

The Meaning of Sex: Genes and Gender
Lecture Four—Sexual Evolution: From X to Y
David C. Page, M.D.

1. Start of Lecture Four (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 fourth lecture is titled "Sexual Evolution: From X to Y." And now, to introduce our program, the senior program officer of the Howard Hughes Medical Institute, Dr. Dennis Liu.

2. Introduction by HHMI Program Director Dr. Dennis Liu (1:05)

Welcome back to the 2001 Holiday Lectures On Science. In just a moment, David Page is going to deliver our fourth and final lecture in this series. Over the past few months, really, we've been working hard to put this show together, and I've got to know David and Barbara quite well, and I'll tell you that they're remarkable people in many ways. They're extremely smart, which is no surprise. They're very skilled, and they're extremely hard-working and as you can see, they take science communication very seriously as well, and if you're thinking about a career in science, it might be intimidating to think about people like David Page and Barbara Meyer, but as you saw in some of the introductory video materials, when they were in high school, they did not know they were going to be scientists. Barbara had an aptitude for math, but David, in fact, wasn't really clear what a scientist did when he was in high school. But what I think got both of them hooked on the idea of science was when they actually started having some research experiences. And Barbara speaks of an addiction for being able to find the answers to questions, to actually being able to come up with an idea and then test whether you're right or wrong. Many different scientists have commented over the years on what it takes to be a scientist. Peter Medawar wrote a book in the late seventies called *Advice For a Young Scientist*, and he, like many scientists, didn't emphasize that you need to be the smartest person around. What you need is diligence, hard work, and a willingness to admit when you're wrong. That's a key aspect of being a good scientist. We hope that these lectures and our web site gives you some insight into what it takes to be a scientist, and, in fact, we've collected on the biointeractive.org web site a feature that we call "On Becoming a Scientist." Because every scientist is an individual and takes an individual path in their career, we think it's helpful for you to hear from a variety of different scientists, and you'll hear that they all have a different story on how they ended up becoming a researcher. Often it's said that a research laboratory is like a family, and it's also like a small business in many ways. Barbara and David each have more than 20 people working in their labs, and David has remarked that science is really a great melting pot, and I think you saw in the videos as well that there's people, all sorts of different backgrounds, working in laboratories. Those people also bring a variety of skills and interests, so there's no such thing as a single right path to get into science. And once again we hope that, if you visit the web site, whether you're interested in a career in science or simply want to learn more about science, which has become an increasingly important part of our economy and everyone's lives, we hope that the things you find on our web site will help that. Well, we'll have a final video to introduce David. David will deliver his lecture, and then, to close the program, HHMI president Tom Cech will return. Thank you.

3. Introductory interview with Dr. David Page (4:31)

I personally feel like I'm very lucky that it's my job to go ask questions and to explore what it means to be a human being at this very fundamental level. Science is not as fundamental as making food or clothing or housing. It's a luxurious activity, and one that I feel extremely privileged to be able to pursue. The part that's most exciting for me is when we put the pieces together, and we sift through a pile of data, and see something that we hadn't even imagined before. I think there's a gap between

science as it's presented in textbooks and science as it lives and breathes in laboratories and in the lives of scientists. And they couldn't be further apart, one from another. I think the decisive thing for me was seeing scientists and getting a sense of their excitement for how scientific knowledge is won, how the battle is fought in the laboratory, and how you gain insights, so that it isn't just stuff that falls out of books. I mean, science isn't in books, it's in laboratories. What makes a good scientist? You have to be a student. You have to be ready to learn new things -- and I mean a student in a very broad sense, somebody who's observant and wants to understand things, is intensely curious. You have to want to understand things. A good scientist has to be pretty skeptical, because a lot of science advances by overturning old ideas. A scientist cannot be faint of heart and cannot give up when something fails. You got to keep on plowing ahead and be fixated on coming to a new understanding or perhaps overturning or modifying an old understanding of how the world works. I think that scientists care deeply about their work. Everybody has things that they really love doing. I think that scientists love the work that they do, and if you're lucky enough to find, in life, something that you love doing and that you feel that you're really good at and that you can pour yourself into, and if science happens to be that, then you're ready to be a scientist.

4. Description of study measuring testosterone levels of participating students (7:04)

Good morning. We were talking about an addiction to science, and I have a problem, and that is that I've been away from my laboratory now for a couple of days, and so I need a data fix. And fortunately, many of you in the room participated in an experiment sometime during the last month or so, and it involved one of these vials. Anybody recognize any of these vials? And, in addition, maybe you had one of these pieces of parafilm, and it turns out that not just many of you students in the room participated in this, but a number of employees here at the Institute also participated. And you were encouraged to take this piece of parafilm and swallow it...mmm. Get this thing out, and then I got to -- Spit in here. OK, you did something like this, probably a little neater than the way I just did it, but anyway, so, fortunately, we've got some data. We got some data back from this experiment, and I haven't really had a chance to study all the data. Remember what this was about? What were we measuring? Testosterone levels. OK, so I haven't really had a chance to study this data, so we're going to put it up here. I'm going to need your help in interpreting it.

