

Challenge Questions

Lecture One

Deciphering the Language of Sex

David C. Page, M.D.

Lecture 1, Question 1:

The *SRY* gene's protein product causes a sexually ambiguous embryo to proceed down the path to becoming male. Using your knowledge of how genes are regulated, discuss a variety of ways *SRY* could cause such a dramatic transformation. How could further research into understanding *SRY* and sex determination help shed light on human biology and on conditions such as sex reversal?

Discussion Points

All cells in an organism contain the same genetic information, yet only a small subset of the entire genome may be active in any particular cell. Explain that scientists studying gene regulation have found a variety of ways that cells keep some genes quiet and turn others on. Review the "central dogma" of how genes work: In order for a gene to become active, the DNA must be transcribed into RNA that is then processed and translated into protein. Ask students how cells might find ways to target each of these steps as a way to regulate cell fate.

Discuss the fact that the *SRY* gene's protein product has been found to activate genes that lead to the formation of testes in a developing embryo. Ask students to suggest some specific ways *SRY* might work to regulate testes formation. For example, the *SRY* protein could be a transcription factor that is necessary for RNA polymerase to recognize and transcribe testes-determining genes. *SRY* could act alone, or it may need to work with other proteins to activate transcription of the testes-determining genes. Alternatively, the *SRY* protein could be involved post-transcriptionally by processing pre-mRNA into the mature mRNA of testes-determining genes. *SRY* could also be an enzyme that modifies and activates other proteins that in turn activate the testes genes. Discuss these and other possibilities with your students.

Discuss the relevance of research into understanding *SRY* and sex determination. *SRY* is one of the most rapidly evolving genes among different species; understanding *SRY* may shed light on how boundaries between new species evolve. Possible medical outcomes of research on sex determination include the development of diagnostic tests and treatments for human infertility and the development of new methods of birth control. Understanding the sex determination pathway should also explain why the sex of some individuals does not "match" their sex-chromosome makeup. Understanding how the *SRY* protein regulates a whole series of genes that lead to male development may provide clues about how other gene pathways operate.

Lecture 1, Question 2:

In the 1960s, the International Olympic Committee (IOC) instituted gender-verification tests to rule out the possibility of males passing as females in Olympic competition. Subsequently, some female athletes were disqualified from competition because of the results of these tests. An early gender-verification test examined the inactivated X chromosome (known as the Barr body). The next step in testing was karyotyping, a way to obtain a snapshot of all the chromosomes in an individual's cell. Later tests looked for the presence of the *SRY* gene. Discuss the validity of using these tests to determine athletic eligibility. Would an XY female necessarily have an advantage over an XX female? Explain.

Discussion Points

XY females are sex-reversed individuals, but they are usually unaware of their genotype. They are phenotypically female. Hormone levels and muscle mass are typically female. Highlight the fact that in these individuals, the embryo proceeded down the path to becoming female because the male sex-determining factor might have been absent—that is, *SRY* could have been deleted from the Y chromosome. Another possibility is that *SRY* was present and functional, but cells were unable to respond to the onset of male hormones (androgen insensitivity). Remind students that these XY females have been raised as females, are psychologically female, and tend to have no issues surrounding their gender. Ask your students their thoughts on the

validity of the genetic tests required of athletes. Many students might agree that the IOC's definition of sex—which is based *solely* on chromosome complement or the presence of *SRY* and ignores physiological characteristics—may unfairly discriminate against XY female athletes. Have students discuss whether these tests should be used at all. Disclose that in June 1999 the IOC decided to discontinue the gender-verification practice on a trial basis for the 2000 Olympics in Sydney.

Lecture Two

Hermaphrodites: The Safer Sex

Barbara J. Meyer, Ph.D.

Lecture 2, Question 1:

Sex in the nematode worm *C. elegans* is determined by the number of X chromosomes inherited in worm offspring. Worms have a way of counting the number of X chromosomes. The *xol-1* gene is considered the "master switch" that determines sex in worms and regulates dosage compensation. Discuss ways that *xol-1* could be regulated by the number of X chromosomes.

