

**Mechanisms of Primary and Secondary Sex Determination**

Reading: 8<sup>th</sup> ed: Chp 17; p. 129; 9<sup>th</sup> ed: Chp 14

**Learning Goals** Be able to:

Compare the general ways that sex can be determined in different organisms.

Explain how the X:A ratio is used to determine sex in *C. elegans* and *Drosophila*, and compare the two systems.

Explain the difference between primary and secondary sex determination in mammals (gonad determination vs. ductal systems).

Predict the outcome of gonadal and phenotypic sex when certain gene products are missing.

Relate to each other the multiple factors in both male and female that ultimately determine gonadal sex.

One of the early major decisions that every animal embryo must make regards its sexual identity; that is, whether it will develop as a male or a female (or hermaphrodite). However, the primary signal for sex determination differs widely among animals, as do the sex determining genes that somehow interpret this signal and control subsequent sexual development. These genes can be regulated in different organisms by a variety of primary signals: environmental, chromosomal, or some combination of both.

**Environmental/Hormonal sex determination**

In general, sex determination mechanisms must maintain an optimal ratio of sexes in the population (generally about equal, although there are exceptions such hermaphroditic species like *C. elegans*). These mechanisms may have also evolved to optimize reproductive capability in other ways. For example, in some fish sex is environmentally determined so that in the absence of males, the largest female can become male. In other fish, the larger animals become females, whose fecundity is directly related to size, while the smaller animals become males, whose fecundity is less dependent on size. In both cases, the determination of sex is dependent on hormone levels, which are changed by the social environment of the fish (the ultimate plasticity!). In some reptiles, sex is determined by the temperature of the water in which the eggs are laid. As in the previous example, the temperature affects hormonal levels; in this case, the enzyme aromatase, which converts testosterone to estrogen can only be active at certain temperatures. This mechanism ensures the production of mostly male embryos while the water is cooler, and mostly female animals when the water is warmer (except in some organisms, where at the two extremes of temperature mostly females are produced, while in the middle, males are produced!). Given these differences, aromatase clearly cannot be the only hormone involved in sex determination in all aquatic organisms, but just as clearly, many organisms' sex depends on hormone levels, not chromosomal makeup.

**Chromosomal sex determination**

In animals that use this mechanism, sex is determined by the presence, absence, or relative number of sex chromosomes. In animals with two different sex chromosomes, such as X and Y in humans, either sex can be *heterogametic*. In birds, for example, males are ZZ and females WZ (heterogametic). In mammals, the presence or absence of a Y chromosome determines sex. In worms and flies, sex is determined by the ratio of number of X chromosomes to number of sets of autosomes (X/A ratio).

This type of sex determination poses two major questions. First, what are the molecules involved in determining the X/A ratio, and how does an embryo assess this ratio? Second, how is the paradox of dosage compensation solved (next lecture)? Sex determination in invertebrates involves developmental switch genes that are components of fairly complex regulatory pathways.

**Sex determination in *C. elegans***

The primary signal is not the number of X chromosomes but the X/A ratio, as shown by sex determination in polyploid animals. In normal diploids, XX animals are hermaphrodite and XO animals are

male. Sex determining genes have been identified by (*homoeotic*) mutations that transform one sexual phenotype into the other. Recessive (*lf*) mutations in the *tra-1* gene transform XX animals into males; dominant (*gf*) mutations in this gene transform XO (and XX) animals into females. Thus *tra-1* acts as a switch gene, and its expression normally causes hermaphrodite (female) somatic development.

Mutations in another gene called *her-1* show the opposite effects: recessive mutations transform XO animals into hermaphrodites, and a dominant mutation transforms XX animals into (pseudo)males. Thus, expression of this gene normally causes male development.

### **Sex determination in *Drosophila***

This process at first appeared superficially similar to that of *C. elegans*, but has turned out to be fundamentally different in several respects. The Y chromosome in *Drosophila* is needed only for spermatogenesis in males; as in *C. elegans*, sex is determined by the X/A ratio. The numerator in this ratio appears to be a small number of transcription factors encoded by X-linked genes that are expressed very early after fertilization. In an XX embryo (2 copies of the gene) twice as much of this protein is produced as in an XO embryo. This "turns on" the first gene, *Sex lethal (Sxl)* in the dosage compensation/sex determination pathway. Like *xol-1* and the *sdc* genes in *C. elegans* (next class!), *Sxl* controls both dosage compensation and sex determination. In contrast to those of *C. elegans*, the sex-determining genes in *Drosophila* positively regulate each other's activities. *Sxl*, *tra*, *tra-2* all encode RNA binding proteins and are all transcribed in both sexes. Their sex-specific regulation is all accomplished at the level of alternative RNA splicing.

### **Sex determination in mammals**

Unlike sex determination in *C. elegans* and *Drosophila*, sex in mammals is determined by the presence or absence of a Y chromosome, and it is determined independently of X chromosome dosage compensation (which we will discuss in the next class).

### ***Primary sex determination: Development of the gonads***

The primordial gonad begins to form from intermediate mesoderm of the genital ridge during the 4th week of embryogenesis in humans. The primordial germ cells (PGCs) actually arise from extraembryonic mesoderm and migrate into the genital ridge area through the allantois during the 6th week of embryogenesis where they populate the primitive sex cords. At this stage, the gonad is "indifferent", and has the capacity to develop into either ovaries, oviduct and uterus, or into testes and vas deferens. During this indifferent stage, the sex of the gonad and the embryo are undetermined, and two duct systems, called the Wolffian and Mullerian ducts are present (see below, secondary sex determination).