5. Testosterone study: Student predictions (8:37)

So, all right, first, I put it up blank because you never do an experiment without a prediction, without a hypothesis. So, what is your prediction as to what we might see? Notice we have plotted along the bottom the age of the subjects. And you're going to see it's going to be plotted into five-year bins, so you can imagine that most of you are going to fall in a bin somewhere sort of between 15 and 20 here, and you can imagine that many of the people who work at the Howard Hughes Medical Institute are somewhat older. OK, so you can expect we're going to see a distribution of ages. But beyond that, what are we going to see? Any predictions as to what we might see when we plot the data? Yes, we've got a suggestion right here. Approximately half, which are the males, will have a higher testosterone level than the females. OK, so, the prediction is there will be a higher level of testosterone in about half the samples -- the males -- than in the females. Another suggestion. We might be able to predict a decline in testosterone as age increases. Ah, you think testosterone might go down with age. OK, well, why don't we go on and see what a little bit of the data looks like.

6. Testosterone study: Interpretations (9:54)

OK, so here are the plots. Now, what do you want to tell me you see? What do you see? What things do you notice about this data? Well, where do you see the highest values? Oh, look, it looks like the highest values are over here in this sort of 15-20 pile, right? And so there does seem to be some evidence of a decline with testosterone in age. Now, there was a prediction that we would see a fairly clean distinction between males and females. Now, let's add sex to this plot. Ah, OK, how's it look? Well, I hear some sighs. What's the problem? Is somebody surprised by something here? Well, can somebody express --

yes? The fact that there's a pink dot on the 15 age, way above most of -- And here's another pink dot. And here are some pink dots, right? And there's a blue dot. You guys thought this was going to be a really good, clean sex marker, right? That would divide the males from the females. Well, it just doesn't look like it worked out that way. I guess -- is the experiment a failure? I don't know. I think we've learned some things from it, right? So, it looks like the distribution of testosterone values, at least in spit -- how relevant is? I don't know -- Testosterone values in spit are clearly overlapping between males and females. We do see some decline, and, of course, if we take the averages for the males and the females, it looks like the averages in the males are about 3-4 times the levels in the females, but clearly, I mean, first of all, some of you might have been thinking that we wouldn't even detect testosterone in females. But anyway, so we see that both testosterone -- and it turns out estrogen, if we had studied that -- play important roles in both sexes.

7. What does the Y chromosome do? (12:14)

So, I want to go back to the larger question, then, of what makes a male. Isn't there more to the Y than SRY? It turns out SRY was actually expressed for just a fleeting moment in that bipotential gonad, at about six or seven weeks of development. Wouldn't you like to think -- at least, wouldn't the males in the room like to think that the Y has some more enduring contribution to maleness? Well, it turns out, the Y chromosome has for many, many years been a badly misunderstood chromosome, and I need to give you a glimpse of that misunderstanding. Ah, but first I want to say, apart from SRY, what does the Y have to do with being male? And that reduces to the question of what other genes are on the Y chromosome, and what are their functions? Now, back to that misunderstood view of the Y chromosome.

8. Cartoon map of Y-chromosome genes (13:15)

Now here's one map of genes on the Y chromosome. I want you to study it carefully. There are a lot of things to be learned here. This is one model of the gene content of the Y chromosome. This is a hypothesis. So, you'll see that -- Please, please, I need order in the room, so I can -- so, you'll see that up near SRY on the short arm, there is the FLP gene, which is located here some of the time, down here the rest of the time. Then there is the BLZ-2 locus, which is self-confidence unlinked to ability. The identifying aircraft, MOM-4U, which is a fascination with spiders and reptiles and such, and then, I don't understand the connection to the testosterone experiment, but we have the P2E locus, and then finally, huh?, the selective hearing loss gene. So, no other chromosome gets cartoons like this made about it. The Y has suffered through years and years of misunderstanding, and it was desperately in need of being rescued by the human genome project -- intellectually rescued.

9. Real map of the Y chromosome: Non-recombining region is passed down clonally (14:35)

And so, it turns out that in recent years, the Y chromosome has been mapped and sequenced, along with the other human chromosomes, and I want to give you a very brief description of what's come out of that genomic study of the Y chromosome. So, here is a version of the Y as we understand it today. So, I want to tell you about the NRY, the Non-recombining Region of the human Y chromosome. And it turns out that that's all the stuff down here, all of the Y chromosome, except the yellow terminal bits, where the X and the Y recombine during male meiosis. Now, in here, in the NRY, this region differs from all other chromosomes in the nucleus, in that, first, it's specific to males, and, second, it doesn't participate in sexual -- that is meiotic recombination. Now, think about that. It doesn't participate in meiotic recombination. Now, there are some males in the room. From whom did you get your Y chromosome? From your father. And who did your father get his Y chromosome from? His father. And so on, and so on. You could trace -- the males in the room could trace the ancestry of their Y chromosomes back to Adam in an unbroken line of male ancestors. Does that sound like sexual reproduction to you? Sounds like clonal reproduction, right? Being passed through one lineage in unbroken form. So, ironically, though we refer to both the X and the Y as sex chromosomes, the Y chromosome actually is reproduced

asexually, clonally, and that has major consequences for its evolution, as we'll see in a couple of minutes.

