In this brief talk, I hope to cover some of the major concepts of mammalian sex determination. Sex determination was called by Erasmus Darwin the masterpiece of nature. And it really is because it combines variation, creation of new variants in a population which is sex with reproduction, making more of the species. Sex determination is often divided into two parts. One is primary sex determination. That's the determination of the gonads. Are they going to be testes or are they going to be ovaries?

Then there is secondary sex determination. That gives you your phenotype, things occurring outside the gonads. This is what the hormones are doing to your body. Mammalian sex determination was discovered in 1905 by two individuals independently, one was E. B. Wilson, the other was Nettie Stevens and they discovered that the male mammal is usually XY. In other words, there are two different types of sex chromosomes, the X chromosome and the Y chromosome. Whereas females were usually XX so that the egg will always be X, the sperm could be X or Y. If an X sperm fertilized an X egg the result would be female, XX. If a Y-bearing sperm fertilized an X-bearing egg, the result would be XY or male.

Now in the 1950s, it was shown that the Y chromosome is critically important in sex determination. Now this came from people who had different amounts of chromosomes. If one had XXY, the person would be male. If one were X but without either X or Y, the person would be female and this was very important. It showed that you needed two Xs to be a fertile female because the people who only had one X had ovaries but then the ovaries became dysgenic, they didn't develop into functional ovaries. Whereas a person who had two Xs and a Y chromosome became a male.

This is different than the case in drosophila. In drosophila, the XXY becomes female and the XO becomes male. That's because in drosophila, sex determination is based primarily on the number of X chromosomes one has, not on whether or not a Y is present. So in humans and mammals in general, the Y chromosome is very important. Each human embryo starts off with a bipotential gonad, a gonad that could go either

way. It's unlike any other organ rudiment that we have in our body. If you have a lung rudiment, it can form a lung. If you have a pancreas rudiment, it can form a pancreas. With the gonad rudiment, it could go either way. It could form a testis or it could form an ovary.

Moreover, we have in our body, not only a bipotential gonad, we have the origins of both duct systems. We have the mullerian duct, which will eventually become the female reproductive system. This is the duct which will give rise to the upper portion of the vagina, the cervix, the uterus, and the oviduct. So we have the female duct system. We also have the male duct system in every fetus. It has the duct which is going to become the sperm transport ducts, the vas deferens, the epididymis, and so forth. So we have the potential to go either way.

So now the transcription factors that are active in the genital ridge, the rudimentary gonad, they are told to become active. And when they're active, if they activate Wnt4 gene, the gene for the Wnt4 protein, Wnt4 starts making beta-catenin, a transcription factor. Beta-catenin activates the gene for R-spondin, and R-spondin will actually cause the creation of more beta-catenin. It makes that pathway more efficient. So in those mammals which activate Wnt4, beta-catenin is made and beta-catenin activates those genes which are responsible for making ovaries. The cells turn into the granulosis cells, or the thecal cells which are the follicular cells of the ovary. Those follicle cells make estrogen and the estrogen is able to convert the müllerian duct into its derivatives, the upper portion of the vagina, the cervix, the uterus, the oviducts. So if you have an ovary, you make estrogen, you get the female reproductive duct system. In the absence of testosterone, the Wolffian duct, which would have given rise to the male reproductive system, degenerates.

Now, going back to that rudimentary gonad again, that bipotential gonad. If there is a Y chromosome there, the transcription factors that are active in the gonad can activate the SRY gene. The SRY gene is the gene which is capable of initiating the development of the gonad into a testis. This is the gene on the Y chromosome responsible for sex

determination. The SRY gene activates a gene called SOX9. SOX9 is a gene found throughout the vertebrates, and it's a male sex determination gene for most vertebrates. It could be activated in numerous ways. In mammals, it's activated by the Y chromosome's SRY gene.

One of the things that the SOX9 gene activates, one of the genes that's activated, is a gene for anti-müllerian hormone. This gene encodes a protein which goes from the gonads, goes from the forming testis, and it destroys the duct that would form the female reproductive system. SOX9 also activates those genes for making the Leydig cells which are active in forming testosterone, which is the second hormone produced by the testis. Testosterone is able to take the Wolffian duct and cause that to differentiate into the various portions of the sperm exit tract. If SOX9 is activated by SRY, then we get a testis forming and we get the Wolffian duct derivatives and not the müllerian duct derivatives.

Now, testosterone has two major forms. One is normal testosterone. The other is a form called dihydrotestosterone. Dihydrotestosterone is active in producing the descent of the testis and the external genitalia. It's actually a more potent form of testosterone. So if the gonad activates the SRY gene, SOX9 is made. SOX9 not only activates the testis-forming genes, it inhibits beta-catenin, which means that the ovary-forming genes are not active. If there's no SRY present Wnt4 is active and Wnt4 will make beta-catenin and beta-catenin blocks SOX9. So you have an either/or relationship here that with an SRY gene one gets the formation of testis. Without the SRY gene one gets the formation of ovaries. Both the testis and ovaries are active gene-made organs. Neither is the default state of the other.

Now, as I mentioned before, the bipotential gonad is then linked to either one of the two organ systems. If the bipotential gonad forms a testis, it gets linked to the Wolffian ducts. If it forms an ovary, it gets linked to the müllerian ducts. And here you see a chart which kind of shows the different pathways that can be taken, the testis with the Wolffian ducts and its external genitalia of the scrotum and penis, the ovary with its

müllerian ducts with the external genitalia of labia and clitoris. So we have this pathway where the SRY and beta-catenin can interact with each other and inhibit each other so that usually, not always but usually, a person has only one type of gonad, either a testis or an ovary. So here we see, for instance in this picture, what happens if a person is XX, has two X chromosomes. One sees that R-spondin, which is in green here, is active. That will create beta-catenin and the beta-catenin will turn on the ovary genes and turn off the testis genes.

