The Meaning of Sex: Genes and Gender
Lecture Three—Sex and Death: Too Much of a Good Thing
Barbara J. Meyer, Ph.D.

1. Start of Lecture Three (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 third lecture is titled "Sex and Death: Too Much of a Good Thing." And now to introduce our program -- the president of the Howard Hughes Medical Institute -- Dr. Thomas Cech.

2. Introduction by HHMI President Dr. Thomas Cech (1:05)

Good morning and welcome back to the 2001 Holiday Lectures on Science here at the Howard Hughes Medical Institute. Yesterday, you heard about how sex is determined from two world experts on this subject who do research in this fascinating area. Dr. David Page talked about the Y chromosome in humans and about a particular region of the Y chromosome that's responsible for determining maleness. Dr. Barbara Meyer talked about the xol-1 gene, which is involved in the switch between sexes in the nematode worm Caenorhabditis elegans. Now Barbara's going to continue a discussion of sex determination in the worm with this lecture, provocatively entitled "Sex and Death: Too Much of a Good Thing." You'll recall from yesterday that if you're a worm and you have two X chromosomes, you'll become a hermaphrodite, and if you have one X chromosome, you'll become, instead, a male. But it turns out that by having two X chromosomes, you get a double dose of all of the genes on the X chromosome, and that can be deadly, and so what you'll hear about today is how the worm deals with having a double dose, in the case of the hermaphrodite, of those X chromosomes. Now, Barbara Meyer began her interest in scientific research when she was a college student at Stanford University, and interestingly, she worked in the laboratory of David Clayton, a professor at Stanford who's now a vice president here at Howard Hughes Medical Institute. I first met Barbara in Boston in the 1980s. It was at a Thanksgiving dinner, and since it was Boston, we were having lobster for Thanksgiving instead of turkey. She was already sort of a legend in her own time because when she was a Ph.D. student at the University of California, Berkeley, she went to Harvard to do some collaborative work with a Harvard professor, and he talked her into not going back to Berkeley and transferring and getting her Ph.D. degree in Boston. But Berkeley ended up sort of having the last laugh because many years later, they recruited her back from her faculty position in Cambridge, Massachusetts, to become a professor at U.C., Berkeley, where she is now. We're going to have a brief video to further introduce Barbara, after which, the stage is hers.

3. Introductory interview with Dr. Barbara Meyer (3:26)

The most important aspects of being a scientist are to have a sense of perseverance and determination to answer a question. Science is fun, but it's also hard because you don't always get the answers you expect. You can do experiments, and they fail. You have to really have the passion to want to know the answer in order to pursue it. Being a scientist does not always allow instant gratification, but in those moments when experiments work and discoveries happen, it is the most satisfying thing you can imagine, and it's an addiction. You just always want to be able to discover something new, and that's what keeps propelling you. Some people think they have to be the smartest people in the world to be a scientist. I thought that, too, but the fact of the matter is, what's most important is to have passion and be willing and able to work hard to pursue your dreams and your goals. I think that most scientists have to have a big imagination. They have to want to know answers to questions. They have to be curious.

They have to want to know how things work, and if they have that combination of curiosity and creativity and discipline, being a scientist is a natural thing to be. So if a high-school student wants to become a scientist...I think it's important to read about science, to be able to join programs that allow the students to do individual research in the summers, but none of that's necessary. One can simply go to college, take courses, find professors willing to take undergraduates in their lab, and start doing research. What's important is to have the desire to be a scientist and then go to the university and be able to work in a lab. I would never have imagined that I could have been a scientist if I had not been given the opportunity to try science myself. What matters is interest and passion, intelligence, and the willingness to try hard. So science is not precluded from anybody if they really want to go into it. It's a question of finding the opportunity, and it's a question of having the desire and interest to do it. I think what's important about my life and illustrative to students...is that one doesn't have to have a certain set background in life. One doesn't have to have thought they wanted to be a scientist all their life. One doesn't have to have wanted to be a doctor all their life. One can discover fairly late in life, even, that the process of doing science intrigues them, and they can do it. And this, you don't even need to have had mentors if you have the ambition to do it yourself, that anything is available in life if you just want to be able to do it.

4. Too many chromosomes: Down Syndrome (6:02)

I'd like to welcome you back to our lectures today. In today's lecture, I would like to begin by calling attention to a syndrome in humans that will help me illustrate the points I'm going to try to make in the nematode. First I'd like to ask, how many of you know about Down syndrome? How many of you have relatives who might have Down syndrome? The reason I want to tell you about Down syndrome and remind you of some of the basic features of Down syndrome...is that Down syndrome is the result of an increase in the number of chromosome 21. Trisomy 21 causes Down syndrome. The problem is, there's a gene imbalance that's the consequence of too many copies of chromosome 21, and that gene imbalance leads to many developmental problems. The most obvious problem is that about 85% of children with Down syndrome die before birth. Those 15% or so who survive frequently have mental problems, frequently have developmental problems, but nonetheless, they can lead happy lives. They frequently live to the age of 40 or so. The point, though, is that because there's too many gene products from chromosome 21, there's serious problems.