An X0 (Turner's Syndrome) person is still female while an XXY (Klinefelter's Syndrome) person is still male. So, **presence or absence of Y is the primary signal for mammalian sex determination**. Testosterone is produced and a male develops only if the embryo receives a Y chromosome from the sperm at fertilization (except in very rare cases, below). However, there are examples of people who appear to be sex reversed; i.e., XY chromosomes, but look female, or XX chromosomes but look male. How does this happen?

### ***Certain regions of the Y chromosome are necessary for male development***

The chromosomal basis for human sex determination was initially explored when karyotyping (looking at all the chromosomes) turned up rare female XY individuals and male XX individuals. Female XY individuals lacked part of the Y and thus appeared female, while male XX individuals had part of the Y chromosome translocated onto an X chromosome. If patients were missing the long arm or part of the short arm of Y, they were still male, but, if they were missing a region of the short arm near the pseudoautosomal region of Y, they appeared female. This indicated that the testes determining gene must be on the short arm outside of the pseudoautosomal region at the tip (which is similar to and recombines with a region on the X). Eventually, a gene was found and named SRY (sex determining region of the Y).

Using reverse transcription followed by PCR (RT-PCR) to test for Sry mRNA, the gene was shown to be transcribed in the genital ridge of males at the end of the indifferent gonad stage (in mice). Later *in situ* hybridization analysis showed temporal and spatial correlation of Sry expression with testis formation.

Insertion of an Sry transgene into XX embryos caused them to develop into male mice (which did not produce functional sperm-- XXY male mice, like their human counterparts, don't make functional sperm)

Cloning of the Sry gene showed that it encodes a factor that opens chromatin, preparing it for transcription. Interestingly, the Sry sequence is not well conserved among mammals and Sry genes have not been found in non-mammalian vertebrates. Another factor called Sox-9 (on an autosome) is much more highly conserved evolutionarily (found in all vertebrates), and is activated by SRY. As is true for Sry, production of Sox-9 protein results in male gonads developing in XX individuals, so Sox-9 has a similar function to Sry.

Originally, female development was thought of as the default state since the mere presence of the Y chromosome determined male gonad development. However, it turns out that both ovary and testes development are active processes requiring the presence of specific proteins. Ovarian development requires Wnt4, which activates high levels of beta catenin, which then blocks production of Sox-9. You can see a summary of the other genes involved in figures 14.4 (9<sup>th</sup> ed).

### ***Secondary sex determination: hormonal production and its affects***

Once the gonad is determined to be male or female, all other sexual characteristics outside of the gonad are "secondary" sexual characteristics, determined by secretion of hormones from the gonad (testosterone from testes and progesterone/estrogen from ovaries).

The difference between "primary" and "secondary" sex determination is important. Primary sex determination concerns only the gonad. Secondary sex determination dictates the eventual phenotypic sex characteristics that differ between males and females, and involves the production of hormones that allow the correct ductal systems to develop. Thus, a person's outward sexual phenotype is dependent on hormone production and reception.

In females (XX), when the gonads become ovaries, each oogonia is surrounded by an ovarian follicle that secretes estrogen. Estrogen causes the Mullerian ducts to develop into the oviducts and uterus, while the Wolffian ducts degenerate due to lack of testosterone.

In males (XY), the gonads become testes. The somatic cells of the sex cords differentiate into Sertoli cells, producing a polypeptide hormone called AMF (Anti Mullerian Factor), which induces degeneration of the Mullerian ducts. The mesenchymal cells surrounding the sex cords differentiate produce testosterone, which induces development of the Wolffian ducts into epididymis and vas deferens. The germ cells remain dormant until puberty within the sex cords, which at that time develop into seminiferous tubules as sperm production begins.

When hormones are not produced, or cannot be bound, sex-reversed phenotypes are seen. An excellent example of this is Androgen Insensitivity Syndrome--phenotypically female individuals that are actually XY. These individuals have no receptors for testosterone (mutation in the gene that codes for testosterone receptor). They can produce testosterone, so the Mullerian duct degenerates. However, because they have no testosterone receptors, the Wolffian duct degenerates as well. Thus they are internally male, but externally female because enough estrogen is produced in the adrenal medulla to generate female secondary sex characteristics. There are several other such sex reversals that are due to the effects of hormones.

In individuals with the wrong number of sex chromosomes, the resulting phenotypes are often due to hormonal levels. For example, in individuals with only one X chromosome (XO, Turner syndrome), the absence of a second X is responsible for infertility and underdevelopment of secondary sex characteristics. These individuals have a female gonad (ovaries), but may not have all the expected phenotypes due to lower levels of estrogen and other factors produced by genes on the X chromosome. Similarly, XXY males are infertile and sometimes have mild female secondary sex characteristics. Both of these phenotypes are best understood in the context of dosage compensation—our next topic.

## Review Questions

1. Name three different mechanisms of primary sex determination in animals and describe how they work.
2. What is the X/A ratio, and how does it work for sex determination?
3. How do *C. elegans* and *Drosophila* accomplish sex determination, and how are the processes similar and different from each other and to mammalian sex determination?
4. What are the events in differentiation of the indifferent gonad into either an ovary or a testis? What hormones are involved, and what determines the secretion of the hormones?
5. What is the difference between primary and secondary sex determination?
6. What lines of evidence indicate that the SRY gene is the testis determining factor in humans and mice?