Gonad development: **Signals for sex**

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The formation of testes or ovaries in the mammalian embryo is critical in determining sexual identity and the ability to reproduce. Recent studies have begun to illuminate the cellular signalling events required for development of functional testes.

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The differences between males and females are beguiling in most species, not least in human beings. And although the sex of the embryo is genetically determined at the moment of conception in mammals, these differences do not start to become obvious until an advanced stage of fetal development. It is the formation of testes that sends XY fetuses off on the tangent of male development. In the last decade, some of the key transcriptional regulators of male sex determination have been identified, beginning with the Y-chromosomal testis-determining gene *Sry*. Attention is now turning to understanding the cellular steps involved — differentiation, migration, communication and morphogenesis — in assembling functional gonads. A recent study by Colvin *et al.* [1] more clearly defines the function of an important signalling molecule during development of the male gonad.

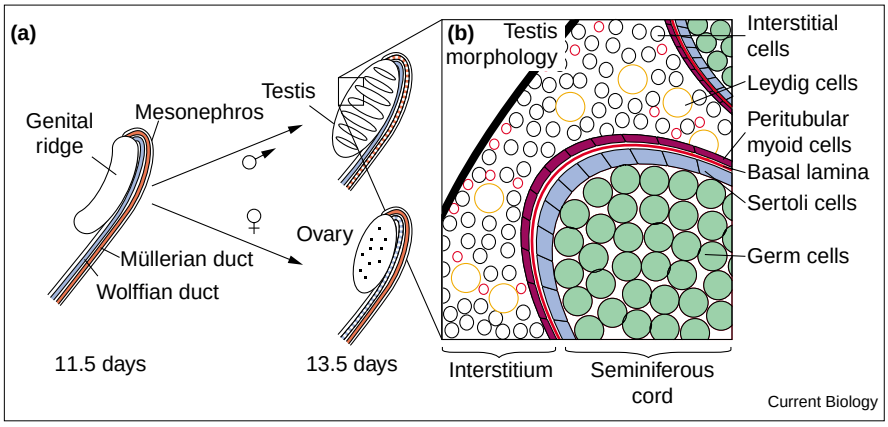
Mammalian embryos go to enormous lengths to equip themselves biologically for two quite different alternative fates, namely male or female development (Figure 1a). The gonadal primordia, called genital ridges, have the ability to develop into testes or ovaries depending on the genetic signals they receive. The primordial germ cells can go on to form prospermatogonia and thereafter sperm, or oogonia and thereafter oocytes, irrespective of their genetic makeup — XX or XY. The path they follow depends on whether or not they find themselves in a testicular environment [2].

The sexual duct system achieves its bipotentiality in another way again: instead of a single, ambiguous duct primordium, both male and female duct primordia are laid down, one of which is later promoted and the other made to regress depending on signals received from the developing gonads. External genitalia, breasts and other important gender-specific features are established as bipotential rudiments whose fate is directed later — sometimes as late as puberty — by hormonal signals.

In all this it is the decision to make testes or ovaries that holds the key to sexual development. This decision is ultimately under the control of the gene *Sry*, which gives the Y-chromosome in almost all mammals its male-inducing properties [3]. One of the results of *Sry* expression is that precursors of the so-called supporting cell lineage in the genital ridges are induced to differentiate as Sertoli cells rather than ovarian granulosa cells. Immature Sertoli cells form clusters enveloping the primordial germ cells, and these clusters later form cords surrounded by a basal lamina through an interaction between Sertoli cells and the enveloping peritubular myoid cells [4] (Figure 1b).