5. How humans deal with having too many X chromosomes (7:20)

This is very similar to the situation with the X chromosomes in any organism that uses X chromosome counting or a chromosomal basis of sex determination in order to decide whether to be male, female, male, or hermaphrodite. This problem is a problem that's pretty universal... even in the case of humans, where there's two X chromosomes for the female and one X chromosome for the male. There's a process in which gene expression from the X chromosome has to be equalized between the sexes because both sexes require X-chromosome products in the same amount. Humans have solved this problem in a particular way, and the way humans have coped with the idea that the single X chromosome of the male must have the same transcript level as both X chromosomes of the female is actually to shut off one X chromosome through the process of X-inactivation. You're probably familiar with the term "Barr body." The X chromosome is simply crumpled up into an inactive state of chromatin where most, but not all, of the genes are turned off. This is the human solution. Now, yesterday, we spoke about the fact that nematodes must count X chromosomes to determine sex, and we spoke about the fact that in humans, SRY determines whether an organism will develop into a male or not. We never mentioned yesterday about X chromosome counting in humans, but I assure you that X chromosome counting does happen in the humans but just for a different process -- namely, for X-inactivation, because in humans, all X chromosomes but one become inactivated. So if a human is born with three X chromosomes, two of those three X chromosomes are inactivated so that one X chromosome remains active. That's how the human manages to deal with this problem of increase in dosage of a chromosome.

6. How the nematode deals with having too many X chromosomes (9:18)

The little worm, C. elegans, has come up with its own solution. It, too, must have dosage compensation, and what it does is slightly different from the human. In the case of the nematode, once again, the two X chromosomes of the hermaphrodite and the one X chromosome of the male ultimately make the same level of product. The worm's solution, however, is to allow both X chromosomes to be active, but each chromosome is repressed transcriptionally by 50%, and that's illustrated here. What we've done in this slide is show you that if there were not the process of X-chromosome dosage compensation, in which transcriptional repression occurs from both X chromosomes, you would see these extra copies of transcripts that could happen if dosage compensation failed. In effect, the point is that transcription of each X chromosome is reduced by half, and what happens if the nematode cannot properly dosage compensate is that the hermaphrodite will be dead. The males will be perfectly normal because this process only works in the hermaphrodites, and not the male. So hence the title "Sex and Death." Too many X chromosomes causes death.

7. Dumpy (DPY) gene, a dosage-compensation mutant (10:31)

Now, if you were a geneticist in my lab and wanted to study dosage compensation, how would you do that? What mutant phenotype might you look for if you were in my lab trying to figure out those genes involved in dosage compensation? Can I have a volunteer? Don't be shy. Yes?

Look for males with an extra X?

That will affect sex determination. That will have a slightly different problem. Have an answer?

A hermaphrodite with three Xs?

We're trying to find a mutation that prevents dosage compensation from occurring, and the question is, what would you look for? Would you look for -- what phenotype? The answer is that you look for death in hermaphrodites. If dosage compensation works by turning down transcription of both X chromosomes in the hermaphrodite and the process of dosage compensation fails because we make a mutation in a dosage compensation gene, then what happens is that the hermaphrodite dies. The hermaphrodite is dead -- those are little dead embryos -- or the hermaphrodite is dpy -- "dpy" means short and fat -- and the reason those nematodes die is that they have high levels of X-chromosome transcription, too high because dosage compensation was not turned on and could not turn those transcript levels down lower.

8. The sdc gene affects both dosage compensation and sex determination (11:53)

However, when we did mutant screens looking for dosage compensation mutants, not only did we find mutations that affected hermaphrodites and caused them to die, we were able to find mutations that caused them to die, and those that escaped lethality were male, not hermaphrodite. In other words, there was not only dosage compensation affected, but also sex determination. How can that happen? In fact, we have two classes of genes here. The dpy genes -- those are involved in dosage compensation only. When you get rid of them, they cause death. The sdc genes, standing for sex determination and dosage compensation -- those are required for both processes. How is that?

9. Demonstration: Branched pathway for dosage compensation and sex determination (12:34)

Well, it turns out that in the worm, there's a branched pathway that controls both sex determination and dosage compensation, and that branched pathway is highlighted right here. Here we have the genes involved in dosage compensation in yellow. Here we have the genes we discussed yesterday involved in sex determination in these colors right here, and, in fact, the sdc gene, which I show up there... is up

here, and that sdc gene has the capability of not only turning on the process of dosage compensation, it has the responsibility of allowing "XX" animals to be hermaphrodite. So we have a branched pathway, and in that branched pathway, we have genes that code for both sex determination and dosage compensation and genes only involved in dosage compensation and genes only involved in sex determination. So the responsibility of these genes is to turn off X-expression.

10. Demonstration: The sdc gene is turned on in normal hermaphrodite development (13:35)

Now I'd like a volunteer to come help me to illustrate for you some more genetic principles by our little demonstration here. Amy, would you come forward, please? OK. So this is going to represent the hermaphrodite state. Every time I push down the dominoes, that means hermaphrodite fate will ensue. And what do you see? You see hermaphrodite sexual development and dosage compensation is turned on. That's because sdc-2 is on. Could you reset these for me now?

11. Demonstration: An sdc-mutant hermaphrodite develops as a male without dosage compensation (14:07)

Now we're going to discuss a situation when sdc is mutant. When sdc is mutant, what are our expectations? Do you want to volunteer? Yes? Could you speak a little louder, please?

They're either gonna die or they're gonna be [indistinct].

When we have an sdc mutation, what happens is -- I'll just speed this process up a little bit. When we have an sdc mutation -- I'm sorry -- what happens is -- I'll just pull this out. Sdc-2 is missing. No flags fly. What happens is, sdc is off, male development ensues because we can't turn on the hermaphrodite pathway of sex determination, and dosage compensation fails, so we have a sex reversal, and we have death. OK. Reset them.

