HHMI's 2001 Holiday Lectures on Science
The Meaning of Sex: Genes and Gender

Lecture One
Deciphering the Language of Sex
David C. Page, M.D.

"It's a boy!" "It's a girl!" shout the banners and balloons that usually herald the arrival of a new baby. They're expressions of welcome and joy, but what do these exclamations really tell us? Just what is a male? What is a female? The questions may seem stunningly simple, but they're actually rather complex. Sex, it turns out, can be defined on a number of different levels, from anatomical to psychological. So before we can discuss-or study scientifically—how we come to be one sex or the other, we need to think more carefully about what we mean by male and female. Examining the distinctions will lead us to the heart of sex determination.

First and foremost, males and females sport obvious differences in external genitalia-penis versus clitoris, for one. Taking note of anatomy is a time-honored method for telling male from female: it's the way an obstetrician traditionally determines the sex of a newborn baby.

Of course there's more to sex than external anatomy. Men and women have different gonads, testes, or ovaries, and produce different gametes, sperm versus eggs. Even their chromosomal complement is different: Females have two X chromosomes, and males have an X and a Y.

How do these differences arise? In one sense, the sex of a baby is determined at the moment of conception. The egg, which always carries an X chromosome, will either be fertilized by a sperm bearing another X-generating a female—or a sperm bearing a Y-generating a male. Other than that single chromosome

difference, human male embryos and female embryos are identical until the seventh week of development. At that point, a sex-determining gene on the Y chromosome—if it's present—sets in motion a cascade of biological activity that results in the development of a male. This genetic trigger directs the bipotential gonad—the primitive tissue from which both male and female sex organs derive—to turn into testes rather than ovaries. The testes then produce the hormones, including testosterone, that prompt the development of all other male-specific characteristics, including the external genitalia and, later, secondary characteristics such as facial hair and a deep voice. In the absence of a Y chromosome, the embryo will develop female structures.

But the process is not flawless. Once in a while, an XY embryo will develop as a female, complete with ovarian tissue instead of testes; likewise, an XX embryo will sometimes develop as a male. Such sex-reversed individuals, unaware of the chromosomal mismatch, often show up at the clinic when they experience problems with infertility. As it turns out, most XX males actually harbor a small piece of the Y chromosome, which has gotten stuck onto the end of one of their Xs; and some XY females are missing the corresponding small fragment from the tip of their Y.

This discovery suggested to researchers that the part of the Y chromosome that directs the development of a male lies in that small region, which is lost or gained in sex-reversed individuals. A genetic manhunt subsequently turned up SRY (sex determining region Y), the gene that acts as the master switch controlling male development in all mammals, including humans.

Why sex chromosomes evolved is still a mystery. Many reptiles, including alligators and certain turtles, develop as male or female depending on the environment. Their sex is determined by the ambient, or surrounding, temperature. Such a system might seem simpler than maintaining special sex chromosomes, but it, too, has its shortcomings. Perhaps some years the climate

will foster the development of many more males than females. Such a situation could hinder reproduction and survival of the species. At least sex chromosomes help to keep the sex ratios relatively stable-two chromosomes, two sexes, usually present in about a 50:50 split.

That raises another interesting question: Why do we need two sexes in the first place? Seems a bit of a waste, really. What if there were only one sex, and each one of those individuals could reproduce an exact replica of itself? In that case, if the population needed to expand in a hurry, every single individual could produce a clone—or a handful of clones. In a way, having two sexes—only one of which can bear young—cuts the reproductive capability of a population in half. So why have males at all?

Further, why have sex at all? One oft-cited benefit of sexual reproduction is that it allows a population to be adaptable, as it promotes the generation of new combinations of genes. But what are the costs—to the organism and to the species? Do the benefits of sex really outweigh the costs? These seemingly simple questions remain hotly debated and enthusiastically explored.