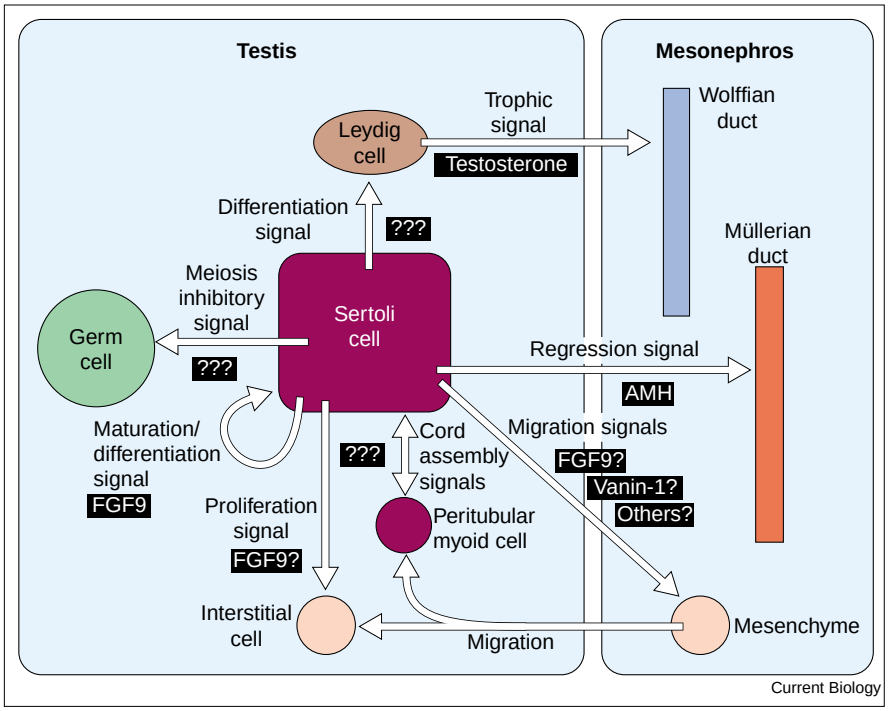
Sertoli cells act as major organizers of further development of the testes (Figure 2). They induce migration of cells from the adjacent mesonephroi [5], which are required for the development of cords and expansion of the testicular

Figure 1



Gonadal development in mice. **(a)** The gonadal primordium (genital ridge) is similar in males and females at 11.5 dpc. By 13.5 dpc, the testis has developed seminiferous cords whereas the ovary is smaller and less organized. In males, the Müllerian duct regresses and the Wolffian duct is promoted, whereas the opposite occurs in females. **(b)** Detail of the cellular structure of the developing testis.

Figure 2



Cell signalling in the developing testis. Known or assumed signalling events are represented by the arrows.

cell population [6,7]. Signals from Sertoli cells inhibit the entry of germ cells into meiosis that would otherwise signal the beginning of oogenesis [8]. And they signal to other cells of the genital ridges, inducing differentiation of Leydig cells that secrete testosterone, a potent inducer of the development of male — Wolffian — duct derivatives and male secondary sexual characteristics. They also signal to cells of the mesonephroi, inducing regression of the female — Müllerian — ducts.

How is all this signalling mediated at the molecular level? It has been known for some time that one of the earliest products of Sertoli cells is anti-Müllerian hormone, the transforming growth factor β-related molecule involved in female duct regression. However, the secreted factors required for histogenesis of the testes themselves have remained a mystery. Recent research implicates fibroblast growth factor 9, FGF9, as one component of Sertoli cell signalling during testicular organogenesis [1].

FGF9 is one of a family of over twenty related signalling molecules important for regulating a variety of developmental processes. In an effort to reveal the developmental roles of FGF9, Colvin *et al.* [1] created knockout mice homozygous for a deletion of exon 1 of the *Fgf9* gene — *Fgf9*$^{-/-}$ mice. These mice die shortly after birth due to lung defects, which led the investigators to examine the etiology of the defects in *Fgf9*$^{-/-}$ fetuses at 18.5 days *post coitum* (dpc), shortly before birth. They observed that females were dramatically over-represented among these fetuses. A large proportion were XY females whose gonads predominantly showed ovarian histology and lacked markers of testicular differentiation. Gonads of the infrequent *Fgf9*$^{-/-}$ XY males contained testicular cell types but were small and poorly organized.