12. Demonstration: Block sex-determination branch only (15:02)

So now I'm gonna try a situation where, this time, we can block one branch of the pathway independent of the other branch of the pathway. So I'm gonna choose to block the branch of the pathway that's involved in sex determination. OK? So I've pulled out a branch involved in sex determination...and what we see is that the flag for hermaphrodite development did not go on -- there's male development -- and dosage compensation -- you want to reset? Dosage compensation is on. So we've been able to separate the two functions of sdc by mutation.

13. Demonstration: Block dosage-compensation branch only (15:42)

The next example we're gonna give you is a case where -- we're gonna give you a case where we block the dosage compensation pathway...but not the sex determination pathway. You want to take this one out? You can see what happens. We get hermaphrodite development. However -- next slide, please -- dosage compensation is off, and we get death. We get a dead hermaphrodite. Thank you. So now we have the illustration -- thank you very much. I'd like to give you a T-shirt. We now see how we can split the two pathways for sex determination and dosage compensation by mutation.

14. How does SDC protein interact with the X chromosome (16:23)

So I'd like to address for you what happens when -- what is dosage compensation? What are the genes involved in dosage compensation? What is the activation of dosage compensation, and what does it mean? Well, the sdc genes activate dosage compensation because they take these dpy proteins and they can recruit them to the X chromosome. So sdc proteins, in combination with the dpy proteins, recruit the dosage compensation proteins to "X." Dosage compensation is activated when these proteins bind to the

hermaphrodite X chromosomes and repress transcription. I'd like to show you now some images of what it looks like when the X chromosomes are bound to dosage compensation protein, to dosage-compensated X chromosomes. In this case, we have an embryo, and this embryo has been stained with a dye that intercalates into DNA. It's called DAPI. We've also stained this embryo with an antibody to an sdc protein. This antibody recognizes the protein wherever it is in the cell, and we have a fluorescent tag on that antibody so that when we look in the embryo, what we can see is the blue marking all DNA, and we can see the red, which represents the X chromosomes. This protein binds selectively to the X chromosomes and only in hermaphrodites, and you can see two red dots for X chromosomes. Now, you might want to see a more rendered version of this, and what we've been able to do in the lab -- can I have the next slide, please? Is to reconstruct that image in three dimensions and give the chromosomes three-dimensionality. You can see two X chromosomes up there, and you can see them in the context of the whole nucleus, which is shown in blue, and then the nucleus by itself. That gives you a feel for what the X chromosomes look like within the nucleus itself.

15. How does the male X chromosome avoid dosage compensation? (18:19)

Now...you might be wondering, if dosage compensation is activated in hermaphrodites... by the sdc proteins associating specifically with X chromosomes, then the question is, how does dosage compensation not occur in the male? How is it that those dosage compensation proteins can never go to the X chromosomes? Does anyone have a guess? Yes?

The presence or absence of xol-1.

Yes. How did you get that right? It's absolutely right. You get a T-shirt. Oh, excuse me. Pass it back. Xol-1, as I told you before, directs male development. Xol-1, when it's on, makes a male and when it's off, makes a hermaphrodite. But xol-1 also controls dosage compensation. Xol-1 is -- we talked last time about how xol-1 is like SRY in it's a master sex-switch gene, but this is an important point. Xol-1 is not like SRY in the sense that SRY does not control X-inactivation. It's even the wrong sex. Xol-1 controls both, and xol-1 controls both because... Xol-1 controls sdc. So in a male, xol-1 is on, telling the animal to be male and to turn off dosage compensation. When xol-1 is on, the sdc proteins are off. When the sdc proteins are off, the sex determination genes are allowed to be in the male fate mode. In addition, when sdc is off, we've learned before that the dosage compensation proteins cannot bind to the X chromosome, so the male is spared. If the male were able to activate the dosage compensation program but have only one X chromosome, what would happen to him? Die. Death. Absolutely. It is lethal to not have xol-1. "Xol-1" stands for "XO lethal." You're doing really well.

16. What happens in an xol-1 mutant? (20:26)

OK. So now... let's see how well we can do. So if we have a xol-1 mutation... xol-1 will be off in an "XO" animal. Because xol-1 is off in an "XO" animal, the sdc genes will be on. When the sdc genes are on, then what's gonna happen?

It's a hermaphrodite. Hermaphrodite.

It's gonna be a dead hermaphrodite because the sex determination genes are shunted to the hermaphrodite pathway. The dosage compensation proteins are on the male X chromosome, and that poor male is gonna die from too little X chromosome gene expression. OK. Now the question is, how can we rescue that male from death? What would we have to do to make that male happy again? Exactly right. You turn up sdc. So let's turn up sdc. If we have a xol-1 mutation and we also mutate sdc, then what happens? We have male development, and the animal is alive because dosage compensation cannot proceed, OK?

17. How can you keep an xol-1 mutant from dying? (21:42)

Now, you're doing so well, I'm gonna ask you another question. You're doing amazingly well. So supposing we have a xol-1 mutation and we want to correct the death. What do we do? Just the death. If we got rid of an sdc gene, then we would correct both the death and the sex transformation problem. We just want to correct the death.

Turn on xol-1. Turn on xol-1.