Key Concepts

- Most animals come in two readily distinguishable forms, a property called sexual dimorphism. In mammals, including humans, these two sexes are male and female.
- The sex of an individual can be classified on a number of levels, taking into account, for example, external and internal anatomy, chromosomal makeup, and the type of gametes he or she produces.
- Generally speaking, the male comes equipped with a penis, scrotum, and testes; he produces sperm and has both an X and a Y chromosome. The female, on the other hand, has a clitoris, labia, and ovaries; she makes eggs and has two X chromosomes.

- Aside from the chromosomal differences, in humans male and female embryos are identical until the seventh week of development.
- Sexual differentiation begins when a sex-determining gene on the Y chromosome directs the bipotential gonad-the primitive tissue from which both male and female sex organs derive—to turn into testes rather than ovaries.
- Hormones produced by the testes or ovaries subsequently shape the development of other sexually dimorphic structures, including the external genitalia, and, eventually, the secondary characteristics—facial hair for males, breasts for females.
- In the absence of a Y chromosome, the embryo will develop female structures.
- The process of sexual differentiation is disrupted in sex-reversed individuals, people whose outward appearance contradicts their genetic makeup. One in 20,000 males lacks a Y chromosome; these XX males are genetically female but have a penis and testes. Similarly, one in 20,000 females has a Y chromosome; these XY females are genetically male but have ovaries and appear to be female.
- Study of such sex-reversed individuals led researchers to discover the part of the Y chromosome that directs male development. Most XX males, it turns out, harbor a tiny piece of the Y chromosome—inherited from their fathers—stuck on the end of one of their Xs. And some XY females are missing this same small piece from their Y.
- Genetic dissection of that fragment of the Y chromosome led to the identification of SRY (sex determining region Y), the gene that acts as the master switch controlling male development in all mammals, including humans.
- Why the sex chromosomes evolved is still something of a mystery. Reptiles, including alligators and turtles, have no sex chromosomes and rely instead on ambient temperature to dictate the sex of their offspring.

- Although all mammals come in two sexes, the benefits of this arrangement remain arguable. Why we have sex—and why we need two sexes—are topics that continue to be debated and explored.

Lecture Two
Hermaphrodites: The Safer Sex
Barbara J. Meyer, Ph.D.

Talk about having it both ways. Worms not only come in two sexes, but one of those sexes is two sexes in itself. *C. elegans*—a slender, soil-dwelling roundworm that measures one millimeter from nose to tail—can be either a male or a "hermaphrodite," a sort of souped-up female that produces both sperm and eggs.

What's more, a single hermaphrodite can procreate all on her own—a trick that comes in handy for the efficient propagation of the species. Although hermaphrodites can (and do) mate with males, they can also self-fertilize.

The hermaphrodite's specialized anatomy allows her to accomplish this biological feat. Not only does she produce both eggs and sperm but she also has a sex-specific set of neurons and muscles that control egg laying. Even her gut is different from the male's, adapted to produce yolk to fill her eggs. Male worms, on the other hand, are slimmer and have elaborate tails equipped with structures that allow the male to detect and inseminate an appropriate mate. The hermaphrodite's tail, in contrast, is a simpler, whiplike structure.

If hermaphrodites can reproduce themselves, then why bother to have males? Self-fertilization would seem to be the most reliable and efficient reproductive method around. It seems, though, that when the environment is unstable or the species is subject to physical or chemical attack, hermaphrodites may find males "useful." When hermaphrodites mate with males, new combinations of genes are introduced into the population. This breath of genetic fresh air can allow the species to weather changing environmental conditions.

So useful are males in times of stress that hermaphrodites start giving birth to them. Normally hermaphrodites spawn other hermaphrodites. But when hermaphrodites are stressed, say if they are exposed to an unusually warm environment, they produce males. And they do it by dropping an X—an X chromosome, that is.

Worms, like humans and other mammals, specify their sex with a sex chromosome. But instead of having an X and a Y (where Y directs the development of a mammalian male), worms have only an X to work with. To determine their sex, then, worms count their Xs: Males have one X (designated XO) and hermaphrodites have two (XX). Thus, when hermaphrodites self-fertilize, they produce other hermaphrodites. Stress a hermaphrodite and she might experience trouble handling these chromosomes during gamete formation or fertilization. And if an X gets lost in the genetic shuffle, a male is born.

