Exploring Biodiversity: The Search for New Medicines
Lecture 4 – Eavesdropping on Tiny Conspiracies
Bonnie L. Bassler, Ph.D.

1. Begin of Lecture 4 (0:15)

[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 fourth lecture is titled, "Eavesdropping on Tiny Conspiracies." 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 our final presentation in this year's HHMI Holiday Lectures. I'm pleased to introduce Bonnie Bassler to give this fourth lecture in our series. Previously, Bonnie introduced you to the fascinating complexity of the microbial world, a world of competition, cooperation, and copious chemical communication, sort of like high school. Early on, some may have viewed Bonnie's work as an esoteric studying of bacteria that uses chemistry to make light. But, as you will see in the lecture today, Bonnie discovered something fundamental, and nearly universal, about the way that bacteria communicate. Having this knowledge, is revealing new strategies for fighting infections and agents that cause disease. And now, a brief video to introduce Bonnie.

3. Profile of Dr. Bonnie Bassler (2:09)

[DR. BASSLER:] This group of young scientists, who have continually joined my lab, because they like quorum sensing, are the ones that really did it. Princeton gave us the time and the space, and the intellectual freedom to do whatever we want, and then these young people, who believed that quorum sensing could be more than whatever we knew at the time, you know, took it to all these new directions. It's been incredibly fun. And then, the original question is, what do I like about this job? What I like the best is that I spend 100% of my days with 20- to 30-year-olds. These young people who are learning the newest things that happen. They're not entrenched in the history of science, you know, because they're just in the midst of learning things, I think their minds are more open to possibilities. And so, for me, it's like always having a great tennis partner, except, I have a dozen of them, you know, that I'm trying to keep up with. They, sort of, come and go every four or five years, and so, it's this constant stream of vibrant, young, unbiased minds. And, I'll tell you what I hate about my job, and that's working with 20- to 30-year-olds. So, don't get me wrong, they drive me nuts. Because they come and they're this raw material. They do all these crazy experiments, and during the four or five years, they refine themselves, and they become better scientists. And their experiments get slicker, and better, and more interesting. And then, as soon as they really get good, they leave, right? And so, it's a sort of, weird enterprise, but it works.

4. Helpful and harmful bacteria use quorum sensing (4:01)

Well, good morning, again. I'm glad to be back to finish up the fourth part of these lectures. And, today I want to try to tell you how one can, hopefully, do something practical by learning about microbes, and learning about quorum sensing. And, of course, you already know what that's going to be, is to try to make new kinds of therapeutics for infectious diseases. And so, I want to start by just reminding you of where we were yesterday. So, we learned, I hope, that most bacteria aren't bad, you know, we're covered in bacteria all of our lives. And, they're doing all of these amazing functions that keep us alive, and keep

nature what we know it to be. And so, that is our normal existence for most of our lives. But, of course, you guys read the newspapers, and you've been ill in your life, it's not always the case. Occasionally, things go awry. And, when one gets an infectious disease, it's because some bacterium got a toehold in us, or on us, that isn't meant to be there. So, it's not part of our normal microflora that lives in the symbiotic relationship with us. And so, on our slide, we've painted these invaders as red, right? And, these bacteria are just natural bacteria that live in the environment, and they have their own sets of genes for coping with whatever niche they live in. The problem is, when they come smack in contact with a human being, they unleash these factors that are good for the environment. But, some of those factors, when they get unleashed in us, infect us, and make our immune system, you know, go haywire, and we have to try to get rid of these infections.

5. Notorious pathogenic bacteria (5:40)

So, what these bacteria are, there's lots of pathogenic bacteria on this Earth, and what we did on this next slide, is just to give a picture, under the microscope, of a few notorious bacteria, that you've probably heard of, or you've of the disease, at least, in the newspaper. So, the top of the slide tells you the name of the disease you've heard. And, what you may or may not know, is that on the bottom is the species of bacterium that causes that particular disease. And so, most of the time, these bacteria aren't on us, and we're healthy. But, occasionally, one might get infected by one of these, and then one becomes ill. And, the reason we get ill, I've already alluded to this, is because these bacteria have proteins and enzymes, and things that give them a competitive advantage in their natural environment. And, they get loose in us, and they make us sick.

6. Pathogenic strategies (6:33)

And so, some of the, sort of, generic strategies, that different pathogenic bacteria use, to infect their host and get to stay, and eat, is that they can release proteins that actually punch holes in your cell membranes. And, remember, we talked, yesterday, that you don't want that because the membrane keeps the outside out, and the inside in. And so, what this does is spill the inside out, and give the goodies to the pathogenic bacteria. And, of course, your cells die when they have holes in them. They can, also, release molecules that just act as poisons, so they shut down vital functions in your host cells, right? And again, this is to allow the bacteria to get the goodies, but, of course, you get sick. Some bacteria are incredibly insidious, what they can actually do, is to wend their way into your cells, and they eat you from the inside by living inside of your cells on the cytoplasmic components. And then, there's finally, numbers of pathogens that, kind of, do this, sort of, slow, not so acute infections, so they can live on us for long times. And, parasitize us, but not actually kill us.