As testes begin to differentiate in mice around 12 dpc, a full understanding of the defects in *Fgf9*$^{-/-}$ embryos rested on extensive analysis of gonadal development shortly after this stage. A problem encountered in this analysis was the variability of XY mutant gonad phenotypes, making it difficult to define the exact cellular consequences of FGF9 deficiency. In general, germ cell migration and proliferation were normal, but defects and deficiencies were seen in testis size, cord formation and organization, Sertoli cell numbers and morphology, Leydig cell differentiation, and mesenchymal and peritubular myoid cell numbers. An analysis of cell division by bromodeoxyuridine labelling showed that mesenchymal cell proliferation was reduced in *Fgf9*$^{-/-}$ testes at 12.5 dpc, suggesting that the mesenchymal depletion is due to a proliferative defect.

Does FGF9 mediate all these processes directly, or is the complex phenotype in *Fgf9*$^{-/-}$ embryos due to a primary defect in Sertoli cell specification, differentiation or maintenance? The latter is quite possible, given the central role of Sertoli cells in regulating testis development. An important point to clarify is whether *Fgf9* is expressed in early

Sertoli cells, and what other cell types in the developing gonad also express this gene.

The observation that Sertoli cell differentiation is impaired in some *Fgf9*$^{-/-}$ XY embryos is surprising and significant in itself. Sertoli cells have been assumed to differentiate primarily as a result of cell-autonomous action of *Sry*, without the need for extracellular signalling [9]. The study by Colvin *et al.* [1] suggests that FGF9 has a role, along with that of SRY, in inducing Sertoli cell differentiation, possibly *via* an autocrine loop (Figure 2). This may explain the observation that XY supporting cells can in some situations develop as ovarian follicle cells rather than as Sertoli cells despite the presence of *Sry* [10,11]. Since *Sry* is a relatively recent evolutionary addition in mammals, FGF9 signalling may represent a more ancient — and widespread — effector of male sex determination. In this light it will be of interest to observe the consequences of inhibiting FGF9 expression or action in non-mammalian model species such as zebrafish, so that the effects of FGF9 can be tested in the absence of *Sry*.

Colvin *et al.* [1] sought evidence that FGF9 might be involved directly in inducing migration of mesonephric cells into the developing testis (Figure 2), a critical process in testicular development [5,12]. Using genetically marked mesonephroi and wild-type testes in an *in vitro* co-culture assay, increased cell migration was observed in the presence of exogenous FGF9. However, while FGF9 was able induce migration in an *in vitro* assay, there is no guarantee that it really does so *in vivo*. Nor is it likely to be the only factor involved. Recently, two groups identified *Vanin-1*, encoding a cell membrane-associated G-protein, as being specifically expressed in male, XY, mouse genital ridges from an early stage [13,14]. Since Vanin-1 protein is involved in attracting migration of circulating thymocytes into the thymus, it follows that an analogous role in attracting mesonephric cells into the testis might be possible. Direct evidence is lacking, however, and how Vanin-1 signalling might relate to FGF signalling is not clear.

So what does this study contribute to our understanding of testis development? FGF9 appears to play an important role in Sertoli cell differentiation, which places the *Fgf9* gene high in the cascade of regulatory events downstream from *Sry*. Attention is now sure to turn to the questions of how FGF9 achieves this role, whether it is directly regulated by SRY, and what molecular events are triggered by FGF9 action. Less clear at this stage is whether FGF9 directly affects differentiation, proliferation or migration of other lineages in the developing testis. It seems certain that FGF9 cannot be responsible for all of the intercellular signalling events that are required for directing the complex development of the testis. Until the other regulators of gonadal development are identified, the true nature of the difference between men and women remains delectably mysterious.

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