Chapter 13
Insights into the Aetiology of Ovotesticular DSD from Studies of Mouse Ovotestes

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In mammals, *Sry* (sex-determining region Y gene) is the master regulator of male sex determination. It induces a cascade of gene expression that regulates the differentiation of the bipotential genital ridges into testicular tissue. In the absence of *Sry*, or if SRY function is impaired, the genital ridges develop into ovaries. Subsequently, hormones produced by the testes and ovaries direct the differentiation of all secondary sexual characteristics (for review, see Wilhelm and Koopman [1]).

Largely as a consequence of the location of *Sry* on the Y chromosome, this gene is subjected to mutations that cannot be repaired by mechanisms that rely on pairing with a duplicate chromosome. These mutations degrade the structure of the encoded protein and also potentially compromise the regulatory sequences required for correct and robust gene expression. This vulnerability of *Sry* to mutations means that the mechanisms controlling male sexual development are fragile, a situation that has profound biological and medical implications.

Sry encodes an HMG domain transcription factor whose primary role is to activate expression of a related *Sox* gene, *Sox9*. Like *Sry*, the activity of *Sox9* is both necessary and sufficient to induce testis development in the genital ridges. Once *Sox9* is activated, it upregulates a suite of genes required for maintaining the Sertoli cell phenotype and directing the development of other testicular cell lineages. *Sox9* also activates signalling pathways that stimulate *Sox9* expression in Sertoli cell precursors that do not express sufficient levels of *Sry* to cell-autonomously upregulate *Sox9*. We used XX–XY gonadal cell mixing experiments to identify prostaglandin D_2 (PGD_2) as a molecule secreted by Sertoli cells that stimulate *Sox9* expression even in XX gonadal cells lacking *Sry* [2]. We also found that SOX9 protein can directly activate transcription of the *Ptgds* gene encoding prostaglandin D_2 synthase, the enzyme producing PGD_2 [3]. Thus, *Sox9* and PGD_2 contribute to a positive reinforcement loop that recruits cells into the Sertoli lineage and operates to ensure testis development even when *Sry* activity is compromised.

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Cases exist of XY sex reversal even in the presence of a normal *Sry* gene. A classic example in mice is B6-Y^{DOM} sex reversal, characterized by the combination of a Y chromosome from some *Mus domesticus* subspecies, such as *Mus domesticus poschiavinus* (Y^{POS}), with the inbred C57BL/6 (B6) genetic background [4]. The severity of the phenotype varies and can include two ovaries, two ovotestes, or one ovary and one ovotestis. Each ovotestis consists of ovarian tissue at one or both poles of the gonad and testicular tissue in the centre. Studies of this mouse model of ovotestis development provide a rare opportunity to study the interaction of the testis- and ovary-determining pathways in the same tissue.

We studied expression of several markers of mouse foetal testis (SRY and SOX9) or ovary (FOXL2 and *Rspo1*) development in B6-XY^{POS} ovotestes by immunofluorescence and in situ hybridization, using normal testes and ovaries as controls [5]. In ovotestes, SOX9 was expressed only in the central region where SRY is expressed earliest, resulting in testis cord formation. Surprisingly, FOXL2-expressing cells also were found in this region, but individual cells expressed either FOXL2 or SOX9, not both. At the poles, even though SOX9 was not upregulated, SRY expression was downregulated normally as in XY testes, and FOXL2 was expressed from an early stage, demonstrating ovarian differentiation in these areas.

Our data show that SRY must (1) act within a specific developmental window to activate *Sox9*; (2) challenge the established view that SOX9 is responsible for downregulating *Sry* expression; (3) disprove the concept that testicular and ovarian cells occupy discrete domains in ovotestes; and (4) suggest that FOXL2 is actively suppressed in Sertoli cell precursors by the action of SOX9. In these respects our findings provide important new insights into the molecular regulation of testis and ovary development.

Our data indicate that compromise of the testis-determining pathway can tip the balance in favour of ovary or ovotestis development. Therefore, regulatory mutations affecting *Sry* expression levels or timing may explain some idiopathic XY DSD cases.

References

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