7. Pathogenic strategies under quorum sensing control (7:40)

And so, those again, are terrible from the humans' perspective. We have to have ways to deal with this. But, what I want to do, to get back to yesterday's talk, is to remind you, if you actually take the perspective of the bacterium, it's important to have these traits, these pathogenic traits, under quorum sensing control. So, just to remind you, bacteria are incredibly tiny, relative to your cells, or to your whole body, and if they release these factors that I was just telling you about, if one bacterium lets out some toxin, nothing's going to happen. But, the better idea is for these bacteria to get into the host, to wait, and to start counting themselves by making and releasing the small molecules, the autoinducers, recognize when they have a quorum, when do they have the right number of cells, that if all of them release these pathogenic factors, they're going to be able to have an impact on an enormous host organism. So now, we have hundreds and hundreds of examples of pathogenic bacteria that use quorum sensing to control the entire battery of traits that are required to make the host sick. And so, what these bacteria are doing, and remember, when they release these traits, those are red flags to your immune

system. Your immune system evolved to see and get rid of invading bacteria. So, what they're doing is they're waiting, they're counting themselves with these tiny molecules, they're recognizing when, if they launch this attack together, your immune system's going to be on the losing side of that battle. Whereas, if they launched it prematurely, they would get wiped out by the host's immune system. So, it's a fabulous strategy, depending on which side you're on, from the bacterium's point-of-view.

8. Structures of quorum sensing signaling molecules (9:25)

So, now we know the bacteria use quorum sensing for good things that takes lots of bacteria, and for harmful things that take a lot of bacteria to make those processes effective. And so, we want to study this quorum sensing chemistry and molecular biology at a really deep level. Because, if we can really understand the details of this phenomenon, both the chemistry, and the biochemistry of it, then we have a hope of pinpointing places in signaling these communication circuits, that just like in Toto's talk, we could learn places that we could disrupt these activites and try to find their Achilles' heel, to make new antibiotics. And so, now you have to learn chemistry and molecular biology. So, first we're going to go through this, and so, the first thing I want to do, you know, on these cartoons, we're showing you these molecules as red circles, but, of course, these are chemicals. These are three-dimensional objects, right, that are made by chemistry. And so, what I want to do, is to just show you a smattering of a few of the molecules that we know that these bacteria talk with. And so, on this slide, what we've done is, we've simply listed a couple of species of bacteria, so the name. And then, the molecule, that each one of those bacteria uses for quorum sensing. And, what I hope you can see, is that the molecules are related to one another. So, the left-hand part of the molecule in every case, in every single species, the left part of the molecule, that ring, is identical. But, the right-hand part of the molecule, the chains, those are just carbons, each of those is a little bit different in every single species. And so, what those tiny differences do, in these molecules, is to confer exquisite species specificities to each of these languages.

9. Demonstration: Quorum sensing molecules (11:14)

And so, we made a model of a few of these molecules, so here's a short one. And, what I hope you can see, this is the ring, and here's the side chain. Here's one that's got a longer side chain, right? So, this part is identical, but the part over here is a little bit different. And, remember, we talked yesterday, how these molecules work, they interact with their partner receptors, and send information into the cell. And so, what these differences in the shapes of these molecules do, is to tailor each of these molecules to its partner receptor, so they fit like locks and keys. So, if I give this molecule to a bacterium that talks with this one, nothing happens, and likewise, this molecule has no effect on a bacterium that has evolved this molecule to talk to, to talk to each other.

10. Autoinducers allow for intra-species communication (12:04)

So, what that means, is that these molecules and these species of bacteria, have evolved these different molecules for intra-species communication. This is how bacteria count their siblings. They count their brothers and sisters, recognize when they have enough of their kin, that if they all carry out these particular tasks together, they're going to be successful, okay? So, these are species-specific, these are private, secret conversations that bacteria have with their brothers and sisters. And, that is encoded in the differences, the slight differences that you see in these chemicals.

11. The LUX signaling cascade in low cell density (12:41)

Okay so, that's the molecules, and so, now I want to talk, again, about the signaling cascade. And so, we talked, in some detail, about this yesterday, but I'm going to add a few more steps to this cascade. And, the reason that I'm showing you this detail, is because I want to reiterate, because we need to know this

deeply, if we're going to be smart about thinking about how to interfere with this. We have to, if we can, know every step of the cascade, how it works, right, in order to be able to intellectually think up strategies to go after the different steps. The cascade that you are looking at, is the one from *Vibrio harveyi*, the read-out, the final output, is light production, right? We're using that as a measure, but as I told you yesterday, these cascades are universal. So, nature has evolved these cascades, they work robustly, with high fidelity, and so, again, even though we started these studies in *Vibrio harveyi* and got the particulars of the steps, we're going to use that knowledge to work on pathogens. Okay. So, the first part of this, you already know from yesterday. So, remember, there's two states, low cell density, and high cell density. So, in the first state, when the bacteria are alone, so they're not doing quorum sensing, those autoinducers, the molecules, aren't there. Remember, the membrane-bound receptors are kinases. So, they put a phosphate on themselves, and then, that phosphate travels from the receptor to that protein LuxU, in the center, whose job is to take that external information, and bring it to the DNA. So, LuxU puts the phosphate, the yellow circle, onto that DNA binding protein, LuxO.

12. Short RNA inhibits the cascade in low cell density (14:18)

And, remember, LuxO's job, at low cell density, when it's phosphorylated, is to stop quorum sensing. And, the way that it does that, is that LuxO sits on the DNA, and it tells RNA polymerase to make a short RNA. And so, this is a new step that we didn't talk about yesterday. So, this short RNA gets made. And, its job is to go around the cell and find the messenger RNA that encodes the special protein we talked about yesterday, LuxR. So, remember, to step ahead, LuxR is that master transcription factor, that when it gets made, it turns on all the quorum sensing genes. So, LuxO's job, at low cell density, is to keep LuxR from being made, so there's no quorum sensing. And so, the way that really happens, is this small RNA gets made, and it makes a partnership with the messenger RNA for LuxR. And, when you get this duplex of the small RNA, sitting on top of the messenger RNA for LuxR, the ribosomes can't get on. And so, that message can't be translated, there's no LuxR, there's no quorum sensing. In the case of *Vibrio harveyi*, no light, okay? So, everybody gets that? At low cell density, short RNA, it stops the long RNA for LuxR from getting made.