We could turn on xol-1, but xol-1's been deleted. There's no hope of ever retrieving it from the genome. We have to do something else. We just want to correct the dosage compensation defect. We want to prevent those X proteins, those proteins from binding to the X chromosome and killing the poor animal. What do you think we do? Yes?

Turn off the compensation pathway.

Exactly. By mutation. Brilliant. We turn off the dosage compensation pathway, and then what happens? What's the sex of the animal?

It would still be a hermaphrodite.

Exactly right. It's a hermaphrodite.

18. How can you manipulate xol-1 mutants to become male? (22:52)

OK. Now let's try the reciprocal. What happens if we want to correct the sex determination defect, but not the dosage compensation defect? We want it to be a hermaphrodite. What do we do? Yes?

Do we still have xol-1?

Xol-1's missing. This poor male is about to die, and it's gonna be a hermaphrodite. We want to make it hermaphrodite, but we're not gonna prevent it from dying. What do we do?

We turn on sdc?

If we turn on sdc, we'll cause a problem. We want to eliminate the hermaphrodite pathway of sex determination. How might we do that? Yes?

Would you block one part of the pathway?

That's right. You can block one part of the pathway. What you can do is selectively knock out the genes involved in hermaphrodite sexual development. The xol-1 gene will still be off. The sdc genes will still be on. The dosage compensation pathway is going to be shunted to the hermaphrodite state, meaning that the male will die, but it will be a dead male, not a dead hermaphrodite, OK? You've done extremely well. I'm very proud of you.

19. Student question: How do you get a 50% reduction in gene activity? (24:03)

At this point, I'd actually like to break for some questions because the next topic is going to involve a wholly different subject, and I think this is a good breaking time. Can I have a question?

Yeah. I was wondering, what happens if the sdc proteins block the same gene on both copies of the X chromosome?

What happens if the sdc blocks -- oh, OK. So the question is, how, in fact, do you get transcription from -- how do you get 50% reduction? So in this case, the sdc genes actually repress both copies, but both copies only by 50%, so it's not that one copy is inactivated and the other is active. It's that both copies get repressed, and so the question would be -- and we've never been able to do this experimentally -- is to get more repression. For example, what happens if we make a super sdc and allow the X chromosome to be repressed more? We don't know the answer to that. We'd assume it's gonna cause a problem, but we've not been able to do that.

20. Student question: Does dosage compensation occur on autosomes? (25:04)

Are there other house -- Yes?

Does dosage compensation ever occur on non-sex chromosomes?

That's a great question. It's a controversy as to whether or not dosage compensation occurs on other chromosomes. For example, in the fruit fly, there's also a system of dosage compensation for the X, but it seems that for the fruit fly, another mechanism of compensation occurs, and it's specifically for the non-X chromosomes. So yes, that does occur in fruit flies. In the worm, we don't yet know of such a phenomenon.

21. Student question: Can a nematode get Down syndrome? (25:35)

Yes?

Uh, like, Down syndrome -- do they catch Down syndrome?

They don't catch Down syndrome, per se. They have problem -- we can't measure the intelligence of a worm, unfortunately, although we try. Worms have developmental abnormalities. It's really a little bit difficult to relate the problems a worm has with the problems that a human has. The way we have of looking at that is very different, so it's not quite the same process, but it's very related.

22. Student question: How can Turner syndrome have effects if X inactivation occurs? (26:07)

If humans inactivate one of the X chromosomes anyways, how come Turner's syndrome has effects?

Yes. The point is that in the X-inactivated -- in humans, when there's X-inactivation, not all genes on the X chromosome are inactivated. In fact, many of them escape inactivation. So that's the answer. It's a great question. Yes?

Are there any applications of turning half-genes off in humans for developmental disorders like Down syndrome that you could find genes that turn off things that -- I guess if humans have three chromosomes at one place?

Yeah. The question is, is there ever a human disease in which loss of one copy, which is the equivalent -- I'm sure there are. I don't happen to know them. David, do you know of them?

Sure. There are a bunch of such diseases, meaning things like -- familial hypercholesterolemia is a good example.

True. Familial hypercholesterolemia. There are many diseases that you need both normal copies to prevent the disease, but having one copy causes the disease. I think we have a question coming in from Moscow. Go ahead, Moscow.

My name is Sutlan Nivera. The question is, you said that when the X chromosomes of a female is switched off, what happens, how this sickness gets transmitted together with the sex, and will it become evident in the sex on which it has been transferred?

OK. The question is, what happens to the X chromosomes in the nematode? In the nematode, both X chromosomes remain active. It's just that both of the X chromosomes are transcriptionally repressed. It's the humans where X-inactivation turns off one X chromosome. So in the worm, unlike in the human, it's proteins involved in both pathways, sex determination and dosage compensation, that controls both, those sdc proteins I spoke about, and those are under the control of the master sex-switch gene xol-1, which controls both sex determination and dosage compensation.

23. Student question: What happens in XXX nematodes? (28:25)

Could I have another question from the house? Yes?

If due to mutation, a nematode is born with three X chromosomes and sdc is still on, leaving 1-1/2 X-activated chromosomes, will the individual die?

That's a wonderful question. The question is, what happens if the worm has three X chromosomes instead of two, but sdc genes are on? The answer is that the animal is very unhappy. The animal turns "dumpy." It's like a partial defect in dosage compensation. The dosage compensation system in the worm can only handle two X chromosomes. If you give the worm three X chromosomes, there's a problem. The animals will live, but they look very unhappy, very sickly. It's a great question.