10. Map of the Y chromosome with its three classes of genes (16:31)

OK, so it turns out, within the NRY, it looks like all the genes are concentrated in this half, the functional part, which spans about 24 million base pairs of DNA, or 24 megabases, a bit shy of 1% of the human genome. Now, within the NRY, we found that there are three classes of genes. Now, the first will come as no surprise. You already know about SRY and its role in causing the gonad to develop as a testis. But that's just one gene on the Y chromosome. Surely, there must be some other contribution to maleness. So, but it looks like the macho part of the Y is not going to come in the second class of genes, because these are cellular housekeepers. It turns out that there are at least 16 genes on the Y chromosome that encode proteins that are involved in basic essential functions in the cell, and just give the name here of one: RPS4. That encodes one component of the ribosome, the machine on which all proteins are translated -- or all proteins are synthesized. Well, now, what did I tell you yesterday was the most fundamental definition of male and female? The most fundamental definition throughout the animal kingdom?

Makes the small gamete.

So, it turns out that -- and I think that was an excellent answer, exactly the one I'm looking for, and so it turns out that the third class of genes addresses this most fundamental aspect of maleness. It turns out the macho function of the Y chromosome, is in sperm production, and there are, in this case, not individual genes, but there are at least nine families of protein coding genes, scattered about the Y, that are involved in making sperm, and one of these is the DAZ gene family that I'll show you a bit about its evolution in a second. Here, I'm showing that there are four virtually identical copies of the DAZ gene. All right. So, it turns out, then, that the Y is a very functionally specialized chromosome. If you looked at most chromosomes, you would find a mix of genes expressed in the liver, the heart, the lung, the skin, and so on, but what we find on the Y is a large fraction of the genes involved in sperm production, and a number of the others, curiously, involved in cellular housekeeping. Now, if those genes are essential on the Y, isn't that a problem? What about females? Don't they need essential functions, too? Well, it turns out that all these cell housekeeping genes have counterparts, have sister genes on the X chromosome.

11. Overview of 300 million years of Y-chromosome evolution (19:27)

And that leads me, then, to... what I want to tell you about, and that is that the human Y chromosome was 300 million years in the making. And so -- And the point I'm going to convey to you is that the X and the Y chromosomes actually, though they are very, very different today -- the X being much larger than the Y, and the Y, which I mentioned here, has on the order of 26 genes and gene families. It has a puny gene content compared with 1,000 or so genes on the X. But I'm going to tell you that if we travel back in time 300 million years, we will see that the X and the Y were virtually identical, and they looked much like the X today. In fact, I'm going to tell you that 300 million years ago -- and this is a progression, a tree of mammalian life. 300 million years ago, when we were reptiles, we existed as males and females, but our sex was likely determined by the temperature at which each of us incubated as an egg. We had no sex chromosomes, only autosomes, and in all these respects, we resembled modern turtles, where sex is likely determined by environmental temperature, and there are no sex chromosomes. So, what I'm going to tell you, and you're going to see on a video that we'll watch in a minute is that 300 million years ago, an irrepressible testis determining, male determining gene arose through mutation on one member of a perfectly ordinary pair of autosomes, and that pair of autosomes became the X and the Y, and the irrepressible testis-determining gene was what we now know as SRY. And so SRY commandeered an ordinary pair of autosomes and took it on a 300-million-year odyssey.

12. Synopsis of the animation on Y-chromosome evolution (21:34)

Now, we're going to follow the course of the X and Y chromosomes down this 300-million-year passage from here down to modern humans. We're going to pass the time when our ancestors parted company with the ancestors of birds. A little later, we'll part company with the ancestors of the duckbill platypus. A little later, we'll part company with the ancestors of kangaroos, dogs, and other primates. And what I want you to look for is that the SRY gene arose by mutation of a pre-existing gene, and for fans of the Y chromosome, this video is going to be a little grisly. You might want to cover your eyes because there's a lot of rotting that went on -- a lot of rotting fruits and veggies -- on this clonal chromosome. And what caused rotting? The Y stopped swapping genes with the X. We're going to look for that stopping of the swapping, and finally, we'll see that the cell housekeeping genes like RPS4 are rare survivors on the Y. They have sister genes on the X, and these XY gene pairs are really living fossils through which we reconstruct the picture. And, finally, I have a little bit of good news for the guys. That is that spermatogenesis genes like DAZ are recent immigrants to the Y chromosomes, so there have been fresh recruits brought into the Y to give it a little bit of life and flow, independent of the X chromosome. So, if we could roll the tape, please.

