The Meaning of Sex: Genes and Gender
Lecture Two—Hermaphrodites: The Safer Sex
Barbara J. Meyer, Ph.D.

1. Start of Lecture Two (00:15)

From the Howard Hughes Medical Institute... The 2001 Holiday Lectures on Science. This year's lectures, "The Meaning of Sex, Genes, and Gender"... will be given by Dr. Barbara Meyer, Howard Hughes Medical Institute investigator at the University of California, Berkeley. And Dr. David Page, Howard Hughes Medical Institute investigator at the Massachusetts Institute of Technology. The second lecture is titled "Hermaphrodites: The Safer Sex." And now, to introduce our program, the Vice President for Grants and Special Programs of the Howard Hughes Medical Institute -- Dr. Peter Bruns.

2. Introduction by HHMI Vice President Dr. Peter Bruns (01:05)

Welcome back to the 2001 Holiday Lectures the fruits and vegetables of scientific research, I guess I should say. My job here is just great. As Tom mentioned, we have an incredibly diverse program that we support of science education. We cover programs in museums and nature centers, medical schools, colleges, universities, and cutting-edge research around the world in select laboratories. The lectures we're listening to today are part of a larger program that we call BioInteractive, and I'm going to make a little commercial plug here of our web site. We have a separate web site for that, called BioInteractive, and there it is with the URL, and please take a note of that. I invite you all to -- when you get home -- go to that web site. You'll find on it, in addition to videos of previous Holiday Lectures -- and, by the way, this Holiday Lecture will be there -- but there are other things there, as well -- virtual labs, mini-lessons on cutting-edge things in biology. It's worth your while. Please go there. That was a commercial message. We've tried to design that web site really to reveal the creative side of research and biology. Our next speaker, Barbara Meyer, really epitomizes exactly that. Her career has been clearly marked by creative energy in both the invention of experiments and even the way she designs her laboratory. Barbara will speak for about 40 minutes, again with breaks for questions, and then we will continue tomorrow with more. The title of her lecture is "Hermaphrodites: The Safer Sex." And so here is another short video to introduce Barbara, and then she'll give her lecture.

3. Introductory interview with Dr. Barbara Meyer (03:04)

I always loved math. I always loved to solve problems because it was very comforting to me to be able to put my mind on solving a puzzle. I thought that in fact I'd become a mathematician, but then I went to high school and discovered biology, which I found actually fascinating when I looked in the microscope and found these microscopic organisms crawling around. And in particular, I became very fascinated with understanding how genes worked, and very fascinated about mechanisms of biology. So suddenly it was possible to take the analytical thinking that I appreciated so much from mathematics and put it to play in biology, where the organisms were beautiful and the processes were very exciting to me. I realized that going into the lab and asking a question that had no answer yet was such an addictive process because it was a process of discovery -- design experiments and then get answers that no one else in the world knew about. And that rush, that addiction, was so exciting to me that from that day forward, I wanted to go into research. So when I was choosing a postdoc, I wanted to work on an organism that had genetics and an organism that I felt that I could handle. And when I learned that this little roundworm, C. elegans, lived on a petri dish and that I could actually freeze the culture and thaw it again and it would live, I realized this was the organism for me. And I wanted to figure out the genetic machinery behind what allowed that worm to make the choice to be male or hermaphrodite. This problem intrigued me because I could not imagine how the worm could count the number of X

chromosomes. The directions that my research took as a consequence of that were totally unexpected, and I'm very happy that it's been such an involved process. And so studying this small, little model organism has given us insight into much larger organisms, including ourselves. The commonality of genetic pathways between a small worm and a human have become so clear that it's now obvious to everybody that research on small, basic organisms is extremely important for understanding human disease. I hope that our Holiday Lectures make students extremely excited about being scientists, about thinking about science. I hope they find that just the process of being able to ask a question and answer it will be so intriguing to them that their textbooks will come alive and that they will feel that they, too, can master a discipline that they didn't think they could, because they'll find that science is a beautiful process, that the answers are very exciting, the imagery is beautiful, and that it gives them power to think about other questions in their life and their environment.

4. Why do we study model organisms? (05:50)

I'd like to welcome you all again to my lecture here. I first would like to ask you a question. How many of you have thought about the idea of studying model organisms, or even looked at model organisms before you walked in here? How many of you have seen fruit flies or nematodes or -- Raise your hands. Shout out. Let me know. What were they? Has anyone ever seen a worm before today? Yes. In your -- did you see them live? Did you see them in textbooks? Earthworms. Earthworms! So you're surprised at the size of these little worms. Our house audience has received petri dishes, which we're going to discuss in a moment, but before I discuss with you aspects of the nematode, I'd like to give you a feel for why we study model organisms. You might all be wondering why we study model organisms instead of larger organisms -- for example, humans. And the answer to that has to do with what's on your first slide. The answer is that there's unity of life. All the organisms you can see out there have common traits -- common body plans, common behaviors, common strategies for life -- and many of the genes that these organisms have are in common, and it's much easier to study those genes in an organism that you can reproduce in the lab, that's tiny, and that you can study at your ease. And that's why people in the world around have chosen to study model organisms, such as our little worm, the nematode. This little nematode shares features with these other organisms up there -- yeast, fruit flies, mice. The important thing is that there are many, many genes in common. There's whole pathways of genes in common, and because of that, we can even take a gene from a human, introduce it back into a model organism, and have it function in that model organism. That means we can understand in detail the mechanisms for organisms that will help us understand man. If we pursue that more carefully, what you can find is the proteins are very similar, and because the proteins are very similar, those proteins can be used from different organisms to understand what happens in humans.

