

## What determines maleness?

### *Sex determination, testis formation and the development of the male phenotype*

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*To be (male) or not to be...  
That is the question (for andrology)*

Being male or female is a fundamental aspect of being human. Becoming male or female is a developmental story that unfolds at three levels (conception, sex determination and sex differentiation) and that involves the interplay of chromosomes, genes and hormones (Fig. 1). In the case of males, the result is an individual with an XY sex chromosome complement, having testicles and male external genitalia (penis and scrotum) and internal accessory sex organs (epididymis, vas deferens, seminal vesicle).

### Chromosomal sex

The definition of biological sex starts at conception. Although the human Y chromosome was reported in the 1920s, surprisingly it was not until 1959, through studies of Turner (XX) and Klinefelter (XXY) syndrome phenotypes, that the Y chromosome was associated with male sex determination. All human eggs contain a single X chromosome as part of their haploid genome. At the time of conception, if a Y chromosome bearing sperm cell unites with the egg, the resulting zygote will have an XY sex chromosome complement and will (normally) be male; if an X bearing sperm cell unites with the egg, the resulting zygote will have an XX sex chromosome complement and will be female. The correlation of the Y chromosome with maleness led to the hypothesis that it contained a “testis determining factor” (TDF); this realization led in turn to a 30 year quest to determine the molecular nature of this factor.

### The male phenotype and the importance of having testicles

Developmentally, the next important decisions for defining sex are the determination of gonadal sex (testes in males, ovaries in females), followed by the acquisition of the secondary sex phenotype (male or female). The go-

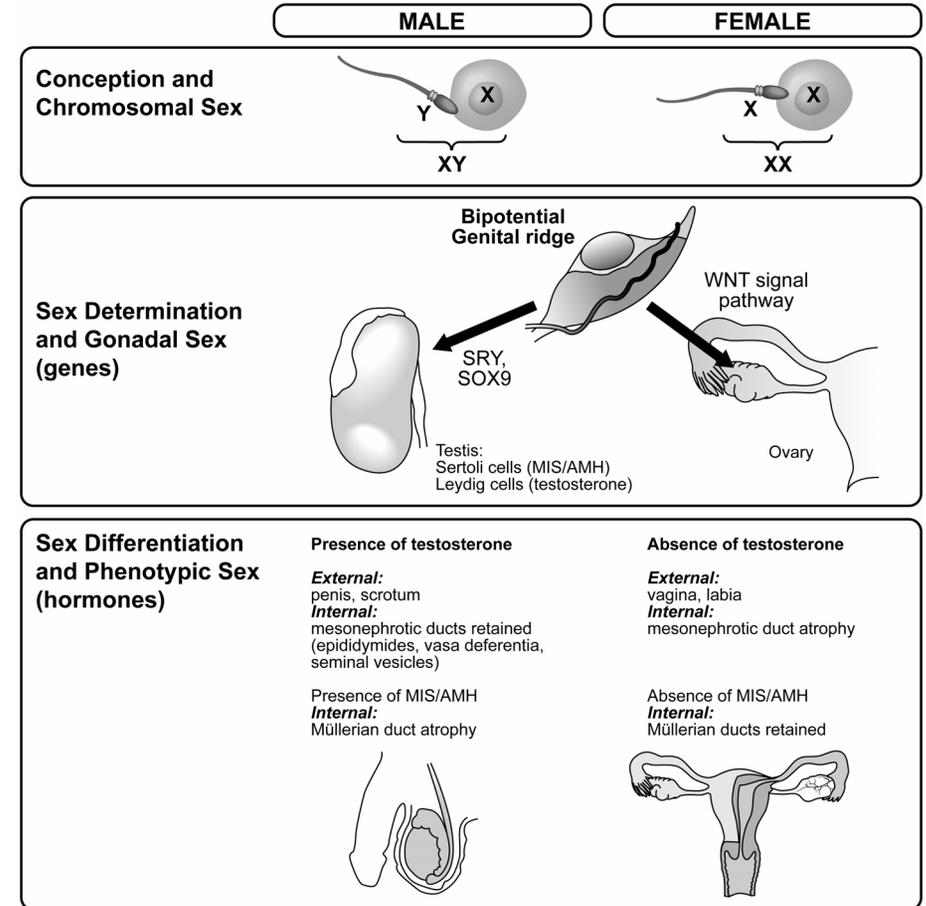


FIG. 1. Overview of biological sex in mammals.