13. Animation: SRY gene evolves and an autosome becomes a Y chromosome (23:10)

OK, so we're going to focus on a set of chromosomes coming down here, and this set of chromosomes is eventually going to make it all the way down to man. And as it comes down here, we'll blow it up. We're going to focus, in particular, on this one pair, an ordinary pair of autosomes that will become the X and the Y. We'll call it proto X and proto Y. Now, we know that in meiosis, things first get duplicated, so we're going to the 4N stage, and this is just a reminder of what you saw yesterday, that this ordinary pair of autosomes destined to become X and Y can recombine and shuffle genes. They can do it at either end, like so. This is all review for you; This is all familiar stuff. And just to remind you that recombination can occur anywhere along the lengths of this ordinary pair of autosomes. And just to simplify, throughout the rest of the animation, we'll just show one version of the X and the Y. Here is an ancestral gene, which has now mutated on the proto Y. This is the initiating event in sex chromosome evolution. We got this tyrannical male determining gene, and here is RPS4 -- again, an ancient ancestor, which survives today on the Y chromosome. Now, how was recombination suppressed between the X and the Y? Well, it turns out the Y underwent a series of inversions, just like the one shown here. You'll see a few more. And as a result of the inversion, the X and the Y can no longer nicely align. And if they can't swap parts, what's going to happen? The fruits and veggies on the Y rot. The ultimate form of rotting is just plain getting lost and deleted, so the Y is going to get shorter. And now recombination is restricted to these regions that are still nicely aligned, nicely paired, out at the ends. All right, we're going to zoom back to our time line.

14. Animation: Y chromosome degrades over time (25:20)

We're now going to cross the point where we part company with the duckbill platypus, and we're going to have some of the same themes. We're going to have some of the same themes reappear. We're going to have this Y undergo an inversion, which is sort of an internal recombination event. Its ability to pair with the X is again reduced. Now, why isn't the X rotting? The X isn't rotting because two Xs can still recombine in female meiosis. So, genes on the X remain healthy because of ongoing recombination on the female side, whereas the Y is rotting because it is only being passed through a clonal line of males. Let the tape roll. Oh, some more rotting. This is just -- cover your eyes if you can't stand the sight of it. Right. It's very hard for somebody who works on the Y chromosome to actually see this because it could very well be continuing to this day.

15. Animation: Y chromosome also gains new genes (26:29)

OK, now we're going to pass the point where we part company with the kangaroo. And now something interesting is going to happen. Another autosome is going to contribute a fresh supply of genes to both the X and the Y. This kind of renews the game, starts the game rolling again. But, look, the Y just did another inversion, even within some of this newly acquired autosomal DNA. So guess what's going to

happen -- more rotting fruits and veggies. This is still happening -- I mean it's probably not happening in too many of the men in the room right now, but XY recombination still continuing just out at the ends. I think we're going to be desperately in need of some new kind of theme here. Unfortunately, as we move now down into primate evolution -- Well, we're not quite there yet. Argh! More shrinking! Really a tough time. The whole last 300 million years have been quite tough. Now we're getting down into primate evolution. And some good stuff is going to happen, guys. It's OK. Now, this other autosome, this other autosome has a spermatogenesis gene. It's called DAZL. And this autosome is kindly going to contribute a copy of this spermatogenesis gene to the Y, without asking the permission of the X chromosome. Let's let the tape roll. So, we're going to see a copy -- as the tape rolls -- we'll see a copy of the DAZL gene insert into the Y, where it's now called DAZ. And an amazing thing happens. The gene gets duplicated on the Y chromosome, and then those two get duplicated again. So, we end up with four copies of DAZ. It turns out the spermatogenesis genes on the Y -- now, fortunately, none of this red stuff got lost. The spermatogenesis genes on the Y in many cases arose through this process of migration in from autosomes. And, so, there we are. We got the sex determining gene SRY, one of the surviving housekeepers RPS4Y, and spermatogenesis DAZ. This gives us the modern human X and Y chromosomes -- of course, in the context of the genome.

16. Student question: Why doesn't inversion occur on other chromosomes? (28:49)

At that point, I'd like to stop and see if there are some questions. Yes?

In terms of the inversion that caused the X and Y to become really incompatible, that kept them from recombining -- Yes. I mean, maybe this is a dumb question. Why doesn't that happen to all of the other chromosomes?

Ah, great question. I think that question is so good, that even before I answer it, I'm going to -- excellent catch. So, why don't all the chromosomes get inverted? Well, you can imagine that inversions do occur on other chromosomes, but they get selected out. Right? Because they're going to lead to eventual decline. Now, the question, then, is why are they tolerated on the Y? So, they do occur on other chromosomes, but those get weeded out of the population through selection. It's a very, very sophisticated question. Why are they tolerated on the Y? It might be that evolution has chosen to package a group of genes together that will always be transmitted to males and never passed to females. Perhaps there's a set of genes that are good for males and bad for females. We think that's why recombination was suppressed, so we'd have this package of genes always transmitted to males never to females.