13. Signaling cascade in high cell density (15:36)

At high cell density, so remember, that's when these autoinducers kick in. So, the autoinducers are in the extracellular environment, and they get bound by the receptor. So, LuxN is the receptor, it binds the autoinducer, and remember, that flips this toggle switch. So now, phosphate goes backwards through the cascade, from LuxO to LuxU, back to LuxN. So, now LuxO is dephosphorylated, so it can't get that short RNA made. So, that short RNA disappears, that frees, the messenger RNA gets freed from that short RNA, the ribosomes can get on, the LuxR master transcription factor, gets made, it sits on DNA, and turns on the entire quorum sensing cascade. it's going to sit on the luciferase operon, that's called Lux, make those two proteins, LuxA/B, that come together and make light, okay? So, it's actually, really simple. At low cell density, there's a short RNA, no quorum sensing, because there's no LuxR. High cell density, no short RNA, LuxR gets made, and there's quorum sensing, okay? But, there's lots of steps that these bacteria have put in place to make that all happen. Okay, so, now we're going to show an animation of the whole thing, and then you are quorum sensing aficionados. Okay,

14. Animation: The molecular cascade in bacterial quorum sensing (16:56)

so starting here, the cells are at low cell density, so they are making the autoinducers, but of course, they live in this big world, and so the autoinducers diffuse away. So, if we hone in onto the bacterial membrane, those are the receptors, and it's very infrequent that they're going to bump into one of these autoinducer molecules, 'cause those have floated off. So, on the inside of this cell, remember, in the absence of autoinducer, the receptor is a kinase. So, it takes a phosphate, puts it on itself, that's what it's

doing now, it phosphorylates itself, and it's going to transfer that phosphate down the line. So, here comes LuxU to take the phosphate from the receptor. LuxU is going to put the phosphate on LuxO, which is sitting on the DNA, waiting to get that small RNA transcribed. So, there it is, that RNA gets made, and then that RNA makes a one-to-one pairing with the messenger RNA for LuxR. So, whenever a LuxR message gets made, which you're seeing here, RNA polymerase make the messenger RNA for LuxR, that small RNA immediately finds it. And, they make a pairing, almost like what you're looking at, and now the ribosomes can't get on. And so, no protein gets made. So, it's a fabulous way to stop that protein from getting made at low cell density. At high cell density, lots of cells, lots of autoinducer, so the frequency of one of those small autoinducer molecules bumping into that receptor increases enormously. So now, these receptors start binding to the molecules, and then, remember, that event, binding the chemical, tells the receptor "don't be a kinase, switch and be a phosphatase." So now, the receptor wants to slurp that phosphate out of the cascade, so there's LuxU stealing it from LuxO, and putting it back on the receptor. So now, LuxO is missing its phosphate, and it is dead. No short RNA gets made. So, the LuxR protein can get made, get transcribed from its gene. So, here comes RNA polymerase, and it's going to transcribe the LuxR message, but that small RNA is gone. That inhibitory small RNA is gone. So, now, when a ribosome comes by, it's just going to get on there, and translate that message, into the protein LuxR, which was the important protein we talked about yesterday, that runs the show. So now, LuxR goes to all the DNA encoding target genes for quorum sensing. In this case, of course, we're showing you my favorite ones, these are the genes for luciferase. It makes messages for all of the proteins that bacteria needs for quorum sensing. In this case, this is the Lux operon, and there's two important proteins we showed yesterday. Those are called LuxA and LuxB, right? So, those are getting made, and then, of course, those two proteins are going to come together and when they touch each other, and make this hand to hand configuration, the bacterium makes light. And so, we learned all this in *Vibrio harveyi*. But, the cascades are similar in pathogenic bacteria. You just have to substitute nasty outputs for that beautiful bioluminescence, okay? But, the idea is the same.

15. Small RNAs also regulate genes in higher organisms (20:16)

And, what else turned out to be kind of interesting was not only are these circuits conserved in bacteria, this is how quorum sensing happens. That small RNA, the reason that I told you about that, it turns out, those small RNAs, in regulating genes, using these small RNA message pairings, is universal in all of biology. So, it turns out all of human development is dependent on those small RNAs. So, bacteria invented that, and again, it's a fabulous strategy, so when you get to higher organisms, those strategies are reused to regulate the genes in your body. So, that's why we put that in there, because we thought you might hear about small RNAs, 'cause they're all the rage right now.

16. Autoinducer 2 handles inter-species communication (21:00)

Okay, so, that's how quorum sensing works. But, if you remember from yesterday, I spent most of the time, telling you about all of this biodiversity in the world, and that bacteria live in these incredible, complicated mixes. And so, what I've just explained is that these molecules are incredibly specific. They are for species-specific communication. The problem is, is that most times bacteria live in these mixes. This is the one I showed you from your elbow yesterday. And so, if it's really true that quorum sensing is about counting cell numbers, you have to have a way to count other species in the environment, or else you're not really counting. If all you can do is talk among your own species, this doesn't work very well. And so, we started to think about the, sort of, biology that bacteria live in, that they live in these mixes, how could these molecules work? And, we thought, we have to go back and hunt for a second molecule. And so, sure enough, we found out that bacteria are bilingual. They have a molecule that says, "Me," and then what they also do, is that they use a generic molecule, which we call autoinducer 2, and this is its structure, it's a five-carbon molecule. So, they use this molecule, the second autoinducer to talk between species. So, what's special about this molecule is that all bacteria use exactly the same

molecule. So, they have a specific molecule that says, "Me," and they have a generic molecule that says, "Other." So, they have two languages, one that's specific, and one that's non-specific. So, we think, now, that autoinducer 2 is, sort of, the trade language of bacteria.