24. Student question: What actually kills when there is no dosage compensation? (29:08)

What is the actual cause of death when there is no sufficient compensation? Is there no protein synthesis, or what?

Yeah. So the question is, what happens when there is no dosage compensation? Why is there death? The answer is that there are many genes on the X chromosome that are important and must be kept in a balance, and that it's probably the action of many genes which, when expressed at too high a level, act together to cause the death. There's no single gene that we know about that's being misregulated that causes the problem. It's more that many chromosome -- many genes on the X chromosome are having too much expression. So it's a cumulative effect, and we don't know exactly the specific genes involved. That's a good question.

25. Student question: How do genes in X chromosomes escape inactivation? (29:54)

There was another question up here.

In humans, when one of the Xs is inactivated, how does some of the parts of it still become activated? Is it not surrounded by a certain protein or something like that?

Yeah. How do genes in the X chromosome escape inactivation? That has to do -- it's, in part, not understood -- but what is clear is that they -- the chromosome in the regions that is inactive forms what's called heterochromatin, a very condensed form of chromatin that prevents transcription from occurring from that segment of X. There's a mechanism that restricts the boundaries for where that formation of heterochromatin occurs so that there are regions that are not subjected to that scrunching up of the chromosome to prevent transcription. What those mechanisms are aren't understood, but there are definitely boundaries for where you can not have that phenomenon occur.

26. Student question: Do autosomes have genes that determine sex? (30:51)

I think we have a question from Moscow. Go ahead, Moscow.

The question is, if -- do autosomes have genes which determine sex?

So, I have cleverly avoided telling you where all these genes that determine sex are located, so I'll tell you the truth now. Xol-1 is located on the X chromosome. A couple of the sdc genes are located on the X chromosome. All the rest of the genes -- all the genes in this part of the pathway here -- are located on non-X chromosomes, on the autosomes. All these genes up here... are located on the X chromosome. So the genes that control both sex determination and dosage compensation are on the X chromosome.

27. Topics to be covered in the second half of the lecture (31:37)

OK. I'd like to thank you -- and these are great questions -- and go on to the next part of my lecture. In this part of the lecture, I'd like to discuss with you more, how does dosage compensation occur? What are the molecules involved in dosage compensation? Where did they come from? And what's the evolution of these molecules? And both David and I, in our second lectures, are going to try to give you a flavor for evolution. In my case, I'm gonna try and give you a flavor of where dosage compensation emerged. In David's case, he's gonna give you a flavor of the evolution of the Y chromosome. So let's just remember that in XX animals, xol-1 is turned off, the sdc genes are on, the animal is a hermaphrodite, and dosage compensation has been activated because the sdc proteins recruit dosage compensation proteins -- these little yellow proteins here -- to the X chromosomes to repress transcription. Now I need to tell you that the dosage compensation process actually has origins in a very different process, namely, chromosome segregation, and what I need to do is review chromosome segregation for you briefly so that you'll have an understanding of my comments about dosage compensation.

28. Review of mitotic chromosome segregation (32:54)

How many of you have studied mitosis in high school? Great. You're all experts. You can help me. As you're probably aware, the purpose of mitosis is to allow DNA to be -- to be properly partitioned to two daughter cells. To do that, the chromosomes have to replicate. They then have to segregate to two different cells. What I have diagrammed for you up here is the process of mitosis, and in this process, there are many different stages to it, and what's shown are the different stages of mitosis. You might remember these names -- interphase, prophase, metaphase. Those aren't so important, but what is important is what happens to those chromosomes. So at the very beginning, just after replication has occurred, we have this fuzzy DNA. DNA is represented in red here, and green shows -- is the color chosen for the spindles that are gonna come in and pull those chromosomes apart. It's called tubulin. So in interphase DNA, the chromosomes are all fluffy, decondensed. When prophase starts to occur, the chromosomes become compacted. By metaphase, those chromosomes have lined up onto that metaphase plate. In anaphase, they start to separate. They're pulled apart by those green fluffy spindles, and by telophase, the chromosomes have become decondensed again, and cell division will occur. So mitotic chromosome segregation is really a complex problem of condensing and decondensing chromosomes.

29. Dosage-compensation proteins evolved from mitotic proteins (34:30)

And what I'm about to tell you is that the proteins involved in dosage compensation evolved from those proteins that were involved in condensing and decondensing mitotic chromosomes. So to make that clearer, it turns out that there's a complex of proteins that are involved in chromosome condensation. This complex of proteins has been discovered in many labs and is conserved from yeast to humans. For your purposes, we color-coded these proteins in these bright little colors. This is the mitotic -- it's called a condensing complex. And what's very interesting is that this complex looks very similar to the complex involved in dosage compensation. So look at the colors of the little balls and look now, again,

at this next slide. Excuse me. Sorry. These proteins are involved in condensing chromosomes. What happens is, when these proteins bind to autosomes, or any chromosome, shown in purple here, these proteins bind, and they cause the chromosomes to be condensed... and that process of chromosome condensation is absolutely essential for normal chromosome segregation. We're going back to the complex of proteins. Next slide, please. The complex of proteins I just spoke about is there, and the complex of proteins that are involved in dosage compensation -- look at them. There are four of those proteins that are very similar to the proteins involved in mitosis. So the dosage compensation proteins include four proteins that have a great deal of similarity with the proteins involved in mitosis from yeast to humans. Not only does the dosage compensation complex have those four proteins, it has these other proteins in gray, and those proteins in gray are your friends -- the sdc proteins. So the dosage compensation complex has two kinds of proteins. The proteins involved just in dosage compensation -- those are proteins very similar to those mitotic proteins I described earlier that are present from yeast to man. In addition, the dosage compensation complex has these sdc proteins that, of course, recruit the complex to X.