17. Student question: How does temperature determine the sex of a reptile? (30:10)

Let's go to Moscow for a question.

In what way does the temperature influence the sex of a reptile?

Right, the question was how does temperature determine the sex of a reptile. Well, I'll give the same answer that's been given many times today and yesterday: We don't know. But let me just tell you what the data are. Contrary to what Aristotle thought -- you recall yesterday, he had some ideas about the role of heat in sex determination -- in most species of turtles, if the egg incubates at about 29 degrees C, it develops as a male. If it incubates at 32 degrees C, it develops as a female. But how that all plays out, we don't yet understand. But that's temperature dependent sex determination, it may well be where we came from 300 million years ago.

18. Student question: Why doesn't the X chromosome undergo inversions? (31:09)

Question, uh, I'll take one more question. Yes?

Why is it only the Y that undergoes the inversions like every single time? Like the first time, the Y underwent an inversion, why did the X just not?

Why did the X not undergo inversions? Because, well, to anthropomorphize, I would say that the X still wished to participate in happy recombination with other X chromosomes in female meiosis. And if it were to undergo an inversion, when it found itself with other X chromosomes, it wouldn't be able to recombine successfully.

19. Deletions on the Y chromosome are a leading cause of male infertility (31:49)

Ok, so we need to move on. Now what I want to say is that this 300-million-year experiment which involved piling spermatogenesis genes onto the Y chromosome, that has a dangerous downside. And that is that deletions on the Y chromosome are the most common known genetic cause of male infertility. Infertility is a very common problem. About one in six American couples is infertile. And in about a third of the cases, the problem traces primarily to the woman, about a third of the cases primarily to the man, and in about a third of the cases, there's some combination of male and female factors at work. Now, when it's the man who's infertile, it turns out that many men are infertile because their semen contain few sperm. Let the tape roll, please.

20. Video: Sperm motility in normal- and low-sperm-count samples (32:43)

Over on the left is a normal human semen sample. You see lots of happy wiggling sperm. And over on the right. You see a fairly wimpy sample with just a few sperm, and they can't even be coaxed into action over here.

All right,

21. Some men with low sperm counts are missing part of the Y chromosome (33:06)

... now, it turns out that some men with low sperm counts, some men with low sperm counts are missing part of the Y chromosome. Now, I have a question for you. What part of the Y chromosome is going to be missing in men with low sperm counts? Is it going to be SRY? Better not be SRY because if it were SRY, you would not be a male. All right. So, a suggestion. I think the men seem to be -- everybody's raising their -- yeah, OK. DAZ? You think that DAZ would be an excellent gene to delete. I think that's a very fine answer. Good. So, let's -- this is like feeding porpoises or something. This is -- So, it turns out that there is a frequent deletion. There's a region of the Y chromosome which is particularly prone to loss. And it contains the DAZ genes, and it turns out that some men with very low sperm counts are missing this region. Now, why are the sperm counts so low in these men? It could be, in theory, because the sperm can't exit the body. But it turns out that in this case, it's because the sperm are not being produced in very substantial numbers.

22. Review of spermatogenesis (34:25)

Now, let's look at where spermatogenesis occurs. Of course it's in the testis. Here we have the internal accessory structures through which the sperm exit the body. But the sperm are produced here in the testis. And it turns out the testis is a collection of tubules in which the sperm are produced. And here is a cross section of one of these seminiferous or sperm producing tubules. Let's look in more detail. So we're going to look at a little piece of one of these tubule cross sections, and it turns out that it takes about two months for a sperm to be produced. We start from cells at the outer edge, out here, and it takes about two months to progress to the middle, where the mature sperm are present. Now, this is where meiosis is playing out, right? Now, you'll recall, and here I'm just showing the gene or chromosome copy number before meiosis. During meiosis when you first double to go to the 4N stage, first division gets you down to the 2N stage, second division gets you down to the 1N, or haploid, stage. These 1N haploids mature physically to become sperm with tails. Well, it turns out that -- Ah, and I should say,

there also are supporting cells. I don't have them labeled in here. There are supporting cells that nurture these spermatogenic cells.

23. Seminiferous tubules of men without the DAZ gene (35:55)

And it turns out that in men who are missing the DAZ genes, and in fact, some other spermatogenesis genes in the immediate locale, it turns out that in those guys, if you look at a cross section of testis tubules, and the guys who are DAZ deleted, you see nothing left, or virtually nothing left, but the supporting cells. The nuclei you're seeing here are not of spermatogenic cells but of supporting cells. Well, and it turns out that men with testis biopsies like this would produce few sperm. They might have some tubules with a little bit of spermatogenesis going on. So they produce very few sperm. And these men would, if out in the wild, where most of us live, they would be infertile. But in the laboratory, it's a different story.