Using the number of Xs to specify sex seems simple enough, but how does a worm count how many Xs he or she has? The animal looks to *xol-1*, the master switch gene that controls sex determination in the worm. When *xol-1* is active, it produces XOL-1 protein, which initiates male development; when the gene is switched off, and XOL-1 is absent, a hermaphrodite develops.

And the amount of XOL-1, it turns out, is controlled by genes on the X chromosome. These X-based regulatory genes encode proteins that turn off *xol-1*. Two Xs can really shut down *xol-1*, but the single X chromosome in the male doesn't make enough inhibitor proteins to keep the master gene fully repressed, so XOL-1 accumulates. Male worms actually produce 10 times more of this protein than hermaphrodites do. Thus, XOL-1 translates the number of X chromosomes into the development of a male or a hermaphrodite.

The worm provides a particularly powerful system for unraveling such complex biological processes. Each worm produces hundreds of offspring in a few days,

and they are easy to manipulate genetically. To find out what a gene normally does in an organism, scientists simply eliminate or alter it and then see how that change affects the animal. Such genetic manipulation has allowed scientists to identify the molecules involved in most aspects of life, including the development of the different sexes. Many of the genes involved in sex determination were discovered when mutations in them reversed the worm's sex, turning a genetic male into a hermaphrodite or vice versa.

As researchers decipher what each of these sex genes does, they fill in a piece of the picture about how a worm develops into a male or a hermaphrodite. The story does not end with *xol-1*—it begins there. Sex determination requires a series of steps within cells and tissues during which one gene's product acts on the next in line. Once the *xol-1* master switch is flipped, it activates a genetic cascade in which subsequent sex-determining genes are turned on or off, as appropriate. Each gene passes a message along to the next until every cell has received the directive: This worm is male or this worm is hermaphrodite. These genes, in turn, trigger the development of particular sex-specific traits—producing the proper sex organs, the correct tail structures, or an appropriately patterned nervous system.

Although the cascade may seem unnecessarily complex, such elaborate designs are par for the course in biological systems. That's because nature doesn't build animals or cells or even molecular pathways from scratch; evolution instead takes whatever biological materials are at hand and cobbles together new molecular systems by tinkering with preexisting ones. The resulting structures and systems are not always the most elegant, but somehow they work. And when all the genes involved in sex determination in worms work together, the end result is a sleek, sperm-producing male or a well-proportioned, self-sufficient hermaphrodite.

Key Concepts

- Worms provide a powerful experimental system for studying many biological processes because they're easy to grow in the lab and can be genetically manipulated. By eliminating or altering genes in an animal, scientists learn how these genes function in the organism.
- Worms have two sexes, male and hermaphrodite; hermaphrodites can fertilize themselves, and they can also mate with males.
- Many anatomical features of males and hermaphrodites differ, including their tail structures, nervous systems, and intestines.
- Scientists still debate why sexual reproduction evolved, but they generally agree that the process can rapidly produce a lot of genetic variation, perhaps benefiting species by generating individual organisms that can survive different types of environmental stress.
- Sex in worms is determined by counting the number of X chromosomes; animals with one X are male and those with two Xs are hermaphrodites.
- The gene *xol-1* acts as a switch early in the sex-determination pathway, interpreting the number of X chromosomes and delivering the appropriate sex signal to all tissues.
- The amount of XOL-1 protein is controlled by regulatory genes on the X chromosome that turn off *xol-1*. Males make 10 times more XOL-1 than hermaphrodites do because the single X chromosome in males does not make enough inhibitor protein to fully repress *xol-1*.
- In response to the *xol-1* master sex gene being "switched on," a cascade of genes is activated to control the process of sex determination.
- Mutations in certain sex-determination genes can turn a genetic male into a hermaphrodite and vice versa.
- Genes that are unique for each cell type translate the common master signal into molecular messages that create sex-specific organs and tissues.