30. MIX-1 protein has roles in both mitosis and dosage compensation (36:46)

Well, it turns out that, actually, nature was really smart. Not only did nature use proteins for dosage compensation that were similar to proteins used in all other organisms for mitosis, the worm very cleverly allowed one of those proteins, called mix-1, standing for mitosis and X chromosomes, to actually retain its original role in mitosis. This protein, mix-1, has two jobs in the same cell. In the same cell, it can either localize to the X chromosome to do dosage compensation, and it does that around the 40-cell stage. Dosage compensation is activated by the sdc proteins around the 40-cell stage. But even before that, mix-1 has played another role. Mix-1 is involved in mitosis, and what you see on the right panel is mix-1 antibody staining complexed with the red, which is tubulin, showing that mix-1 antibody is associated with all chromosomes during mitosis. You can recognize that as a metaphase plate. So mix-1 has two jobs in the cell.

31. How does MIX-1 carry out these two roles and not get confused? (37:56)

And the question is, how does mix-1 carry out two different jobs in the same cell and not get confused? And the answer is that mix-1 has one partner, called dpy-27. Dpy-27 is a protein dedicated only to a role in dosage compensation. When mix-1 binds with that dpy-27 protein, it's allowed to be dragged to the X chromosome for the purposes of dosage compensation. However, when mix-1 is associated with a different protein, called smc-4, mix-1 is allowed to carry out its role in mitosis. Smc-4 plays a role exclusively in mitosis. So the reason mix-1 can play two roles is, it has different protein partners. When it's paired with smc-4, it's involved in mitosis. When it's paired with dpy-27, it's involved in dosage compensation. So when dpy-27 gets together with mix-1... in the presence of sdc-2, that complex of proteins is allowed to bind to the X chromosome to repress transcription. By contrast, when mix-1 associates with smc-4, it can find the fuzzy purple autosomal DNA or the X DNA and cause chromosome condensation. OK. Now...

32. Where and when is SMC-4 protein found? (39:28)

if smc-4 is a protein involved only in mitosis, we have certain predictions for where that protein will be found. So remember I told you that mix-1 is found both on the X chromosomes and on mitotic chromosomes, but smc-4 is only involved in mitosis. So where do we expect to find smc-4? Yes. Or all chromosomes, but when? That's right. The answer is here. Can I have the next slide, please? The answer is that smc-4 is made only at times in the cell that are about to undergo mitosis. What you see here is the top group of shots that represent what you've seen before -- chromosome condensation involved in mitosis. What you see below is those same cells -- they're different cells in the same stage as the cell cycle -- that have now been stained with an antibody to smc-4. What I want you to notice -- that antibody is shown here in yellow. What I want you to notice is that there's very little smc-4 in

interphase. In prophase, you start to see that lining the chromosomes. By metaphase, you see it as little dots along the X chromosomes. It stays in that state in anaphase, and by telophase, it's gone again. In other words, that protein that's involved only in mitosis is only around in the cell during the process of chromosome segregation, and that's why, if it's there, mix-1 is able to carry out its role in mitosis, because it's there only at the time that mix-1 needs to carry out a role in mitosis. So all the specificity comes from smc-4. Now, I would like to show you an animation.

33. SMC-4 is only expressed in mitotic cells (41:27)

Excuse me. One more point, and that is this. If smc-4 is only involved in mitosis, then we should never see it on the X chromosomes, and that is the case. Here we have a little larva that's been stained with a dye for DNA in blue. It's been stained from mix-1, and because mix-1 is able to bind both to X chromosomes and to mitotic cells, we see it in both places. Smc-4 is only able -- is only on in mitotic cells, so we only see it in these two mitotic cells, and so, there's a clear partitioning of smc-4's role in mitosis, and it never shows up on the X chromosome. So I would now like to show you a little animation, but before I do that, I'd like to give you some keys about our animation. The first part of our animation, we're going to see how smc-4 and mix-1 come together to localize to all chromosomes -- autosomes and X -- because all chromosomes have to undergo chromosome segregation. When they bind, the chromosomes become compressed, organized, condensed. Could I have Powerpoint on, please?

34. Video: Embryonic cell division in wild type and SMC-4 mutant (42:33)

OK. This is again a situation where we have an embryo. The embryo has been stained -- it has a histone that is green florescent, protein bound to it. This is a one-cell-staged embryo in which the chromosomes -- maternal and paternal chromosomes -- have come together, and they are about to segregate in the first cell division. We see the chromosomes segregating, just like we saw last time. Now I'm gonna show you an smc mutant. What you see is, the chromosomes fail to segregate. They're stuck together. That's in anaphase. In anaphase, there's no separation. In anaphase, there's a bridge in the case of a mutant. I'm now gonna show you a wild type. It proceeds on. You seek a normal cell division. With a mitotic mutant, what you see is, the chromosomes still never can come apart. They're totally entangled. That's at telophase, where you see a bridge. And now, in wild type, we're gonna go from a two-cell stage to a four-cell stage. First, we're gonna see, before then, the chromosomes compacted in prophase. In the smc-mutant, what you're gonna see is, those chromosomes never really can compact again. And then we're gonna see the chromosomes segregate from a two-cell stage to the four-cell stage... in the wild type, and in the mutant, what we're gonna see is, those chromosomes stay stuck together. Chromosome segregation can never happen.