Discussion Points

Worms inheriting one X chromosome (XO) will develop into males, and worms inheriting two X chromosomes (XX) will develop into hermaphrodites. Ask students to suggest ways that worms might count whether one or two X chromosomes are present in the cell. Discuss how the presence of an X chromosome must be involved in signaling the *xol-1* gene, the "master switch," in some way. High levels of XOL-1 protein lead to the development of a male. Have your students suggest what the signal from the X chromosome might be. Discuss how constitutive genes, or genes that are always expressed at a low level, could act as signals affecting XOL-1 levels. One suggestion might be that these signal genes on the X chromosome produce a protein that inhibits the activity of *xol-1* in some way. In XO worms, signals from the X chromosome would be low, failing to inhibit *xol-1* activity and thus allowing *xol-1* to activate the male-determining genes and to inhibit dosage-compensation genes. In XX worms, sex-determining signals would be high, inhibiting *xol-1* activity, preventing

expression of male genes, allowing the hermaphrodite worm to develop, and causing dosage-compensation genes to turn on. Discuss ways the inhibition of *xol-1* by signal proteins might occur. Your discussion of how signals regulate *xol-1* activity should cover a variety of types of gene regulation: transcription repressors and transcription activators, as well as modification of the XOL-1 protein.

When your class discusses possible models of sex-chromosome counting, it's important to explore all students' suggestions and ideas. Regardless of how *xol-1* is actually regulated, valuable insights into the concepts of gene regulation will come from a thorough examination of each idea. Scientists often develop important hypotheses and experiments from informal "what if?" discussions. Reinforcing this process of science with your students will help them learn and apply these and other concepts to new situations.

Lecture Three

Sex and Death: Too Much of a Good Thing

Barbara J. Meyer, Ph.D.

Lecture 3, Question 1:

Geneticists discover what normal genes do by isolating and analyzing mutant individuals that display abnormal behavior. Finding these mutations in model organisms, such as the worm *C. elegans*, provides a fast and easy way to study how organisms work. How can finding mutations in *C. elegans* that are lethal specifically to hermaphrodites help scientists identify genes involved in dosage compensation? How will the identification of these mutations contribute to the understanding of how dosage compensation works in other species, including humans?

Discussion Points

Remind students that sex is determined in *C. elegans* by the number of X chromosomes inherited in offspring. Hermaphrodites have two Xs, and males have one X. Discuss what might happen if both X chromosomes in hermaphrodites were active. Reveal that without

dosage compensation, double expression of X-linked proteins in hermaphrodites will occur. In fact, it has been shown that if the extra X chromosome is not "turned down," the hermaphrodite will die. Therefore, the hermaphrodite worm must compensate for the double dose of X gene products by turning off one set of the X genes. Ask students what scientists might look for in a worm population to find worms with genes defective in dosage compensation. Discuss that if scientists find that a worm mating produces only male worms or an excess of male worms, they can assume that hermaphrodites with a certain genotype (or genetic makeup) don't form because the presence of the extra X chromosome kills worms with the mutation. Therefore, the normal form of the gene, lost in one of the mutant parents, acts somehow to help lower the expression of the extra X-linked genes in hermaphrodites.

Remind students that there are structural similarities between genes of different species. Oftentimes, genes in one species do a similar job in other species. Once genes involved in dosage compensation in worms have been isolated and identified, comparisons can be made between the worm genes and genes of other organisms, including humans.

Lecture 3, Question 2:

Scientists have discovered similarities between proteins involved in mitotic chromosome segregation and proteins involved in dosage compensation. Outline and discuss how dosage-compensation proteins might have evolved from chromosome-segregation proteins.

Discussion Points

When chromosomes are replicated during cell division, the newly replicated chromosomes must be condensed and separated physically into the two daughter cells. To do this, protein molecules must physically attach to the chromosomes to help with condensation and segregation. (This might be a good time to introduce the concept of a protein complex, in which a number of different proteins physically attach and work together.) Ask students to suggest ways that condensation and segregation proteins might change to cause dosage compensation. One possibility is that a gene or genes that encode proteins involved in condensation and segregation might, by chance, mutate and result in gene products that attach permanently to the X

chromosome and block gene expression. You might imagine this process occurring only when two X chromosomes are present, since blocking X expression in males would be lethal. In *C. elegans*, some proteins involved in dosage compensation have also been shown to be involved in chromosome segregation. These discoveries have the potential to help explain similar processes in humans.

Lecture Four

Sexual Evolution: From X to Y

David C. Page, M.D.

Lecture 4, Question 1:

Many scientists believe that the human sex chromosomes, X and Y, evolved from two homologous autosomes. How could the loss of recombination between the evolving X and Y chromosomes eventually result in such a great morphological disparity in the present-day chromosomes? How does your explanation account for the fact that the X chromosome is bigger than the Y?