17. Two parallel quorum sensing (QS) circuits (22:40)

And so, you've learned how the quorum sensing circuit works for autoinducer 1, right? So, you've seen this many times, right? The autoinducer interacts with this receptor, and a signal gets sent into the cell to It turns out that bacteria have two circuits, running in parallel, that impinge on whether or not they're going to turn on these hundreds of quorum sensing target genes, right? So, all you have to do is take what you learned, and then multiply it by two, that there's a second system of autoinducer 2, and its own receptor that works in the quorum sensing cascade, okay? And so, now, we're going to try to simplify this a little bit for the rest of my cartoons. So, you know the molecular details, but I'm going to draw a cartoon that could represent any bacterium.

18. QS1 signals "self," QS2 senses "other" (23:32)

So, what we're starting to think, now, is that all bacteria have to have some way to know self. So, we're going to call that quorum sensing system 1. So, they have an enzyme that makes a molecule, an They have a second system that is generic. We're going to call that quorum sensing system 2, it's for inter-species communication. They're using the same molecule, autoinducer 2 that we have as those pink pentagons, that interacts with its partner receptor. And then all of that sensory information comes into the cells to tell them to turn on and off quorum sensing, or collective genes that they need when they're in a community, but not when they're alone.

19. QS1 and QS2 differentially affect gene expression (24:18)

And so, now, what we're starting to understand, is the way they do this computation, is we think that the bacteria, sort of, asks two questions. The first, and the simpler question they ask is, "Am I alone, or am I in a community?" So, they simply scan the environment for whether there's any molecule there. And that says something about number. But, then the more sophisticated question that they ask is, "Is it me or is it you?" And so, then what they do, is they look for how much of each of these two different molecules that are there, and by measuring the blend, or the ratios, of autoinducer 1 and autoinducer 2, they can tell, first of all if there's other species present, but also, they can tell, am I in the minority, and you in the majority, or the reverse? And then what they do, is that they tailor this gene expression program at the bottom of the cascade, depending on whether they're winning or losing, in any given environment. So they turn on genes from 1-100, in a precise order, depending on the complicated mixture of molecules that they see in the environment. So, that's what we're starting to think, is that all bacteria have to be, at least, bilingual to manage in nature.

20. How antibiotics kill bacteria (25:30)

And so, now to get to the thing that you've been teased about for two days, is can we take what we know... so now we know that bacteria use these different chemicals to communicate, and to coordinate group activities, so can we actually do something practical with this knowledge? Bacterial and microbial pathogens kill millions of people a year. And, a big problem is, that because we don't have new antibiotics, because we just have a few antibiotics that we've had for about 50 or 60 years, bacteria are increasingly becoming resistant to the arsenal of therapeutics that we have in hand. So, there is a global need for us to develop new antibiotics. And, just in case you don't know, the way antibiotics work, is they kill bacteria. They are chemicals that either pop the bacterial membrane, they make it so bacteria

can't replicate their DNA, they kill bacteria. that makes it resistant, it's resistant, and so are all of its offspring.

21. Can QS be used to treat bacterial infections? (26:30)

And so, we need new kinds of antibiotics, just in general. But, the question for me and for my gang is, about a whole new way to treat bacteria? where we can stop communication, stop quorum sensing?" So, not kill bacteria, but just trick them into thinking that there aren't neighbors around so they don't know, if you And so, we can just try to march our way through the quorum sensing circuit, and see if we can stop it. And so, the simplest idea, is to simply make a molecule that works at the top of the cascade. Could we make a molecule that is an antagonist of the real autoinducer? And so, the idea is, that at low cell density, pathogenic bacteria aren't expressing virulence traits, because they're useless, they're not effective. It's only at high cell density, when the autoinducers kick in, that they launch these virulence attacks, because that's when they're going to be successful. So, the question that we want to ask is, now that we know about these molecules, and we know about these circuits, can we make a molecule that jams the receptor? So, like, make a molecule that locks into the receptor, and prevents the bacteria from recognizing the bonafide autoinducer, and if we did that, we would trick the bacteria into thinking they're alone, when they're really in a group. And so, they wouldn't launch these cascades, it would give your immune system a leg up, just a little extra time to do what it's always doing, which is surveillance and kicking out of pathogenic bacteria. And so, that's what we want to do, okay? And so, I thought that this is a good place to stop, before we do it, and ask if there's any questions. Yes?

22. Q&A: Can pathogens sense when they are too virulent? (28:13)

[STUDENT:] Considering that it'd be just as disadvantageous for a parasitic pathogen to kill its host, can any, or have any pathogens evolved to sense when there are too many cells?

[DR. BASSLER:] So, that's a great question. So, I'm going to take that in two parts, to answer the question I want to answer first is, like, why do they kill us, right? We already talked, we're food, right? And so, in fact, the bacteria never, if I can use these anthropomorphic terms, they don't mean to kill us, right? They have these features in nature that, when they get unleashed in us, are detrimental. But, over time, over evolution, they tone it down, and our immune system gets better, so that what happens is that the bacteria are always working toward a mutualism. They want to be the *E. coli* in your gut that you need to stay alive. And so, the answer to your question is yes, I don't actually know if they've evolved to see when there's too many, but what is happening, is that we see over time that, sort of, pathogens, if you read the newspaper, they come and go. And, that's because they're dampening their virulence system, we're getting better and better at it, and so, first they become childhood diseases, and then eventually they become commensals. So, indeed, bacteria are always working toward reducing virulence, so they get to stay. So, that is certainly happening, you know, the diseases that people died of 200, 300 years ago, aren't the pathogens that kill us now. Uh-huh?

23. Q&A: If you inhibit QS, would bacteria grow uncontrollably? (29:41)

[STUDENT:] By inhibiting part of the, I guess, chain reaction that happens, couldn't that lead to, like, the buildup of bacteria that eventually, the size of the population would be unfavorable to the host also?