24. Intracytoplasmic sperm injection (ICSI) as a remedy for low sperm count (36:54)

It turns out that these DAZ deleted men do produce a few sperm, and that's sufficient to qualify with their partners for a laboratory reproductive procedure that was developed about 10 years ago, and it is called... or ICSI for short. And in this case, a sperm is injected directly into the cytoplasm of the egg. Do not pass go, no normal fertilization, go directly to the cytoplasm of the egg. Now, how does this work? Well, so let's say we've got a couple where the man has a very low sperm count, and they're showing up here at the door of an assisted reproduction clinic. And what will happen is that eggs will be retrieved from the woman. And a few sperm, if they can be found, will be retrieved from the man. And then will undergo, in the laboratory, a procedure will be carried out; the ICSI procedure will be carried out. And let's see what it looks like. If we can run the movie...

25. Video: ICSI procedure (38:13)

OK. So here we are. We got a needle going around. We got a sperm in there, do you see it? Ah, we're going to clean it up a little bit, make sure it's dead. Now, what is this thing? That's the egg. OK. We're going to place it on the end of what's called a holding pipette. You just suck it up on a straw a little bit to hold it. And now we've got one of those sperm in this injection needle. Do you see it right here? As the tape runs, you're going to see this sperm take a jog to the right and then come back and find its way into the cytoplasm of the egg. Let's let the tape run. OK, oomph! You got to poke it through. There's the sperm going back here. And there it is, deposited in the cytoplasm of the egg. And if all goes well, what has to happen then? You take this egg that's been injected, you culture it in a dish in the lab for a day or two, and then return it to the uterus of the prospective mother. And if all goes well, they return to the clinic a year later to display their baby.

26. Problem with ICSI and male babies (39:31)

Now... but a thorny issue arises. You notice, what's the color of clothing of the baby? Blue, OK, so, we're color-coded here. A thorny issue arises because -- Let's think about the dad here. The dad had a deleted Y chromosome, and he was infertile, at least infertile in the wild, as a result. Now, from whom did this man inherit his Y chromosome? His father. Was his father fertile? Yes. Yeah, that seems highly likely. All right, OK. So, now, did his father have -- What did his father's Y chromosome look like then? It was probably normal. OK. So this deletion represents a fresh lightning strike. This is a new -- this is a big chunk -- this is a lot of rotting all at once, right? Missing a big chunk of the Y. So now, if this man produces a few sperm, half of his sperm will carry an X. If those X-bearing sperm fertilize the egg, what would the result be? A normal girl. If the Y-bearing sperm carries the day and is in this sperm and fertilizes the egg, what will the result be? Right. So by ICSI you would expect to see transmission of the deleted Y chromosome. And this is in fact, this has been observed by now, quite a number of times. So you would anticipate that such a boy would, as an adult, have a very low sperm count and be infertile in

the wild, probably able to reproduce in the laboratory, and perhaps you could have a whole sort of lineage that reproduced exclusively in the laboratory this way.

27. Ethical issues with ICSI (41:29)

So, this raises a number of questions. For instance -- now, I said this procedure was developed about 10 years ago. So now there exist a bunch of boys who range in age from 0 to 10, who carry these deletions. So, I just pose to you, among the variety of questions, at what age should the ICSI-conceived son be told about the Y deletion and the likelihood that he will have a very low sperm count as an adult? Or let's turn the question back a little further. What if the couple, what if the man in this couple had had wide DNA testing done in advance of the procedure? So what if the man with the low sperm count has Y-DNA testing done before ICSI and knows he's got the deletion? Well, if you were the genetic counselor, or just a friend of this couple, what would you encourage them to do? Would you say, maybe you should think about adoption? Should you go ahead with the ICSI procedure and just be prepared to tell your child if it is a son that he will be a laboratory reproducer in the future? Should you consider using donor sperm so that at least the woman could make a genetic contribution to the child? Or perhaps the most technologically advanced option would be to take advantage of the fact that when you do this procedure, you don't just inject one egg with one sperm, you collect several eggs and inject each of them with one sperm and then culture them in individual dishes. On average, half of these are going to be XX embryos. So you could -- what if you could identify, and in fact, you can identify the XX embryos, and selectively return those to the womb? And so these are among the difficult questions, whose number grows as human reproductive technology advances. So thinking about everything that we've said today and yesterday, let us pose some broad questions. Should we clone ourselves, or should we stick with sexual reproduction? Should we reproduce in the wild or should we transfer that biological task to the lab? And I just want to close by acknowledging my own lab, which -- we don't actually -- if you're interested in reproducing, don't, I mean -- Anyway, here we are. Here's a snapshot of many of the people in the lab, and you'll notice in particular, down at the bottom, here's an X chromosome, and here's a Y chromosome. And they appear to be undergoing some form of aberrant recombination right here. And I particularly want to draw your attention to the exposed centromere of the Y chromosome. Right here. And with that I'll invite your questions, difficult or otherwise. Yeah? Yes.

