Chapter 26
What determines biological maleness?
Sex determination, testis formation and development of the male phenotype

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

In humans, male and female sex phenotypes are the typical outcomes of the development of biological sex. 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 typically an individual with an XY sex chromosome complement, having testes and male external genitalia (penis and scrotum) and internal accessory sex organs (epididymis, vas deferens, prostate, 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 (XO) 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 (typically) be a biological male; if an X bearing sperm cell unites with the egg, the resulting zygote will have an XX sex chromosome complement and will (typically) be a biological female. The correlation of the Y chromosome with biological 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 testes

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 gonads develop from the paired genital ridges, found on the roof of the abdominal cavity of the fetus during organogenesis. The genital ridges initially have the capacity to form either testes or ovaries: they are bipotential. 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 results 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), which 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 cords structures (in the interstitium). Leydig cells produce two crucial hormones in the developing XY fetus: the protein insulin-like 3 (INSL3) and the steroid testosterone. INSL3 initiates the trans-abdominal phase of testicular descent. Testicular descent into the scrotum (inguino-scrotal phase) is then completed through the action of testosterone. Testosterone and its metabolite dihydrotestosterone (DHT) further 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 also masculinizes the developing brain. In the XX fetus, the absence of testosterone (and presence of maternal and fetal estrogens) ensures that the external genitalia develop as a vagina and labia and that internally, the mesonephric duct atrophies. 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 testis. 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 exclusively found in placental mammals and has very poor sequence conservation between species. As a consequence of *SRY* expression, pre-Sertoli cells express the highly conserved *SOX9*. SOX9 appears as strong as SRY for promoting biological maleness, as overexpression of *Sox9* in mice also forces the development of testes in the XX fetus. SOX9 will also turn on expression of the *MIS*/*AMH* gene. The current picture of 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* and Fibroblast Growth Factor 9 (*FGF9*) expression, and the female pathway which responds to a number of pro-female factors that include the signaling molecules R-Spondin Family Member 1 (RSPO1) and *Wingless*-related MMTV integration site 4 (WNT4), and the transcription factor Forkhead Box L2 (FOXL2). In a typical XY genital ridge, the presence and expression of the *SRY* gene in pre-Sertoli cells tips the balance towards increased *SOX9* and *FGF9* expression and the male pathway—genital ridge development proceeds in the direction of testis formation. In the absence of SRY, the pro-female factors dominate allowing for upregulation of β-catenin, favoring the development of the ovary.

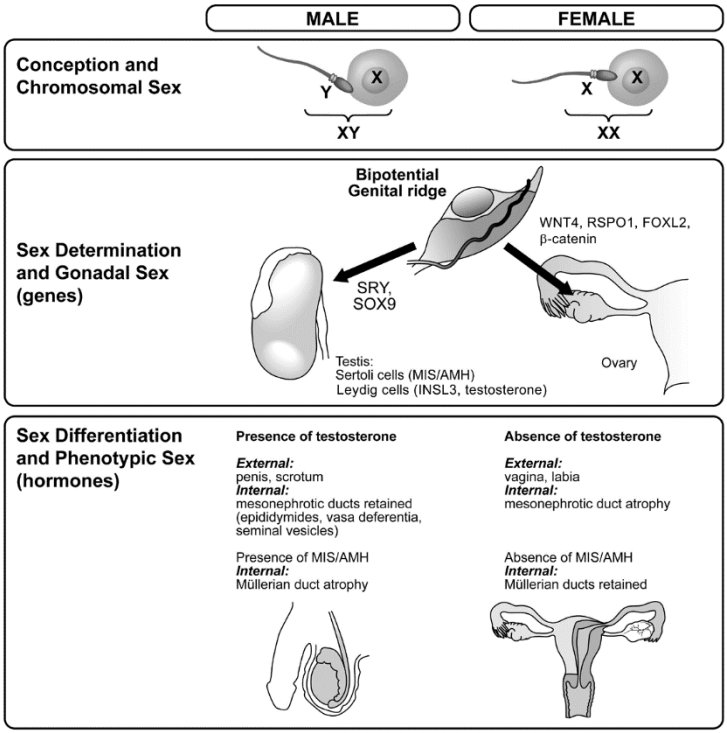

Figure 1. Overview of biological sex in mammals.

Suggested reading

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