Lecture Three
Sex and Death: Too Much of a Good Thing
Barbara J. Meyer, Ph.D.

Sex determination in worms seems one of nature's more simple designs. One X makes a male, and two Xs, a hermaphrodite. Such simplicity, however, comes with a price: Fail to balance the activity of the genes on those Xs and you die.

The conundrum comes from a problem with gene dosage. Because hermaphrodites have two Xs, they also have a double dose of every gene on the X, even the ones that have nothing to do with sex. Genes make proteins, so hermaphrodites stand to produce twice as much X-encoded protein as males. Such genetic imbalance can be harmful in any organism: Down's syndrome in humans is caused by having an extra copy of chromosome 21. For worms, the extra X can be lethal.

To survive, hermaphrodites have to cut in half the amount of X-encoded protein they produce. To equalize their X proteins, hermaphrodites rely on a process called dosage compensation, which allows them to turn down the activity of all the genes on the X chromosome at once. In the hermaphrodite, proteins involved in dosage compensation bind together and then blanket both of the X chromosomes, decreasing simultaneously the expression of all the genes by 50 percent. This level of control is superimposed on the "normal" types of regulation—the ones that switch on or off single genes or small gene clusters.

The need for dosage compensation is not a phenomenon exclusive to worms. Most organisms that rely on chromosomal strategies to determine sex face a similar genetic dilemma. Interestingly, different animals have come up with different solutions to the problem. In humans—where females are XX and males are XY—female embryos undergo a process of X inactivation. In this process, females completely shut down all the genes on one of their Xs. Fruit flies use a

dosage compensation process that is the mirror image of the approach used by worms. Instead of reducing the gene activity in the XX female, flies double the activity of all the genes expressed on the male's single X, bringing levels of X-encoded proteins into parity between the sexes.

Globally controlling the expression of thousands of genes across a chromosome is a mammoth task. Worms, fortunately, could borrow some of the proteins they needed for dosage compensation from the cellular machinery that was already involved in chromosome segregation. During cell division, these molecular complexes decorate each chromosome, where they help the cell dole out its DNA to its daughter cells. Cells that have defects in the genes that encode the proteins in these complexes deliver an unequal number of chromosomes to each daughter. To exploit these dual-purpose proteins for regulating gene dosage, other proteins involved exclusively in dosage compensation simply recruit them to the X chromosome.

When dosage compensation fails, a hermaphrodite will die. This sex-specific link between dosage compensation and death, while tragic for the worms, has actually been a boon for biologists; it has allowed them to discover many of the genes involved in dosage compensation. Mutations that cause death only in a hermaphrodite point to genes that help turn down X gene expression. By studying such mutants, geneticists found the *sdc* genes. These genes encode proteins that physically interact with the X chromosome and recruit other proteins, eventually forming a huge complex that inhibits gene expression.

Although it's less obvious than having an elaborate tail or making eggs, the activation of dosage compensation is another critical difference between males and hermaphrodites—it occurs in only one sex. So how does the dosage compensation machinery know whether an animal is male or hermaphrodite? As it happens, many of the genes involved in sex determination are also involved in

dosage compensation. Although the pathways diverge later on, they share similar genes at the start.

Again, *xol-1* acts as the master switch. Here's how it works: Recall that XOL-1 protein initiates the developmental program that makes a male. It does this by shutting down the *sdc* genes. In addition to their role in dosage compensation, these genes direct the development of a hermaphrodite. So in XO male worms, repressing the *sdc* genes does two things. It makes the animal male by turning off the developmental program that would otherwise produce a hermaphrodite, and it turns off dosage compensation. The latter is critical. A male that does not inactivate dosage compensation will produce half the amount of X-encoded proteins he needs to survive—a lethal mistake. Similarly, a hermaphrodite with defective *sdc* genes will fail to activate dosage compensation and will also die.