28. Student question: Does ICSI always work? (44:57)

I was just wondering, when you use the sperm injection procedure does this ever fail, and if so, why?

Ah, does ICSI always work? Well... about 1/3 of the time, the couple takes home a baby. Why might it not work? Well, it turns out that human reproduction is, at best, a very iffy business. A very large fraction of all embryos are lost or aborted spontaneously. So that problem is not unique to reproduction in the wild. The same applies because chromosome anomalies and all sorts of things can cause an embryo's development to go awry. So, but about 1/3 of a time, and if a couple tries repeatedly, they can, of course, increase the chance.

29. Student question: Does my dog have more genes on its Y chromosome than my brother has? (45:58)

OK. A question in the back. Yes, you in the red.

This is in relation to the... Over time, the mutations having more effect on the Y chromosome.

Yes.

So does that mean that, present day, my dog has more genes in his Y chromosome than my little brother?

Does your dog have more genes? I think that definitely deserves a T-shirt. Oh! Oh! Where did it go? Hey! It's landed in the ceiling. This is incredible. Never happened before. OK, so back to the question. Back to the question. Does your dog's Y have more genes than your brother's Y? Well, maybe I should meet your dog and your brother, and then we'll answer that one. We don't -- It actually takes me to an interesting question, and this is that we need to study the Y chromosomes of other organisms. It turns out the human Y chromosome is far and away the best understood Y chromosome at present. We desperately need to understand the Y chromosomes of flies. You can imagine that we won't learn a lot about the Y chromosome by studying nematodes, right, right? That was one of the ultimate insults to the Y chromosome -- to completely do away with it. But we need to understand more about the Y chromosomes of other organisms. We know something about the mouse Y, but not much about the Y chromosomes of other organisms.

30. Student question: In ICSI, how is the embryo returned to the uterus? (47:41)

A question right down in the front.

You said that in lab fertilization when the egg is injected in the sperm and then cultured and then it is returned back to the uterus -- can you just give a brief explanation how it's returned back to the uterus.

So it's actually placed back in through the vagina and into the uterus. But they're cultured for a day or two in advance. They're actually cultured in dishes and the healthiest looking embryos are those that are returned.

Is there a possibility that it could be damaged by the insertion if the person doing it doesn't have total expertise in what he's doing?

Well, you can imagine -- The question is whether the person doing the procedure can screw up. Absolutely, that's possible, but these are highly trained professionals. I think that the reality is that many of the most important variables and factors are really out of our control. They're things that we can't see about the quality and developmental potential of these embryos. But, again, about 1/3 of the time, about 1/3 is the take-home baby rate.

31. Student question: Will evolution lead to the complete destruction of the Y chromosome? (48:58)

Yes. Oh, I'm sorry. We're gonna go to Moscow for a question.

Can you say that evolution leads to the complete destruction of X chromosomes? I'm sorry, Y chromosomes.

Good correction there. So the question is whether evolution will lead to the complete destruction of Y chromosomes. Well, this is a very profound question. I mean there are multiple possible outcomes. I would actually argue that in the case of *C. elegans*, that's exactly what happened. I would bet that somewhere back there in the evolution of *C. elegans* there was a Y chromosome, and it was dispensed with. Now there are two other possible outcomes for our sex chromosomes and our Y chromosome. Well, it could continue to shrink and rot, but what will keep it vital? If we have ongoing migration of new spermatogenesis genes in from autosomes. And then, remember, there was that time after we parted company with the ancestors of kangaroos when we acquired a whole chunk of another autosome and stuck it on the top of the X and the Y. Well, if we took that theme to its logical extreme, we would simply gather in all the autosomes and stick them on the top of the X and the Y and turn it into one big X and Y chromosome. So I don't think we're about to do away with the Y, but I am a little nervous about it.

32. Student question: Will Y-chromosome deletions always be transmitted? (50:22)

Yeah, a question from the house in the back.

When you gave the example of a couple that would use the ICSI procedure, you mentioned the grandfather had -- probably had a complete Y chromosome.

Yes.

And that the father had a missing portion, DAZ portion. Is it possible that the father could pass his process on to his son and that the son would also lose a portion of his Y chromosome as time went on? And, like, it would go through the generations?

Right, so the question is how is this Y deleted or otherwise transmitted. So when we look at the man who has a very low sperm count and we find a Y deletion, with very rare exception, when we look back at his father, we find an intact Y. But when we look at the boys who are fathered by -- who are conceived via ICSI from DAZ deleted men, using sperm from DAZ deleted men, in every case to this point we've seen a deletion that is identical to that present in the DAZ deleted father. OK, so we're seeing clean transmission of a deleted Y chromosome. That seems to be the rule thus far.

33. Student question: Could we program the sex of a would-be child? (51:37)

Back to Moscow for another question.

Could you program the sex of a would-be child?