In this picture here, we show the importance of the SRY gene. The SRY gene was very difficult to discover but finally in the 1980s, it was found that there was this region of the Y chromosome which in humans and mice was responsible for activating SOX9. There were four human patients who were XX, they had two X chromosomes, but translocated onto one of the X chromosomes was an SRY gene. These XX individuals became male. A similar thing was found in sex-reversed mice. Then what was able to be done was scientists took XX mice and micro-injected into the fertilized egg the SRY gene and its regulatory elements. Those mice became male. They developed testes and the external male genitalia. So it looked like the SRY gene was critical for making testes. If that SRY gene was delayed, if you put that SRY gene on a heat shock protein for instance that you could turn on and off with heat, you can get ovaries or testes, depending on when you activate the SRY gene. There's only a short period of time where the SRY can be active. If that SRY gene is not active at that time beta-catenin is made and the gonad becomes an ovary.

As I mentioned before, SRY will activate the gene for SOX9 and that's its major function. It might be the only function that we know of for SRY is to activate SOX9. Once SOX9 is made, it can generate testes. If you take an XY wild-type male mouse, what you find is that their gonads have morphology of the testis tubes and if you look for anti-müllerian hormone you find it. If you have XY mice and you add an extra SOX9, nothing much happened. If you have XX mice, you don't find SOX9 being made and you find the follicle cells of the ovary. However, if you take an active SOX9 gene, and you put it into XX mice, what you get are testes forming. You get the testes morphology and

you get anti-müllerian hormone. Basically, you have a situation here where XX turns on Wnt4 which turns on beta-catenin, beta-catenin turns on the ovary-forming genes and it inactivates the testis-forming genes such as SOX9. If one has a Y chromosome, one has the SRY gene which activates SOX9. SOX9 activates the testes-forming genes and it inactivates the ovary-forming genes by interfering with beta-catenin.

And I like to liken primary sex determination to digital information. You get an either/or situation, where secondary sex determination is more analog, it has to do with gradients and mixtures, how much of this hormone, how much of that hormone. And so one gets not either/or's but one gets situations which are less distinct than the either/or situation given by just testis or ovary. Now there are major hormones that are being made by the gonads. Females generally make estrogen and estrogen gives you the build and the fat deposition of the female. It also causes the differentiation of the müllerian ducts. Now estrogen is also used in males. In both sexes it's used for bone development but in males it's actually used to concentrate sperm in the testis. So both males and females use estrogen as a sex hormone. Females use it for the differentiation of the müllerian ducts and for body and fat deposition.

Males have other hormones also, they have testosterone, which again gives a male body and fat deposition. It also degenerates the duct which would give rise to the mammary gland and it's useful for the Wolffian duct differentiationto give the sperm exit pathways. So that's testosterone. Then there's dihydrotestosterone which is necessary for the descent of the testes so that the testes come outside the body and also for genitalia development, for the development of the penis and scrotum. The testes also make the anti-müllerian hormone which causes the degeneration of the müllerian ducts.

One can see this starkly in some disorders of sexual development. Here we see a person who has androgen insensitivity syndrome. This person, you might not think to look at this person, this person has testes. This person is making testosterone. However, this person is obviously a woman, not a man. The reason is that the testosterone that is being made by this person's testes is not recognized by their cells.

They have a genetic deficiency in the testosterone-binding protein, what's called the testosterone receptoror the androgen receptor. Their cells cannot respond to testosterone. Their cells can respond, though, to estrogens and as a result get the female body type. So here the primary sex determination is to be male; however, secondary sex determination is to be female. This is called androgen insensitivity syndrome. It calls into question our notions of normalcy. Is this person a mutant male or is this person a normal but infertile female? And one can find out much more about this by reading the website at the Intersex Society of North America.

There are some countries now which are allowing a third category, X, in addition to M and F on their government documents. Now another disorder of sexual development is the deficiency of the enzyme called 5-alpha testosterone reductase which converts testosterone into dihydrotestosterone. Here we have a situation where the person is born and is making a testis and makes testosterone. However, the person cannot make dihydrotesosterone and as a result the scrotum and the penis remain relatively undifferentiated. The testes do not descend and the penis does not enlarge. However, at puberty when more testosterone is made, these deficiencies are overcome and the testes descend and the penis does enlarge. As a result, the person who had been raised as a girl is found to be a young man.

So, going back to this scheme that we showed before, you have a genital ridge which is a bipotential gonad. It can go either direction. With two X chromosomes, beta-catenin is made, the genes for ovaries are active, the genes for testes are inhibited. If one is XY, the genes for the testes are activated, especially SOX9, SOX9 will activate the other testis-forming genes and it will inhibit the ovary-forming genes. Once the hormones are made, the hormones will make the secondary sex determination characteristics. And here one has the anti-müllerian hormone which will be made from the Sertoli cells of the testis, this destroys the müllerian duct, testosterone coming from the Leydig cells of the testis will allow the Wolffian duct to differentiate. In females, the müllerian duct is allowed to differentiate through estrogen and in the absence of testosterone the

Wolffian duct degenerates. In that way, the ovary gets to be connected with the müllerian duct and the testis gets connected to the Wolffian duct.