[DR. BASSLER:] Yeah. So, the question is, right, and I want to get back to this at the end of the talk, too. The question is, if you get the logic of this, you're not doing anything to growth. The bacteria are growing fine, they just don't recognize they have neighbors. So, let's say you really could do this, does that mean that when you stop taking your anti-quorum sensing drug, you have this massive infection because they're just there? And so, it turns out that using mutants in bacteria that can't talk, can't hear —

that's not what happens. It is essential that the bacteria launch this virulence attack to get to stay. So, if they can't do quorum sensing, and mutants tell us this, they're there, they're growing fine. But, because they don't get this toe-hold, they don't make the biofilm, they don't make the poison, your immune system gets rid of them. So, it turns out, they're not just hanging around. All we're doing is tipping the scales, we hope, in the favor of the immune system, and it turns out that these bacteria get taken care of. I mean, you guys eat gobs of bacteria all day, everyday, and your immune system is constantly finding them, and getting rid of them.
And so, that's what we're doing to these bacteria is just making them much more like the innocuous bacteria that we encounter every day, that don't ever gain a toehold. Yes.

24. Q&A: Would a QS-based treatment require a functioning immune system? (31:12)

[STUDENT:] So, kind of, a follow-up question, would that approach only work for people who have a fully-functioning immune system?

[DR. BASSLER:] Right. So, I do not want to pretend, and we're going to get to this later. There is a place for traditional antibiotics, absolutely, right? So, you get that, right? People who would be immune-compromised, this is not going to work, right? And so, I don't want to pretend, this is not a panacea, right? These are really complicated problems. We need to increase the number of technologies that we have to fight against bacteria. But, absolutely a physician would never use this for an immune-compromised person. They would get a traditional antibiotic where the bacteria get killed, absolutely. That's a great question. Yes?

25. Q&A: Do any diseases come from inter-species QS? (31:55)

[STUDENT:] I know you're talking about inter-species quorum sensing. Are there any diseases that we might know about, that require two bacteria quorum sensing simultaneously?

[DR. BASSLER:] You know, that's a really good question. So, it turns out, so nobody could imagine this a while ago. But, it turns out, on your teeth, how we talked about yesterday, they actually need that molecule to make the mixed species biofilm. So if we make mutants... so, I should tell you, teeth bacteria have been worked on a lot, because you can get them, right? And so, and that's a polymicrobial infection, 600 species, right? And so, now that we know about this molecule, so people have made mutants, so they can't do inter-species communication, each mutant can make a biofilm by itself, but they can't make the mixed biofilm, where they take on the specific jobs. And so, that's the first example we have.. I of course, think there's millions of examples, but that's the first example that we have, where they really do need to cooperate to make an infection. And, I think there will be more, but it's very early days.

26. Developing drugs for blocking either QS1 or QS2 (32:56)

Okay, alright. So now, all this big talk for two days, how do you actually do this, okay? So, I hope you guys are believers in quorum sensing, and so now we want to make a new antibiotic. Okay. So, this is our simplified version of the quorum sensing circuit. You guys know there's lots of steps, but what we're going to try to do, is to interfere at the top of the cascade, make an antagonist. So, there's two, sort of, general strategies that you could take. The first one, is to try to make an antagonist of the intra-species quorum sensing system 1, the private language. So maybe, you need a therapeutic that is bacterial specific. You are a cystic fibrosis researcher, you want a drug for *Pseudomonas*. If that's your goal, then what one would do is to target the specific system, try to find an antagonist that works on that bacterium. The alternative strategy is to go after this generic system, with autoinducer 2. So, oftentimes a person is ill, they go to the hospital, doctors don't know what you have, and so you get treated with broad-

spectrum antibiotics that work against lots of different bacteria, while they try to pinpoint the source of the infection. So, we need broad-spectrum antibiotics, as well. with autoinducer 2 quorum sensing, since that molecule is works against lots of different species, okay? So, the logic to go from that idea to getting the molecules, is identical, no matter which of these you choose. So, for today's lecture, we're going to go with the second system, but it's exactly the same steps if you wanted to make an antibiotic against a species-specific system, okay? So, everybody gets where we're going.

27. Two ways to generate candidate QS1 and QS2 (34:40)

We want to try to find an antagonist that blocks autoinducer 2, which the cartoons show us, the pink pentagon, from interacting with its receptor, and sending the quorum sensing information in. So, how do we do it? So, there's, sort of, two ways to do it. The first, is that we know what that molecule looks like, I've already shown it to you. So, we can use synthetic chemistry, and just make molecules that, kind of, look like the real one, you'll give them bumps and knobs, and hope that we can, by using the scaffold, So, that's called rational design. The second way, is that we can just massively screen every chemical that we can get our hands on, and try to find something by luck that locks into the receptor. So, it turns out that chemists make molecules for all kinds of different reasons, with all kinds of different shapes. And so, what they have done, is to put them all into big libraries, and they've given us access to these libraries, to screen for all kinds of different therapeutics. So, they've put hundreds of thousands of molecules into these libraries, and we just want to try to find the one that, maybe, works for our system, okay?

28. Rational design of anti-QS drug candidates (35:51)

So now, to go through these a little bit. So, the first way, is this rational design. So, remember, autoinducer 2, so we're going after this species non-specific system, autoinducer 2 is that five-carbon molecule, that we're showing as a pentagon, for the cartoons. So, what we can do is, using chemistry, is we can simply, one by one by one, go through those atoms and change them. And so, we put three examples on the second side of the slide. And, what we've done now is highlight what's different between the analog and the original molecule. And so, in our cartoons, what we do is we take a pentagon, and we make it, kind of, pentagon-shaped, but a little bit different. So, remember, these are not flat, these are three-dimensional molecules, so we're just putting a bump, or a groove, into the original molecule, changing its shape just a little bit, okay? So, that's the rational design, you have to make more than three, we made hundreds of these, so they get kind of fancy, but if you actually look closely, they are all based on that original molecule. So, we can make these one by one, a few hundred of them.

