." So, in fact you can squirt all the autoinducer you want on *Vibrio fischeri* and they won't make light in this tube. And that is why we had to switch to this bacterium to understand that. That's a great question. Uh-huh? Sure.

43. Q&A: What benefit is there to making light in the ocean? (52:46)

[STUDENT:] What benefit do *Vibrio harveyi* get for making bioluminescence in free-floating ocean?

[DR. BASSLER:] Right, so that's a great question that has driven us bananas all of my life. So, I got into this 'cause I thought these were pretty and I wanted to know why they did that, and this is what we've found out so far. So, what a scientist can ask is how and when. And I always thought we'd get closer to why and we haven't. And so, what is amazing about *Vibrio harveyi*, you've seen it, it makes a gob of light free-living, right? It takes 20% of the total energy of this bacterium to make this light, and we understand the biochemistry perfectly. They get nothing back except the light. And so, we have never understood what the benefit is to a free-living organism. What we do know is that this bacterium, if we go to those trees, is older than *Vibrio fischeri*. So, the idea is that *Vibrio harveyi* had this function, bioluminescence, and then *Vibrio fischeri* got smart and got a free ride for it. But bioluminescence was there earlier. So, I'm making a long answer to say I have no idea, but what I do know is that in the ocean, almost everything makes or uses light. So on land, we have light, so there's only a few things that make light. In the ocean it's estimated that 75% of all of the organisms in the ocean are either making their own light or using somebody else's, because light's a big deal down there. And so, I think we're going to eventually find that out, because there's lots of other organisms, free-living bacteria, that make light, but the answer, the long, long, long answer is we don't know. But we don't know what experiment we would do to ask why is it making light, you know, especially in the lab, you know, what would we do? I mean, it must give it, it absolutely gives it an evolutionary advantage or they would have lost it, right? Because it's a huge amount of energy committed to that one reaction. Yes?

44. Q&A: Can you make a bacteria unable to detect autoinducer (54:39)

[STUDENT:] Can a mutation occur in the bacteria that can prevent it from, like, making the light?

[DR. BASSLER:] So, could we immunize the bacteria...

[STUDENT:] A mutation occurs...

[DR. BASSLER:] Would make them so they, what? Can't do quorum sensing? Absolutely. And that we're going to get to tomorrow. So, we can do that or it can happen in nature and then they're deaf or they're mute. So, we can make bacteria that can't talk, you know, can't make the molecules or can't respond to them, and those we're going to use for great tricks tomorrow. In the wild, those mutations happen, but because quorum sensing must give a selective advantage, you know, if you just think about it from the human perspective, it's good to know when you're alone and when you're in a group because we

behave differently. They need to do that, too. So, even if those mutations happen in the wild, they get selected against because you lose fitness by not being able to do these community behaviors.

45. Q&A: Does quorum sensing lead to specialization of bacteria? (55:35)

[STUDENT:] I was wondering if quorum sensing leads to specialization of bacteria.

[DR. BASSLER:] Absolutely. So, the bacteria... so the idea of quorum sensing is universal, this "I'm alone, I'm in a group."
The molecules you're going to see tomorrow are quite different one bacterium to the next. So, these are private, secret conversations, right? And the tasks that lie at the bottom of the cascade are absolutely specific to the bacterium. So there's, you know, *E. coli* in your gut is not making light, but it has quorum sensing to do other group functions. So, absolutely, even though the signaling circuit, sort of the architecture is the same, the inputs and the outputs are tailored through evolution for what a bacterium needs to occupy a particular niche, and we're going to get into that tomorrow.

46. Closing remarks by HHMI Vice President Dr. Peter Bruns (56:26)

[DR. BRUNS:] Thanks, Bonnie. That was a great talk and asking and answering great questions is what science is all about. And although 90% of the cells in this room, I guess, are bacteria, you still asked some great questions. But I know there are lots more questions that you probably have thought of, and certainly the people out there in the cyber-world have thought of and couldn't ask, but we have a way to get around that, too. Again, if you go to our website, HHMI.org, and go to Cool Science, there's another site there called Ask a Scientist, and you can pose a question and we have a panel of volunteer scientists who will answer your question. And if it's a really good one with a good answer, we'll post it on the site and archive it so that there are other good questions and answers you can seek there, too. So, Ask a Scientist is a good way to keep this dialog going. So, please join us tomorrow morning for two more great presentations by Toto and Bonnie, and in those talks, they'll sharpen our focus on how understanding nature leads to very practical medical benefits. Thanks for coming.