5. Introduction to the nematode *Caenorhabditis elegans* (08:08)

In particular, I'd like to show you some details about the worm. The worm is a very small multicellular organism. It’s only one millimeter in size. You can tell from the slide that the lifespan is 2-3 weeks, but actually what that means to us in the laboratory is that every three days, we can see a new generation. We can perform genetics, do crosses, find answers in only three days. Because this organism has been so popular to study, this organism was one of the first higher organisms to have its entire genome sequenced. And when its genome was sequenced, the New York Times was especially proud to take our tiny little worm...and display it all over the front section of the Science Times. And it touted the fact that the dainty little worm tells the secret of the human genetic code. I must say that this dainty little worm had its history in one man, Sydney Brenner, who decided to study this worm, and he was such a charismatic person that we've all followed in his footsteps to try and understand them. Now I'd like to go to the little plates you're all holding. In these little plates, this is going to allow me to help you understand what a hermaphrodite means. Could some of you tell me what you think a hermaphrodite is? Could I have a volunteer to tell me, what is a hermaphrodite? Yes? It has tissue of both male and female

reproductive organs. And what do you think that means? In humans, a hermaphrodite is not fertile. What do you think it means for other organisms? Yes? They can produce both sperm and egg. They can produce their own sperm and eggs. That's correct. These hermaphrodite worms can produce their own sperm and eggs. And we've given you two little plates here -- one that's marked with blue tape and one that's marked with yellow tape. The one with blue tape had one single worm placed on it several days ago, and if you look with your magnifying glass, you'll be able to see little critters crawling around. The other plate, which you don't have to look at now, has only one worm placed on it. What we want you to do is take that home, and we want you to study it over the next few days with your little magnifying glass, and notice how quickly this population can reproduce. This little plate can hold several thousand worms. There's a whole population down here.

6. Anatomy of *C. elegans*: every cell is known (10:39)

For you to understand better what a hermaphrodite is, I'd like to show you this giant worm that we've made for you. This worm -- five feet long, compared to its normal size of only a little millimeter -- lets me tell you all the features that I would like to expose you to. First, you can see the worm in gray up above, and then you can see the color-coded worm here. The first thing I'd like you to understand is that to be a hermaphrodite, this little worm is capable of first producing sperm. It takes its sperm and it stores them up in this little yellow tissue called the spermatheca. Those sperm are just waiting for those oocytes -- that are in light blue -- to come running through, be fertilized, and become embryos. That's a huge portion of what the hermaphrodite does. She's a reproductive organ. The other features of the worm are shown and color-coded. There is a red intestine, there's an orange pharynx, and the nervous system is surrounding the rest of it. The gonad is shown in green. This worm is a very intriguing organism to study because when you put this little worm in a microscope, you can actually see every single cell. This worm has 959 cells. We know every single cell, we know the daughter of every single cell, we know every tissue every single cell makes, and in fact we can manipulate the worm. So, for example, we can take a laser microbeam, shine it through our microscope objective, find a cell that is destined to become the gonad -- the precursor cell -- we can shine that laser microbeam, kill that one single cell, and the entire tissue will disappear. The worm will have every tissue normally, but it will be missing its gonad. We can do that with every single tissue. And the importance of that is we're able to study gene interactions, and we're able to study interactions among cells that let us understand more completely how this little organism works.

7. *C. elegans* has two sexes: male and hermaphrodite (12:39)

Of course, as I said, you know that nematodes are usually hermaphrodite, but that's not truth in practice. In fact, the little nematode has two sexes -- a self-fertile hermaphrodite and a male -- and those are shown up in the slide. And the importance about having males and hermaphrodites, as you've understood from Dr. Page, is that we need to be able to swap out material -- genetic material. And, in fact, the little hermaphrodite is good at reproducing, but she's not so good at cleaning up her genetic material, so a male is needed. So I've shown for you the structure of the male and hermaphrodite, and you notice that the hermaphrodite gonad is large, it's bilobed -- it's really a tube of reproduction. If you look closely at the male gonad, it's long. It goes out to the tail. The sperm are right there. And what you notice is that the mail is a lot smaller than the hermaphrodite. That's because the male only has to provide sperm, whereas, of course, the hermaphrodite has to provide eggs, as well. So let's look in more detail at what the male looks like compared to the hermaphrodite. Up there are the tails of the hermaphrodite and the tail of the male, and what I want you to notice is that the hermaphrodite has this sort of boring whip tail, but the male's tail is very intriguing. In fact, it's his mating organ. This is what the male uses to inseminate the hermaphrodite. What you'll notice are the fan -- this bright, large fan -- and the rays. The rays are sensory structures that enable the worm to find the hermaphrodite, to run his body along the hermaphrodite, and find her vulva. The spicules, which I'll show to you in a little while,

are these large claws that allow the male to hang onto the hermaphrodite while he's mating.