Discussion Points

Homologous chromosomes—for example, all the autosomes—recombine, and in doing so, they repair mistakes that sometimes occur during replication. Brainstorm ways that homologous chromosomes might stop recombining. If students have difficulty, highlight the fact that if a mistake occurs where a large region of a chromosome flips position (an inversion), then the region that moved may no longer be able to physically line up with its homolog during recombination. Discuss that if recombination stops between two autosomes, mistakes in those regions will no longer be repaired and will remain in the DNA sequences for generations to come.

Apply these concepts to the case of X and Y. Ask how they might explain why the Y is so small. For example, if long ago an inversion occurred on the chromosomes that would become X and Y, then over time the nonrecombining one of these chromosomes might have lost entire regions to deletion mistakes. As long as the Y was never able to recombine and its partner X chromosome contained backup copies of essential genes, then the Y chromosome could have acquired more

and more mutations and deletions and gotten smaller and smaller. Ask why the X chromosome did not experience the same fate as the Y. Though the X chromosome cannot recombine in males, any given X chromosome spends two-thirds of its evolutionary history in females, where it can recombine normally and therefore get the mutation-weeding benefits of recombination. Today, therefore, we see a large X chromosome containing many essential genes, and a small Y.

Lecture 4, Question 2:

Scientists have found evidence that the evolution of the Y chromosome occurred in distinct steps over time. Discuss how data from the Human Genome Project might be used to support this theory.

Discussion Points

Provide an overview of the Human Genome Project. Most likely, students have some knowledge of the project. Start by asking students to share what they know with the class. Highlight that the project is an international effort to find the exact sequence of the three billion DNA nucleotide bases that make up our genetic blueprint. Discuss how knowing the DNA sequence of all our genes will enable scientists to identify genes involved in many human conditions. Each gene can be studied to find out its function and how it interacts with other genes. From this information, new diagnostic tests and treatments may be developed.

All the human genome sequence information generated from the Human Genome Project is stored in computer databases. Scientists can search for particular parts of every human chromosome, including the X and Y chromosomes, and study and compare them. Discuss that scientists have found that the X and Y chromosomes share 10 or so genes in parts of those chromosomes that don't appear to recombine. Remind students that when regions of chromosomes stop recombining, mistakes can't be repaired and homologous sequences slowly tend to become different (diverge). Ask students what would happen to unrepaired sequences generation after generation. Students may determine that the longer ago a pair of sequences stopped recombining, the more mistakes they might have and the more their sequences might differ.

Ask your students how the Human Genome Project might shed light on the evolution of the X and Y chromosomes. Discuss how comparing DNA sequences between shared regions of X and Y allows scientists to estimate how long ago they diverged. If the present-day Y evolved (lost homology with X) in distinct steps, then sequence data would reflect this. Indeed, this is what scientists have observed. In other words, sequences that diverged long ago show greater differences than sequences that diverged more recently.

Lecture 4, Question 3:

Oligospermia is a condition in men where very few viable sperm are present in their semen. Intracytoplasmic sperm injection (ICSI) has been used to help subfertile men with oligospermia have children. This condition is often caused by the deletion of genes on the Y chromosome that are necessary for sperm production. Identify and discuss ethical and social implications of ICSI using sperm from men with oligospermia.

Discussion

Infertility occurs in approximately 15 percent of couples, and one-third of infertile couples have male-related infertility. Discuss how ICSI has been used to help couples that want to have children but cannot conceive on their own. Explain to students that ICSI is a form of in vitro fertilization (IVF), which involves using drugs to induce superovulation of the female partner to provide eggs for the procedure. After ICSI, the embryos are implanted in the female partner's uterus. As you discuss issues surrounding ICSI using sperm from men with oligospermia, you may wish to include the following points:

1. Most causes of infertility are unknown.
2. Male infertility may be the result of a damaged Y chromosome, which will be passed on to any male children conceived by ICSI. Those male children will most likely be infertile as well.
3. Other genetic factors may be involved in infertility, and they would also be passed on to children conceived through ICSI.

4. Fertility clinics promote procedures from which there is a financial benefit. What is their role in providing informed consent?

Ask your students to consider implications for both parents and children. You may want to extend these discussions by having students suggest other new technologies that have created social issues. Ask students for their thoughts on how society can best prepare to make informed decisions regarding health and family.