The study of worms has revealed an elaborate and sophisticated system for determining sex and controlling the activity of genes on the X chromosome. These simple organisms have helped scientists elucidate processes that share many features with events in mammalian cells. Some of the findings are directly applicable to humans. Many of the worm genes that are involved in chromosome segregation, for example, resemble those in organisms from yeast to people. Already, studies on numerous cellular processes have revealed similarities between worms and humans, and the surprisingly small number of human genes revealed by the Human Genome Project suggests that we may be even more like our nematode neighbors than researchers anticipated.

Key Concepts

- Because a genetic imbalance can be deadly, hermaphrodites—which have two X chromosomes compared with the single X of males—must turn down the production of proteins encoded by genes on both Xs. The term for this process is dosage compensation.

- In worms, dosage compensation operates by gene repression, the ramping down of expression of genes on the X chromosome by 50 percent in XX animals. The results are similar to the outcome of X inactivation in humans, whereby females shut down most of the genes on one of their two X chromosomes.
- To repress all the genes on the X chromosomes of hermaphrodites, a complex of several proteins work together to bind along the Xs and reduce gene activity.
- Defective forms of genes normally involved in dosage compensation hurt hermaphrodites but not males; this characteristic allows scientists to identify mutations in the process by seeking mutations that cause hermaphrodite-specific lethality.
- Dosage compensation evolved by borrowing genes from the more ancient process of chromosome segregation. Some proteins in the worm have retained their original role in chromosome segregation and added the new role of regulating gene expression.
- Proteins specific for dosage compensation recruit other gene-silencing proteins onto the X chromosome preferentially.
- Dosage compensation, like sex determination, is a sex-specific process and is controlled by the same master switch gene.
- A single pathway controls the initial stages of dosage compensation and sex determination but later branches to execute these distinct tasks.
- Chromosome segregation in unrelated organisms such as yeast, worms, and humans use genes that resemble each other. Some of these genes are also involved in dosage compensation.

Lecture Four
Sexual Evolution: From X to Y
David C. Page, M.D.

It's not much to look at. Under a microscope, the Y chromosome is a tiny rod only one-third the size of all the other human chromosomes, including its partner, the X. But for a puny scrap of genetic material, the Y chromosome packs a real biological wallop. It sets in motion the development of everything needed to make a male—directing the production of not just the proper external equipment but also the internal accessory organs and a trillion or so sperm.

But the Y chromosome, as important as it is for sex determination, didn't always exist. In fact, both the X and the Y derive from what was once a perfectly normal pair of chromosomes that had nothing to do with sex. Recall that reptiles come in two sexes, even though they do not have sex chromosomes. So the X and Y were born sometime after mammals and reptiles diverged, about 300 million years ago. In a sense, turning a pair of autosomes (the term used to describe all non-sex chromosomes) into the X and Y has been a remarkable 300 million year experiment—one that is still ongoing.

What did these ancestral chromosomes look like? The answer lies in the genes that are common to both the X and Y. Although the Y houses a handful of genes that make a male, about half the genes that reside on this chromosome are also found on the X. They encode proteins that take care of general housekeeping and cellular maintenance tasks, needs shared by both sexes. At first researchers handled these shared genes as oddities. Now these genes are recognized as living fossils, representatives of the genes that were present on the pair of identical chromosomes from which X and Y sprang.

Somewhere along the way, though, the X and Y parted company—each to follow its own evolutionary path. The Y most likely came to be when it somehow

acquired *SRY*, or a gene that performed a similar role in sex determination. Then, at some point, X and Y lost the ability to recombine—a process in which chromosomes pair off and swap bits of genetic information. Such swapping is necessary for maintaining chromosomal integrity. In females, the two Xs can still partner with one another and exchange DNA. But with no proper partner, the Y began to unravel, losing many of its genes. Such genetic decay would explain why the Y chromosome has only 30 or so genes while the X supports thousands.

But the Y chromosome is more than a rotting X. It also harbors genes that are male specific. Some of these genes, it appears, used to be located on various autosomes; they relocated to their home on the Y some 30 to 50 million years ago. This genetic migration may represent an opportunistic move—perhaps the Y provided a safe haven for genes that benefit males but are inconsequential or even somehow harmful to females. Sequestering such genes to chromosomal regions that are present only in males may have helped keep harmony within the species.