8. Video: *C. elegans* mating (14:30)

I'm going to show you a video of males and hermaphrodites mating. Here we have the male rubbing up and down the hermaphrodite body. You can see that he's searching all over for that vulva. He oftentimes isn't so smart in how he does that. He looks around for a long time. What you'll be amazed to find, if you watch worms for a long period of time, is this is what they always do, and in fact this is genetically programmed. Every single male does the same thing. And this genetic program can be studied in great detail. Now, if you look right now, that male is successfully finding the vulva, and he's beginning to inseminate the hermaphrodite with his sperm. What you just saw is that process, and what I want to focus your attention on are these large little structures here -- the spicules -- that are inserted into the hermaphrodite vulva and allow him to hang onto her while he injects his sperm.

9. *C. elegans* sperm (15:26)

Now, you've heard about sperm from David Page, and perhaps you've even seen sperm in the live demonstrations we've shown you today. I'd like to show you what worm sperm look like. Worm sperm are rather different. They are ameboid. That means they're big. They're not just simply little packages of DNA that have this jet propulsion system. In fact, they have their own proteins that they can introduce into the hermaphrodite, and one of those proteins actually induces ovulation and meiosis, so these are not just passive moving partners. These play an important role, and I'd like to show you a video of how these sperm crawl.

10. Video: Amoeboid sperm of C. elegans (16:07)

You can focus your attention on this light that's showing the little sperm with its ameboid motion crawling down.

11. A behavior gene in *C. elegans* is related to a human gene important in kidney function (16:17)

So one of the reasons I wanted to show you this mating behavior was not just because it's fun to watch, but rather, one can actually describe the process of mating in genetic detail. One can make mutants in the process. In fact, Paul Sternberg's lab -- a Howard Hughes investigator -- looked very carefully at the process of male mating, and what he was able to do was find mutations that caused the male not to be able to find the vulva at the same rate as other males might. They cloned the gene and discovered that that gene was very similar to a gene required in humans to make kidneys. This is an important point I want to make -- that the building blocks used in different organisms are frequently the same, but those building blocks might be used in different ways. In this case, the gene involved in making a kidney -- it causes polycystic kidney disease, large cysts of the kidney -- have in fact been used in the worm for the neurobiology of male mating. This is just one of the many examples that illustrates that point.

12. Life cycle of a hermaphrodite (17:22)

So now I've told you that males and hermaphrodites can mate, and I've also told you that hermaphrodites can self-fertilize, and you might be wondering when they do which and what it means to do both. So what I'd like to show you is the life cycle of the hermaphrodite. The hermaphrodite, of course, can produce both sperm and eggs. She can self-fertilize, and she can reproduce herself. She does go through meiosis -- I'll tell you about that in a little while -- so she's not just a clone of herself. She's not a lizard. She's a hermaphrodite worm. That self-fertility allows her to produce mostly herself, but occasionally

there's an error in meiosis, and she can produce some males. One in 500 of her progeny are males. Once there's a male in the population, the male produces sperm, the hermaphrodite produces an egg, they cross-fertilize, and then they produce themselves -- both males and hermaphrodites -- in a 50-50 ratio. So that allows the population to have a large number of males at will.

13. Fertilization mechanism in *C. elegans* (18:21)

So let's go back now to the process of fertilization. The hermaphrodite can receive sperm either from herself or from a male, and once she's received those sperm, she actually stores them in a structure called the spermatheca, which is shown up here. This spermatheca has either her sperm or her mated sperm. The oocytes, as they mature, pass through that spermatheca. They become fertilized, and they become embryos.

14. Early embryonic development of *C. elegans* (18:53)

What I'm going to show you is what happens in the first cell division. Using nematodes -- one of the great advantages of studying nematodes is that one can actually look at live organisms and look at them from the first cell division onwards. And what I'm showing for you up here is a picture of a one-celled embryo. That one-celled embryo has green nuclei. The first one I'm showing you is a sperm nucleus. The reason those nuclei are green is that we've taken a protein that binds to the chromosomes -- a histone protein -- we've tagged it with a green fluorescent protein from the jellyfish. That jellyfish protein fluoresces bright green -- that's why jellyfish are bright green in the ocean -- and we can follow chromosomes. The sperm, when they enter the oocyte, produce the polarity of the animal. When the sperm enters, the side it enters is going to become the tail. The side that it doesn't enter is going to become the head. The oocyte nucleus is right next to it, and what you see -- these green spots on the outside, called polar bodies -- those are the products of meiosis that aren't used in making gametes when an oocyte forms. An oocyte, once she develops -- it develops -- only makes one gamete, not four, unlike sperm.