29. Screening massive chemical libraries for drug candidates (36:56)

The second strategy that I told you about is this massive screening. So, you guys know that autoinducer 2 is this pink pentagon, right? So, what we can do is just get all these molecules that the chemists have donated, right? And, they look like all kinds of crazy structures. They have nothing to do with autoinducer 2. Chemists make molecules for tons of different reasons, they're all in this library, and what we want to do, is to find, out of these 300,000 molecules, the few that might be pentagon-shaped, just by chance, right? And so, the idea is that, maybe, in this massive library of differently shaped molecules, there's a molecule that, by chance, slots into the autoinducer 2 receptor and acts as a quorum sensing antagonist, okay?

30. Video: Screening chemical libraries with robotics (37:40)

So, we have hundreds of thousands of molecules, whether we made them ourselves, or we got them from this library. And so, the question is how do you do that? And so, what you do, is to one by one by one, array those molecules, 300,000 of them into plates like this. This has 300 wells, so you need 1,000 of these plates to separate those molecules one by one by one. And then, what we want to do is, one by one by one, we want to test, does any of those molecules block quorum sensing? Okay? So, first you have to get them arrayed, and so, I am not doing that, that is B-O-R-I-N-G, boring, right? And so, what we do is, we take advantage of the fact that we work with engineers and colleagues and the technology that's available today, robots do this for us. This is not, you know, smart work, right? We just have to get them arrayed precisely. And so, what we have are robots that will dispense in an ordered fashion, all the molecules into the different wells of that plate. They'll put the cells in, and they'll actually measure the read-out for you. So, we can do all of this, arraying the molecules, doing the entire screen of hundreds of thousands of molecules in a couple of days. And, that's because we have robotics to use, as opposed to human beings to one by one pipet these molecules. We could never do it, if we didn't have this kind of technology, okay?

31. Bioluminescence as an assay for QS block (39:04)

So now, we have these molecules in our hands, we have them arrayed, the question is, you know, we have now 300 and some thousand molecules, how do we find the very few that ever have a hope of being made into an antibiotic, right? Most of those molecules are duds for our purposes. So, how do we get what might be the winners? And so, I hope you can guess what I would do. What I would do, is use bioluminescence, right? So, remember, bioluminescence in *Vibrio harveyi* in this harmless bacteria, is a read-out of quorum sensing. It's a visible readout that we can see. And so, now I'm going to go through the experiment that we did, to get these molecules. So, what we did is, of course, we used *Vibrio harveyi*, and we made a mutant in *Vibrio harveyi*, where we deleted the genes that encode the first system. So now, this bacterium makes light, only if it sees autoinducer 2, right? So, everybody gets that? It's deaf for autoinducer 1, but it makes light, if it's provided, autoinducer 2. So now, what we can do is, one by one by one, take that mutant strain, and add these molecules. So, one by one by one, we can add a molecule and ask, "Do you make light? Do you make light? Do you make light?" Right? And so, most of the molecules, hundreds of thousands of them do nothing, right? They don't have anything to do with the shape of that molecule and its partner receptor.

32. Differentiating QS2 versus poison (40:31)

But, every now and again, when we look into these wells, there's going to be a well where no light is coming out, right? Maybe, because there's a molecule, in this case, it looks like a yellow goldfish, that is pentagon-shaped and slots into the receptor, and shuts down quorum sensing. So, does everybody understand that? So, some molecules slide into the receptor, they do what we want, those are few, they shut down light. But, any molecule in that vast library of cockamamie molecules that's toxic, that kills the bacteria, that's a poison, no light is going to come out of that well, because the bacteria are dead, okay? Right? They're dead, they don't make light. So, anyways, so what we need then, is a way to distinguish... all we have are these wells giving off light or not, right? So, I'm going to show you what it looks like

33. First screen finds potential QS2 blockers (41:19)

in the experiment. So, in the experiment, there's one of these plates, right? Each well has one of these different of the hundreds of thousands of molecules, right? We put our *Vibrio harveyi* mutant that doesn't have quorum sensing system 1, it only has system 2 in there, and we let the machine tell us how much light is coming out of those wells, and we're going to see a result like that. So maybe, ten of them, there's no light. But, what we can't tell is whether there's no light, because we did what we want, which

is to block autoinducer 2 reception, or we just killed the cells. Okay, so everybody gets that right? That's a problem with this screen.

34. Second step screens out toxins (41:54)

So, what we have to do, is to do a second screen that distinguishes between those two outcomes. And so, of course, we're going to use bioluminescence. And so, what we did was we made a mutant that doesn't have system 2. So, now it has the intra-species system 1 circuit, it turns on light, if you give it autoinducer 1, okay? So, what's going to happen with this is, you have a bacterium that's making light, exclusively in response to intra-species quorum sensing system 1 molecule. If we add the good molecule, the molecule we want that blocks quorum sensing system 2, nothing's going to happen, right? That molecule looks like this one, it's not going to fit in the receptor for that one. So, even when we add our autoinducer 2 antagonist, this strain will make light. If one of those molecules was a poison, that strain will be killed. The poison works irrespective of which quorum sensing system is there, right? So, now we can do this second screen, and take only the guys that still make light in the second screen, but not the first. And so, if we put the two experiments together, so here's our first screen, we had these dark wells, right? They're either dead, or there's our golden molecule. Then, what we do, is we put the quorum sensing mutant, that only responds to autoinducer 1, into each of those dark wells, and ask, "Light or not?" And, maybe, in one case, you'll get bioluminescence. So, everybody gets that? In all the cases where the wells are dark in both screens, those bacteria are dead, something's wrong with them. In that well, hopefully, what we have is a molecule that specifically shuts down quorum sensing system 2, but not quorum sensing system 1. That's our candidate for the drug. So, to summarize, we have a molecule now, we've gone from 300,000 molecules in a few days, to a molecule that appears to specifically shut down light in *Vibrio harveyi*, if it has system 2, but not if it has system 1.