Could we program the sex of a child? Well, we can't actually flip the switches for SRY and that sort of thing, but it is very possible, in the case of assisted reproduction, where you have XX embryos and XY embryos having been produced by this laboratory introduction of eggs and sperm one to another. In that case, it is possible to identify the XX embryos or the XY embryos and to selectively reintroduce one or the other. Now of course there are -- Immediately there arise ethical and other issues that need to be considered, but, in theory, it is possible to do that. In this case, with the DAZ deletions, I think that most medical professionals would be quite comfortable with selectively reintroducing the XX embryos if that's what the couple wanted to do.

34. Student question: What is considered a normal sperm count? (52:47)

OK, another question down here in front.

In terms of the sperm count, you had a low end and we had a normal. Is there a high end also?

So the question is how much do sperm counts vary. Sperm counts vary tremendously and the actual counts, a normal sperm count is considered anything above 20 million per milliliter, but the counts can-- typical counts are 50 or 100 or 150 million per milliliter. So there's tremendous variation in sperm count in human populations. What causes that variation we really don't yet understand.

35. Student question: Why do flies have the Y chromosome but reptiles don't? (53:33)

Back to Moscow for another question.

You talked about chromosomes in animals, but you know that such chromosomes one can come across in *Drosophila* fruit fly. How would you account for that?

Well, there are, OK -- So the question concerned the Y chromosome in flies. It turns out, and it's a point I'd love to emphasize, that -- so I made the point that the human X and Y chromosomes arose from the

commandeering of an ordinary pair of autosomes and turning them in to X and Y. It turns out that that experiment of nature, to take an ordinary pair of autosomes and have them become sex chromosomes, that experiment has occurred repeatedly throughout nature. So it turns out birds have the Y chromosome in females, and it turns out the bird, what are called the sex chromosomes in birds, are actually the counterparts of what is autosome 9 in humans, OK? So an autosome that lives on in us today, chromosome 9, has become the sex chromosome in birds. And back to flies, it looks like the X and the Y chromosomes in fruit flies represent, yet again, an independent experiment of nature with the same theme: picking a pair of autosomes and turning them into an X and a Y chromosome. This game is played out repeatedly, presumably starting from the situation where there are no sex chromosomes, environmental sex determination.

36. Student question: Could we reinsert DAZ genes in sperm with DAZ deletions? (55:16)

One quick last question. Yes?

No pun intended, but is there any conceivable way that you could reinsert the DAZ gene into the Y chromosome of the infertile father so that they baby that -- the embryo that is implanted in the uterus is not infertile and has a normal sperm count?

OK, so the question is could we conduct some kind of gene therapy on the sperm from the DAZ deleted man so that his son would receive, let's say, some DAZ transgenes in addition to the Y chromosome. Well, in theory that might be possible. I think we're technically a long way off. We're also ethically a ways off because we'd be talking, in that case, about so-called germline gene therapy. Well, all these questions have been terrific. It's been extremely fun to spend these two days with you and thanks for all your great questions. I'll say good-bye. [applause]

37. Closing remarks by HHMI President Dr. Thomas Cech (56:31)

I know from my own teaching how challenging it is to take these really complex scientific topics and to make them understandable and interesting to a variety of different audiences, and I think that David Page and Barbara Meyer have done just a remarkable job, obviously after having put in a lot of effort preparing the visual aids and preparing their remarks. I think that all of you, through listening to these lectures, have gotten more of an appreciation for the process of science, and how scientists go about asking and answering questions, and the uncertainty that still lies in many of these fields. So we hope that that's something that all of you take away with you. On the other hand, we do hope that a few of you will be inspired by having heard these lectures and by your other experiences to take more courses in science and to consider becoming a research scientist, a research clinical physician, and to engage in these sorts of questions directly with your own hands. Are we going to continue these Holiday Lectures in 2002? Absolutely. We have a very different topic in store for you. It's something that you read about in the papers all the time. You heard a little bit about it from David Page's work with the Y chromosome: The Human Genome, The Area of Genomics. The bioinformatics that the computer programs that people use to analyze genomic sequence data, and also the emerging area of chemical genetics. The speakers are going to be Stuart Schreiber, who's a professor at Harvard University in Cambridge and also a Howard Hughes Medical Institute investigator, who's well-known for his ability to merge the science of chemistry and organic chemistry with biology to address important medical questions, and secondly, Eric Lander, who's a professor in the same department that David Page is in at MIT. He set up the Genome Center at the Massachusetts Institute of Technology, founded Millennium Pharmaceuticals, and it's a name that you've seen in the New York Times and in Time and Newsweek Magazine and in all the popular press over that last couple of years. So we look forward to hearing both of their lectures. Please let us know how you felt about the program -- whether there's anything that we should be sure to keep in it or anything that you think we could improve. Our mailing address is going to be flashed on the screen by the end of the program. And now, from all of us here at the Howard Hughes Medical Institute, I'd like to wish you a happy, healthy holiday season. Thank you. [applause]