15. Video: From fertilization to egg laying in *C. elegans* (20:09)

What I'm going to show you now is a video. First you're going to see ovulation. You're going to see the oocyte here passing through the spermatheca and becoming fertilized to become the embryo. Soon you're going to see a video in which the oocyte nucleus and the sperm nucleus come together. You can see the chromosomes condensing as the nuclei come together. You can see that as the chromosomes condense, the chromosomes come together, align together -- the chromosomes from the hermaphrodite mother and the chromosomes from the male partition. They then resegregate, and you can see cell division. So once you see cell division, this embryo continues to be growing inside the hermaphrodite. It grows for quite a while, and then what happens is that hermaphrodite lays an egg, and you're going to see the next video of an egg-laying hermaphrodite. Next video, please. Watch quickly. Watch where that arrow is, because that embryo is very small. It's 1/20 the size of the animal. That happened very quickly, so we're going to run it back in slow motion so you can see the egg coming back through the embryo -- through the hermaphrodite. There you go.

16. Using mutations to study sex determination in *C. elegans* (21:22)

So now we have an egg being born. Here we have a new life. The question is, what is its sex? David Page has just told you that nematodes count the number of X chromosomes to determine sex. What happens is, if a sperm has an X chromosome and an oocyte has an X chromosome, the animal is XX, and it becomes a hermaphrodite. If a sperm has no X chromosome and the oocyte has an X chromosome, that's XO, and that's going to become a male. So how do we study the process of sex

determination in the worm? We study it by being able to make mutations. This is a great advantage of studying model organisms. We do mutagenesis. We put a mutagenized worm on a plate like this. We then look in her progeny for mutants. And what we find is that if we have mutations that cause an animal to change its sex -- for example, we can have mutations that cause an XX animal to develop as a male, be sex transformed -- we can have mutations that make an XO animal be transformed into a hermaphrodite. That's sex transformed. And by finding a whole series of mutations like this, we can find the genes involved in sex determination.

17. Activation of sex-determining gene (22:39)

And we know there are many genes required for male development and many genes required for hermaphrodite development, and the important thing is, both sets of genes are in all organisms. What's important is what genes are turned on and what genes are turned off. When there's an XX animal, the genes required for hermaphrodite development are turned on -- they're shown in white there -- and hermaphrodite development ensues. When the genes required for male development are turned on, male development ensues. And this demonstration here is going to be an example of how this pathway works, but I want you to take a quick notice that in the case of the XX animal, one set of genes are on. In the case of the XO animal, the complementary set of genes are on. It's never both the same set of genes. And what we're going to study in the next time is a demonstration about how this pathway is set up. But before we go to this demonstration, I'd like to take questions from the house. Yes?

18. Student question: Why can’t human hermaphrodites self-fertilize? (23:34)

The question is, why can't human hermaphrodites self-fertilize if they consist of both tissues?

Sorry. "Why can't self..."

A human hermaphrodite self-fertilize itself?

This is a question for David Page, but I'll pretend I'm David Page. Hermaphroditic humans rarely have their reproductive organs correctly formed, so a hermaphroditic human has parts of males and parts of females, but they are not fertile. They don't make the appropriate sperm and oocytes. They are not capable of making them, so they're not fertile at all. Is there another question from the house?

19. Student question: Why aren’t there any female worms? (24:18)

Why aren't any of the worms only female?

Great question. In fact, the truth is that hermaphrodites can be females. A hermaphrodite is simply a female that can make sperm transiently. There are mutations -- some in that pathway up there -- that selectively prevent the hermaphrodite from making sperm, so she becomes a female. So there are females, there are hermaphrodites, and there are males. Another house question? Yes?

If asexual reproduction isn't as beneficial to an organism as sexual reproduction, why did this worm evolve to do asexual reproduction?

See, this little worm has it both ways. This little worm can go out in desolation, have no males for months at a time, reproduce...but again, she produces a male one in every 500 progeny she produces, so she herself can produce a male. That male can scout out a hermaphrodite that doesn't -- isn't a lineal descendant of his, and he can fertilize her. So, in fact, it works both ways. Yes?

Hermaphrodites are shes?

Hermaphrodites are shes.

OK.

20. Student question: Do hermaphrodites use male sperm preferentially? (25:36)

Yes?

Do hermaphrodites preferentially mate with males if males are present, or will they asexually reproduce regardless?

Yes. The question is, do hermaphrodites...Whose sperm do the hermaphrodites use? Will they use their own, or will they use the male's? The truth is, they'll use a male's. And this has taken a lot of years to figure this out, but the reason is, even though the male's sperm look like the hermaphrodite's sperm, the male's sperm is bigger, and the male's sperm can displace the hermaphrodite's sperm from the spermatheca, so that always wins out. These are great questions.

21. Student question: Do male sperm permanently displace hermaphrodite sperm? (26:11)

In the case where a male has mated with one of the hermaphrodites, does the male's sperm permanently displace the hermaphrodite's sperm?

The male's sperm lasts for a very long time, so she produces cross-progeny for a long time. Once the sperm supply has been depleted, she can then return to using her own sperm and self-fertilize, so she can produce both cross-progeny and self-progeny. Yes?