35. Preclinical testing of new drug candidates (43:54)

Okay, so now we think we have our hands on it. The problem is, is that we're shutting down light in *Vibrio harveyi*, right? That's not a pathogen, so that doesn't mean we have the drug yet. But, what we have done is gone from hundreds of thousands of molecules to, maybe, ten, maybe a dozen. And, that is a number of molecules we can deal with in this, kind of, complicated test. So, now we take the few molecules that came out of our requirements in those screens, and we put them in animals. So, we take a pathogen that requires quorum sensing to kill that animal, right? And then, we add our candidate, our lead molecules, and we ask, "Can we get a molecule that saves the animal?" And, the answer is, yes we can. So, we have a few molecules, and again, remember, this has been done for all the different quorum sensing systems, that appear to save the animal from a quorum sensing mediated toxic infection. These are now lead molecules for drugs. They're not drugs yet. So, these are molecules that simply came out of these screens, but they're the leads, they have some effect. And so now what we do, is we team up with the chemists, with the medicinal chemists, and we iterate, we start with the molecule that's pretty potent, and then we start doing what we've done before, which is to change its shape to make it have more and more and more higher potency. And also, we build into it with synthetic chemistry, features that make the molecule not toxic to the humans. We make the molecule able to get where it needs to go, so medicinal chemists have all kinds of tricks, and features they like drug-like molecules to have that are good for the humans, but we can keep the features that are bad for quorum sensing. And so, that's the state we're at, these are the lead molecules. So, we're very far behind Toto, in our drive to get there, but at least we have our hands on a few molecules that have the potential, and we think there's actually merit to this strategy, now, because even with these, sort of, crummy molecules, it works.

36. Looking to nature for other ways to interfere with QS (45:48)

So, if we're so smart and we can do that, of course, Toto and I have been telling you that nature has already thought up everything we can think of. So, of course, bacteria have had billions of years to actually think up these strategies. So, now that we realize we can interfere with quorum sensing, we thought, maybe we should just go look and see if the bacteria already figured that out. Remember, bacteria have to compete for a living. And so, sure enough, what lots of colleagues of mine in the field have done, is to go out into nature and dig up dirt, or get sea water, and look for these natural anti-quorum sensing therapies. And, we have three kinds of things that happened, so far. So, bacteria that are in competition with another bacteria, actually secrete molecules that act as antagonists, just like what we're trying to do. So, those exist in nature. We also have bacteria that live in communities with each other, and they eat each other's autoinducer. So, one guy is trying to talk, and the guy next to him, is eating its autoinducer, and using it for carbon to grow. And so, the first guy can't talk, so it's made mute, right? So, that's a good strategy. The other strategy that's been found, is that bacteria secrete enzymes that will clip the chains like that, off these rings, and that makes neither of these molecules work. So, there's enzymes that can cut these molecules in half, and stop quorum sensing. And so, of course, these strategies that were made by the bacteria, not by the humans, have been tested over billions of years of evolution. And so, maybe, we should just go and find them, instead of trying to do them ourselves, because these are the potent strategies. And so, now the field is changing to this idea of just going out and looking for anti-quorum sensing therapeutics.

37. Multicellularity, bacteria, and human cells (47:27)

I'm done. I hope what you've learned today and yesterday, in my two lectures, is that bacteria talk to each other, they don't have words, they use chemicals to talk. And, what quorum sensing, or chemical communication allows bacteria to do, is to count neighbors, and recognize when they're alone, and when they're in communities, so they can do something different under those two situations. And, what I hope you believe in a further, sort of, intellectual leap, is this is the invention of multicellularity on this Earth. Bacteria have been here billions of years. Human beings, maybe 200,000. The way these circuits work, the way the information gets transduced, how these circuits work in a noisy environment, those features are built-in to the cells of your body. And so, we really believe that by studying these simple bacterial systems, we're going to be able to help scientists that study higher organisms, and diseases of organisms that aren't bacteria, just by learning about quorum sensing. What I would also say is there's two molecules, and so that means, bacteria can tell self from other. And, of course, that's what happens in your body. It's not like your kidney cells and your heart cells get all mixed up, everyday. And, that's because they have different jobs, they use different chemistry to tell them what they're supposed to be doing, each of the groups of cells. We think bacteria invented that as well. This allows bacteria to be multi-cellular. It allows them to take on complicated tasks, and to behave in many, many respects, like the cells of eukaryotic organisms. So, we think that we can find out how cells work together by studying quorum sensing, and shed light onto processes in the human body. And, then, of course, there's this practical application, which is, we could try to find anti-quorum sensing strategies. So, I talked about antibiotics, but there's an industrial need. People want to make paints that have anti-quorum sensing molecules in there, so like, cooling towers don't get all gunked up. They want to put them in tubes of catheters, and dental things, in these heart stents, salves, lotions, there's all kinds of places, both for biomedical and industrial uses of anti-quorum sensing strategies. But, then to finish, of course, with a plug for all the good bacteria, remember most bacteria on this Earth, either don't matter to us, or they're good for us. And so, maybe the real strategy we should be doing is to be trying to make pro-quorum sensing molecules. So, if we could give molecules to your commensal bacteria, and make their conversation better at the expense of these invaders, maybe that's the next strategy for treating infections, is to actually work with the bacteria that live in us, or on us. But, of course, as I told you yesterday, we don't know so much about them. We have to learn about them, in order to be able to make these pro-quorum sensing strategies realistic. And also, of course, you guys probably know that in industry we use bacteria to make all kinds of products that human beings use. And so, if we could make

them better at quorum sensing, maybe we could make them better machines at making products that are useful for human health.

