<Further Development 6_03>

Genes Controlled by Sry and Sox9

Once made, the Sox9 protein has several functions. First, it appears to be able to activate its own promoter, thereby allowing it to be transcribed for long periods of time (independent of Sry). Second, it blocks the ability of □-catenin to induce ovary formation, either directly or indirectly (Wilhelm et al. 2009). Third, it binds to *cis*-regulatory regions of numerous genes necessary for testis production (Bradford et al. 2009a; Rahmoun et al. 2017). These genes include those encoding anti-Müllerian hormone (which causes degeneration of the uterus-forming duct; Arango et al. 1999; de Santa Barbara et al. 2000), Dmrt1 (needed for testis maintenance), and Fgf9, a paracrine factor critical for testis development. Fgf9 is also essential for maintaining *Sox9* gene transcription, thereby establishing a positive feedback loop driving the male pathway (Kim et al. 2007).

The *Dmrt1* gene is needed to maintain testicular structure, throughout life. The deletion of *Dmrt1* in adult mice leads to the transformation of Sertoli cells into ovarian granulosa cells. Moreover, overexpression of *Dmrt1* in female mouse ovaries can reprogram the ovarian tissue into Sertoli-like cells (Lindeman et al. 2015; Zhao et al. 2015). Dmrt1 protein is probably the major male sex inducer across the entire animal kingdom, having been found in flies, cnidarians, fish, reptiles, and birds (Murphy et al. 2015; Picard et al. 2015). In mammals, *Sry* has taken over this function. However, these recent results show that *Dmrt1* has retained an important role in male sex determination, even in mammals.

Having the right genes doesn't necessarily mean you'll get the organ you expect. Studies have shown that the *Sry* gene of some strains of mice failed to produce testes when bred onto other genetic strains of mice (Eicher and Washburn 1983; Washburn and Eicher 1989; Eicher et al. 1996). The *Sry* gene is activated by transcription factors and nucleosome-modifying enzymes that are present in the bipotential gonads, but the allele of *Sry* found in one strain of mice may respond differently to these activating proteins than the *Sry* allele in other strains of mice does. Failure of the *Sry* gene to produce testes can be attributed either to a delay in *Sry* expression, or to the failure of the Sry protein to accumulate to the critical threshold level required to trigger *Sox9* expression and launch the male pathway. By the time *Sox9* gets turned on, it is too late—the gonad is already well along the path to becoming an ovary (Bullejos and Koopman 2005; Wilhelm et al. 2009).

The importance of timing was confirmed when Hiramatsu and collaborators (2009) were able to place the mouse *Sry* gene under the control of the regulatory sequences of a heat-sensitive gene, allowing them to activate *Sry* in the XX gonad at any time in mouse development by simply raising the embryo's temperature. When they delayed *Sry* activation by as little as 5 hours, testis formation failed and ovaries started to develop (Figure 1). Thus, there appears to be a brief window during which the testis-forming genes can function. If this window of opportunity is missed, the ovary-forming pathway is activated.

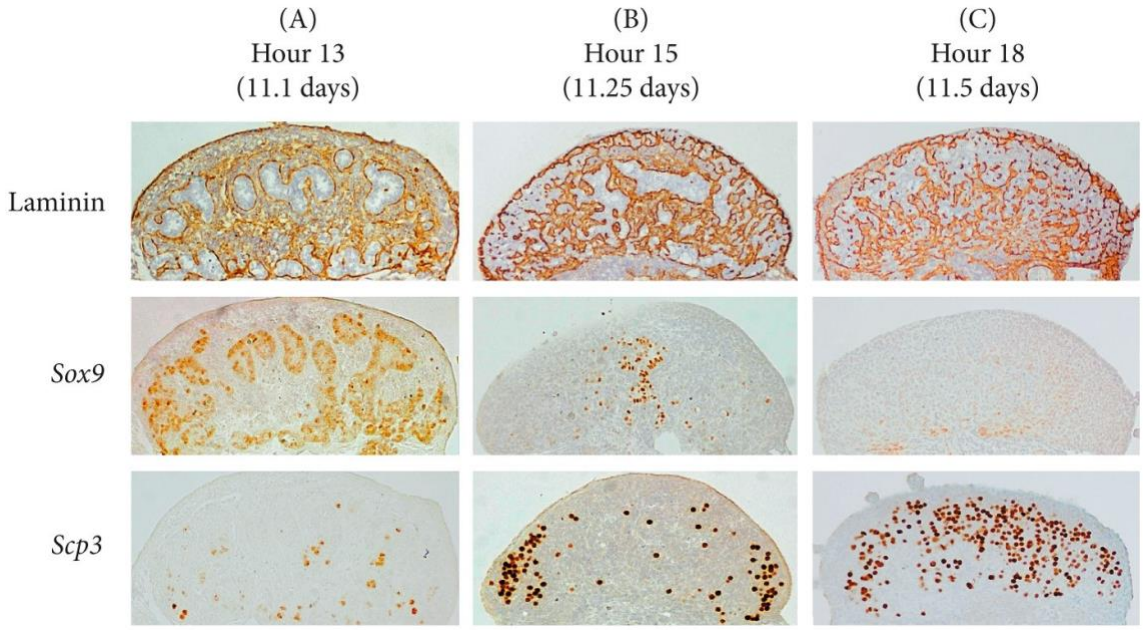

Figure 1 Experimental delay of *Sry* gene activation by 5 hours leads to failure of testis development and the initiation of ovary development. Genital ridges were removed from XX mice carrying a heat-inducible *Sry* gene. These tissues were then heat-shocked at different times to activate *Sry* and then allowed to mature. (A) Those genital tissues experiencing *Sry* induction at 11.1 days of development (when *Sry* is normally activated) produced testes. Their laminin distribution and Sertoli cells indicated testicular tissue, as did the presence of Sox9 (a marker of testis development) and the absence of Scp3 (a marker of ovary development). (B) Two hours later, the activation of *Sry* caused a central testicular area to form, with ovary-like structures forming peripherally. Sox9 was present in the central testicular region, while Scp3 was found in the periphery. (C) If *Sry* was activated in the genital tissues 5 hours later, the structures formed ovarian tissue, Sox9 was absent, and Scp3 was seen throughout the tissue. (After Hiramatsu et al. 2009.)