You mentioned that when a hermaphrodite produces a male worm that he will go out in search of a hermaphrodite that was of a different worm. Can they mate with their brothers and sisters?

They can mate with anybody they choose to mate with. Are there any more questions from the house? Yes?

How often do they mate?

How often do they mate? Continuously. And what you saw on the screen is pretty realistic. Yes?

22. Student question: Did hermaphrodites evolve from females? (27: 14)

Did the worms evolve into hermaphrodites? Like, was there at first just females and males, and they developed hermaphroditic traits?

That's a great question. We believe that the species were first male and female, and that the hermaphrodite condition is a more recent adaptation.

23. Student question: Can hermaphrodites mate with each other? (27:35)

Could you tell us, can there be a cross between hermaphrodite and hermaphrodite? So if we cross and mate one hermaphrodite with another hermaphrodite, what will happen? What will be the consequences of this crossing?

Our worm hermaphrodites can only fertilize themselves. They don't have the genitalia and reproductive structures that are external. They're only internal, so they can only reproduce with themselves and not with a neighboring hermaphrodite, so it's really much more a self-oriented process.

24. Student question: At what stage can you determine the sex of the worm? (28:14)

I'd like to take another question from the house. Yes?

If they produce offspring, can you tell right then what they are?

Can you tell whether it's a boy or a girl immediately? Sex is determined six hours after fertilization, and six hours after fertilization, you can look with a microscope at certain cell types, and you can sex the animal, so it takes only six hours to get sex determination going.

25. The basis for sex determination in C. elegans (28:44)

OK. I'd like to continue on with my lecture, and this time, I'd like to discuss with you the basis for sex determination and how worms count chromosomes. I have to remind you that worms have no Y chromosomes, so any of the deleterious effects you're going to hear from David Page about will not apply to the worm. OK. How to the worms count chromosomes? I have to remind you that the worms -- hermaphrodites -- have two X chromosomes, and the male has one, but hermaphrodites and males share in common all non-X chromosomes. Those are called the autosomes. One set comes from the sperm, and one set comes from the oocyte. The animal is diploid, and on your slides and all succeeding slides, there's going to be a light purple copy and a dark purple copy of those autosomes. This organism has two copies. It's diploid. How sex is determined is by the ratio of the number of X chromosomes to the number of sets of autosomes -- the ploidy. And in particular what happens is when one X chromosome is present in a diploid organism so that it's one over two -- a ratio of 0.5 -- that allows a male to develop. When instead there's two X chromosomes and two sets of autosomes, that's a ratio of two to two, or 1.0, and a hermaphrodite development ensues. It's not just the absent number of X chromosomes, however. It really is the ratio, and the evidence on the screen right now let's me tell you why. It's possible to make worms that are tetraploid -- four sets of autosomes. In that case, an animal with two X chromosomes is a male, whereas a tetraploid animal with four X chromosomes is a hermaphrodite. It's always the ratio that's important.

26. Activity of xol-1 sex-determining gene is dependent on the ratio of X chromosomes to autosomes (30:40)

Now, you might wonder, how is that ratio assessed? How are those chromosomes counted? And the answer is the gene called xol-1. Xol-1 is the first gene in the pathway. When the ratio is half, xol-1 is turned on, and that allows male development to ensue. When the ratio is one, xol-1 is turned off, and hermaphrodite development ensues. Remember, both males and hermaphrodites have xol-1. It's only a question of whether it's on or it's off.

27. The balance of X signal elements (XSEs) and autosomal proteins regulates xol-1 activity (31:12)

Now, you might wonder how the signal actually works. How does counting work? And we've drawn for you a little cartoon that lets me illustrate this idea for you. What you can see is that the X chromosomes produce proteins. They're these little musclemen right here. These proteins are called the X-signal elements. These proteins turn xol-1 off. Opposing those proteins are the autosomal proteins. Those

proteins turn xol-1 on. There's a competition. Which proteins win -- the wimps or the musclemen? When there's one X chromosome, what do you think? When there's one X chromosome, who wins -- the wimps or the musclemen? The wimps win. That's right. Now, when there's two X chromosomes and 10 musclemen and 10 wimps, who wins?

The musclemen.

Yes. Xol-1 is turned off, and you get a hermaphrodite.

28. Demonstration: Balance of molecules in XO (32:13)

We're going to show you now a demonstration. I need three volunteers. They've been actually selected? Come on up. This is a very sophisticated demonstration that requires some work. In this demonstration, the scale represents xol-1. One pan is going to have the X-signal elements, and one pan is going to have the autosomal elements. These lines here represent the genes that I described before, here and here, and what we're going to show you is what happens when we unload the chromosomal content first of a perm that has no X chromosomes. So the sperm with no X chromosomes is going to be loaded, and we're going to load it into the autosomal holder. Would you place those in the holder, please? And Leslie is going to produce -- put in the oocyte contribution, and the oocyte contribution, which you can load in later while I'm describing these things, is going to have two parts to it -- the autosomal part...Now, what you're going to see is that we're going to have autosomal components and X components, and we're going to demonstrate for you whether the musclemen win or the wimps win. So not only we have the autosomal components -- Could you just place that on there for me, please? And could you place the oocyte -- oops. You want to place the oocyte contribution here? So...What do you think will happen? Let me just help this balance a little bit. What do you think will happen? This is a scale, and this is xol-1. Now I'm going to release the scale. This is the active fertilization. What happens? It's a boy. Now let's reset the scales.