nads develop from the paired genital ridges, found on the roof of the abdominal cavity of the fetus soon after gastrulation. The genital ridges initially have the capacity to form either testes or ovaries: they are bipotential. Historically it was known since the 1940s that the sex of the gonads is important for determining the phenotypic sex of the individual. More specifically, the presence of testes is required to insure a male phenotype, since removal of the genital ridge in an XY embryo was shown to result in a female phenotype. Hormones secreted by the developing testes are involved in these sex differentiation decisions. Sertoli cells of the developing testes

secrete the protein Müllerian inhibiting substance (MIS, also known as anti-Müllerian hormone or AMH), that causes the atrophy and loss of the paramesonephric (Müllerian) ducts in the XY fetus. Sertoli cells, along with germ cells, are now organized into cord like tubules that will become the seminiferous tubules. Leydig cells develop outside of these cord structures (in the interstitium). Leydig cells produce the steroid hormone testosterone. Testosterone and its metabolite dihydrotestosterone (DHT) cause the fetal external genitalia to develop into a penis and scrotum, and the internal mesonephric (Wolffian) ducts to be retained and develop into the epididymides, vasa deferentia and seminal vesicles. Testosterone produced by the male fetus will also masculinize the developing brain. In the XX fetus, the absence of testosterone (and presence of maternal and fetal estrogens) insures that the external genitalia develop as a vagina and labia and that internally, the mesonephric ducts atrophy. At the same time, the absence of MIS/AMH in the female allows the paramesonephric (Müllerian) ducts to be retained and develop into the oviducts (Fallopian tubes), uterus and cervix.

### **SRY and the molecular mechanism of testis determination**

Studies of rare deletions within the Y chromosome that resulted in discordance of chromosomal and phenotypic sex (i.e., XY females), along with the advent of molecular genetic techniques, culminated in 1990 with the cloning of a Y chromosome gene, named *SRY* (Sex determining region Y). When it was reported a year later that the introduction of *Sry* into an XX mouse genome resulted in a male phenotype, *SRY* was accepted to be the long sought-after TDF. How does it accomplish this role? *SRY* is expressed in pre-Sertoli cells of the developing XY genital ridge, just before the ridge starts its histological transformation to become a testicle. *SRY* was the first identified member of a larger family of DNA binding proteins, the SOX (SRY related HMG box) proteins. SOX proteins are important in making a number of key developmental decisions in animal embryos. Curiously, *SRY* is not a typical SOX gene: whereas other SOX genes are structurally well conserved between vertebrate and even invertebrate species, *SRY* is only found in placental mammals and has very poor sequence conservation between species. As a consequence of *SRY* expression, pre-Sertoli cells express another SOX gene named *SOX9*. In contrast to *SRY*, *SOX9* is highly conserved in vertebrates, and overexpression of *Sox9* in mice can force the development of testes in the XX fetus. *SOX9* will also turn on expression of the *MIS/AMH* gene. The picture now emerging for the molecular mechanism of mammalian sex determination is that the bipotential genital ridge, either XX or XY, is poised in a delicate balance between two competing developmental pathways, the male pathway dominated by *SOX9* expression,

and the female pathway which responds to the extracellular signal molecule WNT4. In the normal XY genital ridge, the presence and expression of the *SRY* gene in pre-Sertoli cells tips the balance towards increased *SOX9* expression and the male pathway—genital ridge development proceeds in the direction of testis formation. In the absence of *SRY*, WNT signaling dominates in cells of the genital ridge, favoring the development of the ovary.

### **Suggested reading**

- DiNapoli L, Capel B. *SRY* and the standoff in sex determination. *Mol Endocrinol.* 2008; 22: 1-9.
- Koopman P, Gubbay J, Vivian N, Goodfellow P, Lovell-Badge R. Male development of chromosomally female mice transgenic for *Sry*. *Nature* 1991; 351: 117-21.
- Sekido R, Lovell-Badge R. Sex determination and *SRY*: down to a wink and a nudge? *Trends Genet.* 2009; 25: 19-29.
- Sinclair AH, Berta P, Palmer MS, Hawkins JR, Griffiths BL, Smith MJ, Foster JW, Frischauf AM, Lovell-Badge R, Goodfellow PN. 1990. A gene from the human sex-determining region encodes a protein with homology to a conserved DNA-binding motif. *Nature* 1990; 346: 240-4.
- Vidal VP, Chaboissier MC, de Rooij DG, Schedl A. *Sox9* induces testis development in XX transgenic mice. *Nat Genet.* 2001; 28: 216-7.
- Wilhelm D, Palmer S, Koopman P. Sex determination and gonadal development in mammals. *Physiol Rev.* 2007; 87(1): 1-28.