38. Inspired by nature and biodiversity (50:33)

And then, finally, this is because I get the last word, between Toto and me, I hope that, what we were trying to say, in a very general way, is that Toto got into his job, because he liked these beautiful patterns on these shells. I like things that glow in the dark, right? We were just looking at what nature had put out there, these crazy, seemingly, non-medical objects, you know natural things. Just because we were curious about why and how they did the things that they do. But, nature is filled with these, right? These aren't just one-offs, right, that Toto and I have landed on. And so, what we hope is that you'll go out there, right, and find a system you like. We think nature has zillions of these, and that all kinds of mysteries and secrets, and they're just waiting to be discovered by people like you, simply by following your nose, and asking, and being curious about the natural world. And, how it does all the magical, and wondrous things that it does. And so, I hope that you guys have some appreciation for the biodiversity on this Earth, and that you go find whichever critter on that slide, or not on that slide, that you like, right? And, study it, because if there's some secret there that nature's given, then chances are, it's going to be useful for many different things. And so, that's where we are. you know, while we're here, and we are having a ball. And, we're so glad that you guys want to come and hear us, and we're really lucky to get to give this, so we thank Howard Hughes, and we thank you guys for actually wanting to hear this science. Yes?

39. Q&A: How do bacteria attack other specie's QS molecules? (52:14)

[STUDENT:] You said that, find that a single bacteria may be in competition with this variety of different species of bacteria, and may secrete enzymes that may try to degrade autoinducers, so is there a single set of enzymes that targets one single area to clip the autoinducer?

[DR. BASSLER:] Yeah. What's really cool, what's really cool... See, they have to have immunity. So, these enzymes are quorum sensing controlled, right, because you don't want to put them out there if there's not a lot of you. And then, we have found, this is not my work – colleagues – there's one set of enzymes that cuts the ring open, this molecule is dead. There's another set that different bacteria use to cut the chain off the ring, and they all have to have immunity. You can't let your own quorum sensing molecule get clipped, and they built that into these systems. It's very beautiful. Yes?

40. Q&A: What are some of the side effects of antibiotics? (53:04)

[STUDENT:] What are the side effects of using antibiotic and why?

[DR. BASSLER:] The side effects of traditional antibiotics? And so, a global side effect is that we have multidrug-resistant bacteria, because antibiotics, for the past 70 years, have been used on industrial scale. So, that is the biggest problem and threat facing humans. The individual side effects, when one takes an antibiotic, is that if you have an antibiotic that just, you know, pops bacterial membranes, makes it so bacteria can't replicate their DNA, it doesn't just seek out the invader, it actually works against many of the bacteria that you have in your gut. And so, typically, people who are taking a course of antibiotics will get a stomach ache, because you've messed up your personal microflora. Women get yeast infections, because their microflora is skewed. And then, what is amazing about the human microbiome, your bacteria, is that once you... so you'll have those effects while you take the drug. But, once you finish the course of antibiotics, we have what are called persisters, so we have bacteria, our commensal bacteria, that aren't affected by the drug, and once you stop taking the antibiotic, your own microflora, you know, the invader is gone, and your microflora repopulates. So, within a couple of

weeks, you're back to where And, that's why we can actually use antibiotics. I have to say it's amazing they work, given that we started using antibiotics before we ever knew we had all this bacteria in us or on us. Yes, up in the back.

41. Q&A: How many bacteria constitute a quorum (55:35)

[STUDENT:] I was just wondering if there were levels of quorum sensing? I mean, if the bacteria knew if there were five bacteria near it, or like, ten?

[DR. BASSLER:] Right, so yes. So, the question is what's the quorum? So, each species, this is again what evolution has that a particular species needs to do, right? So, species A's tasks are different than B's and C, they count to different numbers. So, each system has evolved to let that species count to the right number, right? And then, they launch the task, and then within the species, absolutely, they count, it's a threshold, there's no quorum sensing, and then it turns on. And then, remember the steps on the inside take time, and so, genes still turn on in a precise order, right, from 1 to 100. But, it's not, but it's a threshold, right? There's nothing, nothing, nothing, then they flip their bit, and they launch the cascade. So, it's not, sort of, like I'm going to do this when there's ten of me, this when there's 20, you know, they wait until they have the number, and then all these things start ticking off. I'm going to ask you, yes?

42. Q&A: Can we remove rather than block the QS signal? (55:22)

[STUDENT:] I just want to ask if, can we like, find a method to actually reduce the number of the signals, instead of, like, just for the bacteria?

[DR. BASSLER:] So, like, if we could just get rid of the signals?

[STUDENT:] Yeah.

[DR. BASSLER:] Yeah. These are all strategies, like if we could think of, like a molecule that had a really high affinity for the signal and you could add that, and the signal would disappear, that would work, too. You don't have to make an antagonist, you just, if you could make something that inactivated the signal that would also work. Those are strategies people are trying to do. I have to stop. Thank you.

43. Closing remarks by HHMI President Dr. Robert Tjian (56:28)

[DR. TJIAN:] Bonnie, do you think you could be more enthusiastic next time? That was fantastic. This is an incredible close to our series this year. And, Bonnie, I'm sure you've heard this before, this is an amazingly brilliant talk, with flashes of insight. And, thanks again for Toto Olivera, for his wonderful talks. I want to thank everyone who was involved in the production of these engaging and entertaining Holiday Lectures. And, of course, next year, we're going to have an equally interesting and exciting series, that we're going to be featuring HHMI Investigator Joe DeRisi from the UCSF, of San Francisco, and Eva Harris of UC Berkley. And their theme will be, teaming fieldwork with technology to catch emerging viral diseases. And now, from all of us at HHMI, thank you, goodbye, and have a great holiday.