35. Animation: SMC-4/MIX-1 protein in mitosis (44:04)

OK. Now we're gonna go to an animation, but first, I want to show you, after this completes, that -- what I'm going to show you. First we're gonna see is that the smc-4 protein, when bound to the mix-1 protein, associates with chromosomes and condenses. When you first see this animation, you're gonna see about a 30-cell embryo -- a real shot of a 30-cell embryo. It's gonna then go to a cartoon form, where the individual nuclei -- you can roll the animation now -- where the individual nuclei will appear in cartoon form. So here you see an embryo. You see those holes? Those are the nuclei. Those nuclei are now red circles, and the cytoplasm is blue. You see the chromosomes coming up. The first thing that happens at the beginning of -- or just before mitosis -- is that the chromosomes are gonna have to replicate. The reason we show you dpy-27 and mix-1 there is that those proteins are around -- they just can't associate with DNA yet. The chromosomes are divided. We see this burst of synthesis of mix-1 in blue and smc-4 -- mix-1 in green and smc-4 in blue. That protein begins to appear in the cell cycle. The protein associates with all chromosomes in preparation for cell division. You see the dpy proteins not localized to X. You see mix-1 and smc-4 localized to condensing chromosomes. The chromosomes now

condense... and we're about to see chromosome segregation. The chromosomes line up on the metaphase plate. The spindles come zooming in. The chromosomes will now segregate. OK. Next what we're gonna see... I'm going to the slide now.

36. Animation: SDC/MIX-1 and SMC-4/MIX-1 proteins in dosage compensation and mitosis (45:50)

We're gonna see what happens when dosage compensation is activated. We just saw a round of mitosis prior to the point where dosage compensation is activated. Dosage compensation is gonna be activated at about the 40-cell stage because mix-1, dpy-27 associate with sdc-2. We're gonna open the animation right now by showing you sdc proteins associating and coming on. Can we have animation, please? So we go back to the cell. We see the dosage compensation proteins sitting in the cytoplasm. It's all there, provided by the mother. Dosage compensation is gonna start to be activated because sdc proteins come in. At this point, sex has been determined, the sdc proteins are present, and you suddenly start to see... dosage compensation. The chromosomes have become compressed at some form. Now we're gonna compare transcription between the X chromosomes of a male and the two X chromosomes of the hermaphrodite. What you should notice is that there's the same number of transcripts coming from the two Xs plus the single "X." That means dosage compensation has been successful. We now have another round of mitosis, and what I want you to notice is, the dosage compensation complexes now still there -- are allowed to stay there -- during this next round of mitosis. We see chromosome segregation. OK. So what we've now seen is a round of mitosis. We've seen a round of dosage compensation being started. Dosage compensation continues. We see the cell go back to its normal stage. Can I have the next slide, please? OK.

37. DPY-28 protein is involved in meiosis (47:45)

I've told you that there are many components in dosage compensation that have retained their original roles in mitosis. I want to make one further point before closing, and that is that some of the proteins are also involved in meiosis. The protein called dpy-28 carries out a role in meiosis now, not just mitosis. So dpy-28 has two roles -- dosage compensation and meiosis -- and the pattern you see on the right is meiotic chromosomes, and those meiotic chromosomes, I'm going to describe to you in just a moment. But before I do that, I want to review, very quickly for you, meiosis. David Page has already given you a very lucid explanation of meiosis, and I just want to remind you that in meiosis, a diploid organism manages to make sets of gametes such that you have four gametes, in the case of sperm, coming from the diploid organism. What happens is, during meiosis, you get replication of the two chromosomes. Those chromosomes are shown in red and blue. Those two chromosomes then synapse, in the process of chromosome pairing, and once those chromosomes synapse, what I'm gonna show you is that dpy-28 is between those two homologous chromosomes. It's at that time, when those two chromosomes are put together, that that process of recombination occurs, and that process of recombination allows the two chromosomes to come together. You can see the little recombination event here. The little chromosomes separate and you get four products of meiosis. The chromosomes are now haploid. Well, it turns out that dpy-28 is that glue that's between those two homologous chromosomes. So we show you DNA at the stage of packeting, when the chromosomes first pair. And what you can see here is this tiny little area of darkness. That area of darkness is between the homologous chromosomes. If you look at where dpy-28 is, dpy-28 is right between those homologous chromosomes, and you can see that in the merged image on the right as a yellow stripe. So what happens when dpy-28 is missing? Surprisingly, when dpy-28 is missing, recombination is changed in the worm. Normally in the worm, recombination that occurs during meiosis is extremely carefully regulated. There's only one crossover event per chromosome. There's only one recombination event. It's very unusual. Dpy-28, however, is part of the process that must be involved in restricting the number of recombination events to just one, because in a dpy-28 mutant, you get multiple different recombination events, all across the same homologous chromosomes. So, clearly, dpy-28 plays a very important role in meiosis.