29. Demonstration: Balance of molecules in XX (34:26)

This time, we're going to have a sperm that has an X chromosome and an oocyte that has an X chromosome. We don't need to reorder the autosomes, because that stays the same. The only thing that differs is the X chromosomes, and I'll have you put the X chromosomes on. So now we're going to find out what happens when there's twice as many X-signal elements. Our scale is set, and what do you think's going to happen? Here we go. It's a...hermaphrodite. OK. Now we're going to have to reset everything.

30. Demonstration: Balance of molecules in XX with lower levels of XSEs (35:17)

The question we're going to ask next is, what we've just seen is that when we have two X chromosomes, hermaphrodite development ensues, and when we have one X chromosome, male development ensues, and that's because the number of X-signal elements differs by a factor of two. Now we're going to do an experiment. We're going to ask what happens when we start to remove X-signal elements. Which will win? And first what we're going to do is remove an X-signal element. You want to remove an X-signal element? Let's set our scale. So what do you think's going to happen? If we get rid of one X-signal element out of 10, do you think the wimps will win, or do you think the autosomes will win? Well, let's take a vote. Wimps? Musclemen? Musclemen. OK. OK. We have the scales ready. We're ready? This is a very elaborate system here. The whole point of this demonstration is to illustrate to you what happens when you selectively remove X-signal elements, because the question is, what's the boundary between male and hermaphrodite? When is a hermaphrodite able to develop, and when is a male able to develop?

We're going to fertilize -- fertilization -- and see what happens. It's going to be a hermaphrodite. OK. Now

31. Demonstration: Balance of molecules in XX with much lower levels of XSEs (36:53)

…now what we're going to do is remove selectively more X-signal elements. Do you want to reset this for me? And when we remove more X-signal elements, this time we're going to remove two more. The first time, we removed one. The next time, we're going to remove two more, and the question is, who wins? The answer is -- the moment of truth. Who wins? Yes. It's a boy. The male wins. Well, this time, we've shown that if you remove more signal elements, the male wins. I'd like to have you give a hand to our participants. [applause] I'd like to give you all Berkeley T-shirts. Thank you very much. OK. Now, if you've really been understanding the genetics lesson, then the question is, if we take those same X-signal elements and just those X-signal elements, and we introduce them into a male, what's going to happen? We give the male five extra signal elements -- X-signal elements. Will that XO animal, now with 10 X-signal elements instead of five, be a male or a hermaphrodite?

Hermaphrodite.

Yes. You got it right. Excellent. OK.

32. Review of the central dogma of genetics (38:17)

Now I'd like to begin to give you a flavor for how the molecular mechanisms work for X chromosome counting. What I need to do first is give you a review of the genetic code -- the genetic dogma, the central dogma. And you probably have all remembered and learned from school that a gene is first transcribed to make an RNA -- a complementary copy -- and that RNA has many components to it. It has parts of the gene that are going to code, and those are called exons. It has parts of the gene that will code for nothing, called introns, and those introns must be removed through the process called splicing. Splicing occurs, the RNA gets exported to the cytoplasm, and once in the cytoplasm, it produces protein. A gene can be regulated at any one of those steps. It can be regulated at the level of transcription. It can be regulated at the level of splicing. It can even be regulated at the level of translation. And our X-signal elements could, in principle, repress xol-1 by any of those mechanisms.

33. An X signal element (XSE) represses transcription of xol-1 (39:26)

And they review for you transcriptional repression. Normally, a molecule called RNA polymerase runs down the DNA and makes a complementary copy in the RNA -- the pre-mRNA transcript. That's what gets spliced. A repressor molecule is able to block that polymerase from either binding or from moving down the DNA to allow transcription. Repressors can come in varying strengths. Some repressors can completely prevent transcription. Some repressors are wimps in and of themselves and only partially prevent transcription. And what I'm going to tell you is that the X-signal elements of the worm are wimpy repressors -- at least, some of them. These wimpy repressors, when they're present in the two copies and they're present in the single dose in the male, still allows transcription of xol-1 to proceed. However, when the double dose of those transcriptional repressors is present...then transcription is greatly slowed down. We have a stop sign here saying that the transcript is slowed down. It's not a permanent stop. It's just that very low transcript levels are made. That's what happens in an XX situation, but that's not the whole story. In fact, having to tell the difference between one X chromosome and two X chromosomes is so difficult that it can't rely on just one mechanism.