Many species, from the fruit fly to the mouse, carry genes essential for male fertility on their Y chromosomes. In humans, the genes involved in sperm production have also made their way to the Y. This evolutionary trend has clinical ramifications: Deletions on the Y chromosome can cause infertility, a problem that affects about 3 percent of men.

Thanks to the mapping and sequencing of the Y chromosome, researchers are now unraveling the mechanism by which these deletions may occur. In some cases, it appears that recombination can take place between sets of matching sequences present on the Y chromosome. So a single Y chromosome might fold over, line up these similar patches of genetic sequence, and then accidentally delete everything that lies in between—including genes that are important for sperm production and development.

Because infertile males cannot pass these defects along to their offspring, such deletions represent new mutations—changes that occur very early in a male embryo or even in the single sperm that donated its Y on fertilization. But there have been rapid advances in reproductive technologies. Now infertile males who produce at least a small amount of sperm can become biological fathers by a procedure called ICSI—intracytoplasmic sperm injection. Doctors can obtain isolated sperm cells from these otherwise infertile individuals and then inject them directly into a female oocyte to achieve fertilization.

Unfortunately, any sons conceived by this method will also possess the defective Y chromosome, making them infertile as well—unless they opt to undergo a similar procedure when they decide to raise a family. This raises the eerie possibility that someday entire lineages may reproduce only with the aid of a laboratory technician. In time, society will need to grapple with the long-term consequences of such interventions.

Until then, scientists will continue to study the sex chromosomes—particularly the Y, which despite its small stature continues to make important contributions to our understanding of sex, chromosomal evolution, and human biology.

Key Concepts

- The X and Y sex chromosomes did not always exist. They evolved some 300 million years ago from a pair of identical autosomes—chromosomes that have nothing to do with sex.
- Today the sex chromosomes look quite different. The Y is one-third the size of the X and contains only 30 or so genes, as opposed to the thousands present on the X.
- Half the genes that reside on the Y chromosome also have a counterpart on the X. These common genes can be considered living fossils; they provide a snapshot of the ancestral autosomes from which these sex chromosomes derived.

- The X and Y chromosomes most likely parted company, evolutionarily speaking, when they lost the ability to recombine—a process in which chromosomes pair off and swap bits of genetic information. Such swapping is necessary for maintaining chromosomal integrity.
- Without a recombination partner, the Y chromosome decayed, losing many of its resident genes. With every passing generation, this tiny chromosome continues to evolve.
- X inactivation evolved to compensate for the fact that most of the genes on the X chromosome are present in a double dose in females. This process shuts down one copy of these genes in all the cells of the female, so that she does not end up producing unnecessary—or indeed toxic—amounts of the proteins they encode.
- The Y chromosome harbors many genes that control male development and fertility. These male-specific genes appear to have relocated to their home on the Y some 30 to 50 million years ago.
- Because genes that control the development of sperm are located on the Y, deletions on that chromosome can cause infertility.
- Mapping and sequencing of the Y chromosome have revealed a mechanism whereby such mutations may occur. It seems possible that a single Y chromosome might fold over, lining up patches of matching sequence, and accidentally delete all the information in between—including genes relevant to sperm production.
- Males that are infertile cannot pass these defects along to their offspring by the conventional means. Thus, these deletions represent new mutations that occur very early during embryonic development or in the single sperm cell that donated its Y during fertilization.
- Rapid advances in reproductive technologies allow some infertile males to become biological fathers. Using techniques such as ICSI (intracytoplasmic sperm injection), doctors can insert individual sperm cells obtained from these otherwise infertile men directly into an egg. Any

sons produced by such a procedure, however, will inherit their father's defective Y chromosome and will themselves be infertile.

- Studies of the X and Y chromosomes will continue to bolster our understanding of sex, chromosomal evolution, and human biology.