38. Summary of Lecture Three (50:33)

So I'd just like to sum by saying that dosage compensation has evolved from chromosomal processes that were present in the worm way before the worm even thought about dosage compensation. Those processes are mitosis and meiosis. So we can see that those building blocks of life that were used for one process have now been recruited to a new role, the role of X chromosome gene expression. And ultimately, the message I'd like to leave you with these Holiday Lectures is that, as I started to say at the beginning of my first lecture, there is unity to life -- that many of the molecules, the building blocks involved in many processes in smaller organisms like the worm -- are very similar to processes that are involved in humans, and therefore, one can study both processes simultaneously by looking in a smaller organism like the worm. And moreover, that even within the worm, you can see evolution in action because proteins involved in one role have been recruited from their previous older role. At this point, I'd like to show you my laboratory, and I'd like to acknowledge three very important people. First, Jennifer Powell, who's been showing you these demonstrations in the last several days on the worm. She's a Howard Hughes Medical Institute pre-doctoral fellow, and she's also on the Ask a Scientist team for Howard Hughes. I'd also like to point out Annette Chan, who helped make the videos, and I'd really like to thank Ed Ralston because he's done a fabulous job helping us get these demonstrations together. Thank you very much, and I'd like to turn to house questions.

39. Student question: Can you tag SMC-4 and use it as a marker for mitosis? (52:02)

Yes.

If smc-4 only occurs during mitosis -- if it is only present -- can you tag that and see which cells, exactly, are going through mitosis and use that to catalog the growth of the organism?

That's exactly right. You can use smc as a tag for mitosis. In fact, the way we know that smc for itself is mitosis is if there are other mitotic tags. Histone proteins are modified specifically at different times by phosphorylation, and those phosphorylated forms of histone are also markers for mitosis, as is smc-4. It's absolutely a marker for mitosis. That's a great question.

40. Student question: At what developmental stage does dosage compensation start? (52:41)

I believe we have a question from Moscow. Come in, Moscow.

At which stage of the development of the organism does the complex of dose compensation start to operate? At which stage?

Thank you. The dosage compensation complex associates with X chromosomes at about the 40-cell stage. At that point, sex has already been determined. Xol-1 is definitively repressed. The sdc-2 gene is now activated, and the protein is present, and it can recruit the proteins to the X chromosome. So around the 40-cell stage. It's very early.

41. Student question: Is the dosage-compensation mechanism similar to cell division in any way? (53:19)

Do we have a question from the house? Yes.

You talked about the evolutionary, like -- the ancient role of those proteins. Is there any similarity among the -- like, the actual process of dosage compensation other than the fact that it just happens to do both dosage compensation and... help with mitosis?

The question is whether or not, because the dosage compensation proteins are very similar to the proteins involved in mitosis and meiosis, is not only -- is their function conserved? In other words, does a dosage compensation protein carry out its role in dosage compensation by the same mechanism it carries out its role in mitosis? It's an excellent question. So it boils down to whether or not the X chromosome is condensed by the dosage compensation complex in order to prevent a reduced transcription, and the answer is, we don't know yet. It's one of the question -- actually, a very active part of research that's going on in the lab now. It's a great question. We just don't know.

42. Student question: How does DPY-28 control the number of crossing-overs? (54:29)

I think we have a question coming in from Moscow. Go ahead, Moscow.

How dpy-28 control the number of crossing overs in the chromosome? Crossing overs? Maybe I didn't get it right. How does it control the number of these coursing overs or crossing overs? Yeah. Whatever.

Right. The question is, how is it that dpy-28 somehow, mysteriously and miraculously, helps restrict the number of crossing-over events between chromosomes in meiosis? And the answer is, we don't know yet. That's yet another area of active research. You're all getting the right questions.

43. Student question: Without SMC-4, would the chromosome stay tangled? (55:06)

House question, please. Yes.

In the absence of smc-4, will cells continue -- will chromosome condensation continue in mitosis?

In the absence of smc-4, what happens is that the chromosomes fail to condense properly, and they stay entangled. So if the sisters are supposed to split apart like this, with an smc-4 mutant, they kind of stay tangled, so they can never come apart. So what happens is that they fail to come apart, but there's a new round of replication. So then we get more chromosomes still stuck together, and then the chromosomes still can't come apart, so cell division tries to happen, but that doesn't work, so then we get another round of replication -- I need David Page's hands by now -- and we get more extra -- more and more chromosomes. They still don't come apart, and the animal proceeds in that fashion until you get, basically, one cell that has all the DNA, and the embryo dies because there's many cells that have no DNA or partial pieces of DNA. That's a great question. Thanks for your questions. I've had a lot of fun talking to you. It's been -- it's been great. You've been wonderful. [applause]

44. Closing remarks by HHMI President Dr. Thomas Cech (56:33)

Thank you, Barbara, for another great lecture. One of the things that I liked about it was that it illuminated the fact that a lot of these questions -- you have some understanding, but there's still a lot of areas where there isn't understanding. I also really like your model over here. I think the students should think a little bit more about what it means when one of these blocks hits another one and knocks it over. Barbara gave one example. It can be interaction. It can mean that one of the proteins interacts and binds to another protein and forms a complex that has a different activity. But that isn't the only sort of molecular event that can happen when -- that we're illustrating by the blocks knocking each other over. Sometimes one of the proteins actually chemically changes another protein in the pathway, and in other cases, a protein goes into the nucleus, sits down on a gene, and activates another gene or another set of genes. So there are numerous ways in which these sort of cascades can occur. We're going to now take a break. When we return, David Page is gonna switch back from the nematode worm back to the mammalian and human systems. He's gonna talk about two issues: how do the sex chromosomes -- how do they evolve in the mammals, for one, and then, secondly, how are some of these genes related to the medical problem of male infertility? See you then. [music plays]