34. An XSE also acts to prevent proper splicing of xol-1 mRNA transcript (41:00)

Instead of just one transcriptional mechanism of repression, there is another mechanism that's added on top of that transcriptional repression, and that mechanism is one of splicing. One of those muscly X-signal elements is, in fact, a factor that prevents proper RNA splicing. When there's only one copy of that splicing factor, then the functional transcript is allowed to be made. Xol-1 has one intron that is controlled by this X-signal element, and this one intron is very sensitive to the dose of that X-signal element, so that when only one copy is present from the male, then splicing can occur, and as a consequence, a normal, full-length xol-1 protein can be made. By contrast, when there are two signal elements, then that splicing event is stopped, and in fact there's no functional transcript. Now,

35. Summary of repression mechanisms of xol-1 (42:09)

can you imagine how that could work? It's a real challenge to try and understand how just a twofold difference in concentration of molecules can either repress or repress poorly, splice or not splice. And that's the important trick here. The importance is that it's not just one mechanism. It's multiple mechanisms simultaneously. It's an extremely important decision to determine sex. If sex is not determined properly, then reproduction doesn't occur properly. And so two mechanisms have been used, and I want to then put this together for you. So, normally, the xol-1 gene is present in both XO and XX animals. In XX animals, there's twice the dose of X-signal element repressors as there is in -- XX animals have twice the dose as XO animals. This leads to low levels of transcripts in XX animals and high levels of transcripts in XO animals, and what I want you to notice here is this little yellow intron. This little yellow intron is different from the other introns. These little purple introns are spliced out by the normal splicing machinery of the cell. What's different about that yellow intron is that it and only it is subjected to splicing control by an X-signal element. When that X-signal element is present in only one copy, as in a male, that X-signal element is not sufficient to be able to block splicing. The result is normal transcripts in the male. In the hermaphrodite, when there are two copies of the X-signal element that block splicing, what happens is that that intron is not removed. This binding protein is actually an RNA-binding protein. It binds to that sixth intron, and it prevents the normal cellular machinery from being able to splice out that intron. The consequence is that in the male, there's high levels of xol-1, and in the hermaphrodite, there's low levels of xol-1, and that leads to high levels of -- the high levels of xol-1 lead to male development, and the low levels of xol-1 lead to hermaphrodite development.

36. Does xol-1 at low levels have any functions? (44:38)

So now I have a question for you. Given that it's a differential dose of xol-1, I want you to think about the fact that if hermaphrodites have a little bit of xol-1, is that a bad thing? Do you think that's a bad thing? A good thing? Do you think it matters? How sloppy is nature? It's a good thing because...? Do you know why it's a good thing? It is a good thing. It turns out that xol-1, although it's required for a male, does have other roles in development, and this tiny level of xol-1 in a hermaphrodite is important for other things. So this is another example of how nature uses one molecule more than one way, and in so doing, it has great economy in coding for genes in a genome.

37. Student question: What is the purpose of the low level of xol-1 in hermaphrodites? (45:29)

At this point, I would like to ask for questions to go over this material. First, from the house. Yes?

What do hermaphrodites use xol-1 for if they have it in small amounts?

The actual state of being a hermaphrodite requires low levels of xol-1 much later in development, way after the time when xol-1 is used as a switch. So xol-1 is an on/off switch. It's thrown. And many -- actually, two days later, after sex is already determined and these genes in the pathway are being used,

xol-1 comes in and helps promote the female fate at that point. It's used again for reasons we don't fully understand, but it has two roles -- two apparently contradictory and even opposite roles.

38. Student question: Is *xol-1* comparable to *SRY* in mammals? (46:19)

House question. Another house question. Yes?

Would you say that xol-1 accomplishes the same basic purpose as SRY in mammals?

That's correct. Xol-1 is the master sex determination switch gene. It is the comparable gene to SRY. When it's active, you get a male, and when SRY's present, you get a human male. That's correct.

39. Student question: What is the difference between the blue and yellow XSEs in the demo? (46:39)

Yes? Another house question.

On one of your slides, there were two different versions of XSE -- the yellow one with the little...What's the difference between the two -- the yellow and the blue?

The yellow and the blue one?

Yeah.

The yellow ones are the transcriptional repressors, and the blue ones are the splicing factors. The yellow ones act first -- make the transcripts -- and then the blue ones act to splice out or not splice out the introns.

40. Student question: What do we know about human hermaphrodites? (47:09)

OK. We really now have a question from Moscow. Go ahead, Moscow.

I have a question. What do we know about human hermaphrodites? What do we know about human hermaphrodites?

What do we know about human hermaphrodites? Again, David, I'm going to have to imitate you. I'll have to get a little taller. We don't know a lot about why the hermaphrodite condition occurs, so, in worms, hermaphrodites are the natural species, and by mutation, you get females. We have detailed understanding of hermaphrodite development in the worm because we have found many of the genes involved in that development. In humans, the genes that are responsible for causing the hermaphrodite condition when mutant are not really known. In fact, I think none have been cloned. Is that correct, David? None have been cloned, so it's quite a mystery why humans can develop as hermaphrodites.

41. Student question: Is it possible to produce a worm that lacks xol-1? (48:07)

House question next. Yes?

Is it possible to produce a worm without any xol-1, and what would be the consequences of that?

It's absolutely possible to produce a worm without xol-1. Basically, the hermaphrodite doesn't care too much. The male is very upset. The male is so upset that it's the whole topic of my lecture tomorrow.

The male will die, because not only does xol-1 control the process of whether an animal becomes a male or hermaphrodite, it controls a process that's referred to as dosage compensation that controls X gene expression, and that's a whole huge topic. House questions?

42. Student question: Do all organisms require a gene to be switched on for male development? (48:55)

Is it usually the case that a switch like SRY or xol-1 has to be turned on for an organism to develop into a male? Are there others where a switch is turned off, and it's usually female?

The question is whether it's always the case that you must turn on a gene for male development. The case of the fruit fly is the opposite situation. There's a gene called Sex-lethal, and that gene must be activated in the female. The male is the ground state, and the X-signal elements in fruit flies serve to activate Sex-lethal in a positive transcriptional loop that then allows female development. So by no means is sex always determined by a gene that can either -- that when active makes a male. It's very heterogeneous what happens in the population -- in organisms. Another house -- yes. House question.

I have a question. With animals that can change from hermaphrodite to female to male -- can go through all three -- what's going on there, as far as the genes and those chemicals?

In general, we have hermaphrodites or females. The female state we get by mutating a gene required for sperm development. We can turn that female, which now has one mutation that's prevented it from making sperm -- we can give it another mutation that prevents it from being a hermaphrodite. Instead, it makes it into a male. So we can do that by two mutations. But it's always a progression of mutations. An animal does not spontaneously, without mutation, go from hermaphrodite to female to male. That's only by our manipulation in the laboratory by mutations.

For other animals? Yeah, like I know there's a type of frog that frequently switches between male and female. Is it constantly making that mutation within their lifespan?

So a lot of those systems are actually environmental sex determination, and what happens is the temperature at which the embryo is developing -- usually at high temperature, the animal can develop into a female, and at low temperature, a male. But there is an example of a swordtail fish, and that swordtail fish is a little confusing because there's only -- in a tank of fish, there's usually only one male. It's the dominant male, and everything else looks like a female, and if you remove that swordtail male, what happens is, all of a sudden, a fish that looks like it's a female suddenly becomes a male, and the truth is that that fish was never really a female. It was a juvenile male, and as a juvenile male, it just simply didn't develop external structures to let you know it was a male because you couldn't really sex it at that stage. So, in fact, I don't think it's transformation of an overt fertile female into a male. I think we all get fooled because that "female" isn't really a female. It's an undifferentiated male.

43. Student question: Can you add xol-1 to a hermaphrodite and make it male? (52:07)

Yes?

By adding xol-1 to a mature hermaphrodite, can you change the sex to a male?

To an adult? If you add extra copies of xol-1 to an embryo before sex is determined, 6 hours into development, then yes, that XX animal will develop into a male. It'll actually die, for reasons we'll talk about in my next lecture. If you add xol-1 after gastrulation later in development, it doesn't matter. The hermaphrodite has already determined she's a hermaphrodite, and she doesn't change her sex,

so xol-1 must be acting early and briefly to determine sex.Yes?

Could you do the same thing in the first six hours by removing xol-1 from a male and making it a hermaphrodite?

Yes. We can do that by making xol-1 genes that are temperature-sensitive. So, at will, we can inactivate the gene, or we can activate the gene. And so, for example, if we take an XX embryo that has xol-1 to be activated by high temperature -- we produce xol-1 at high temperature before that 6 hours -- she will actually develop into a male. If instead what we do is we remove xol-1 by reducing the temperature in an XO animal, or if we reduce in an XO animal, then that XO animal will develop into a hermaphrodite, because XO animals need xol-1 in that first 6 hours. So, yes, we can completely change back and forth by altering the activity state of xol-1 in an embryo. OK. Our last question is going to come from Moscow. Go ahead, Moscow.

So when hermaphrodites mate, who gets born?

When hermaphrodites mate, who gets born? So when a hermaphrodite mates with a male, what you get out is a 50-50 sex ratio. Fifty percent of the progeny will be male, and fifty percent of the progeny will be hermaphrodite. These have all been great questions. I'd like to thank you very much. You've been a wonderful audience.

[applause]

44. Closing remarks by HHMI Vice President Dr. Peter Bruns (54:29)

Thank you, Barbara. That was a terrific lecture. You redefined the domino effect, and I think we learned a lot, and those were wonderful questions, too. Now, it turns out the thing about science, of course, is that usually one question leads to another, and so some of you, I'm sure, have some unanswered questions here in the audience, and a lot of you will be watching this in a different time and a different place on the web. I will remind you with another commercial message that we can accommodate that. Here again is the web site of BioInteractive, and on this site, you will find a thing called "Ask a Scientist," and so we will be able to field your questions not only now, but in the future on this, and so please go to that site. So that is it for today. We'd like to thank you all for coming and hope that you will come back tomorrow, when Barbara will talk about what happens when gene regulation goes wrong, and David will talk about the evolution of human sex chromosomes. Thank you very much for coming.

[applause]

[music plays]