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Cell fate commitment during mammalian sex determination

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The gonads form bilaterally as bipotential organs that can develop as testes or ovaries. All secondary sex characteristics that we associate with 'maleness' or 'femaleness' depend on whether testes or ovaries form. The fate of the gonads depends on a cell fate decision that occurs in a somatic cell referred to as the 'supporting cell lineage'. Once supporting cell progenitors commit to Sertoli (male) or granulosa (female) fate, they propagate this decision to the other cells within the organ. In this review, we will describe what is known about the bipotential state of somatic and germ cell lineages in the gonad and the transcriptional and antagonistic signaling networks that lead to commitment, propagation, and maintenance of testis or ovary fate.

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Formation of the gonad

Gonads form as paired, bilateral organs that are composed of several lineages of somatic cells as well as the population of germ cells. Precursors of many of the somatic cells in the gonad arise from proliferation of the SF1 (steroidogenic factor 1, aka NR5A1)-positive cells in the coelomic epithelium (CE) overlying the region of the intermediate mesoderm called the mesonephros. The CE begins to thicken in this region at approximately embryonic day (E) 10.0 and contributes to at least two distinct somatic precursor lineages that are bipotential: first, supporting cell precursors, which give rise to Sertoli cells in the testis or fetal granulosa cells in the ovary, and second, steroidogenic progenitors, which give rise to Leydig cells in the testis or theca cells in the ovary [1,2]. Genes including *Wt1* (Wilms tumor 1 homolog) [3], *Lhx9* (LIM homeobox protein 9) [4], *Emx2* (empty spiracles homeobox 2) [5], *Sf1* [6], *M33* (Cbx2, chromobox 2) [7,8], *Gata4* [9] and *Six1/4* (sine oculis-related homeobox 1/4) [10•] are essential to establish the bipotential population of somatic cells in the gonad.

The bipotential stage

The early somatic progenitors are capable of adopting either male or female fate. In accord with classic theory in the field, the transcriptomes of whole XX and XY gonads are nearly indistinguishable at E10.0 through E11.2 [11••,12]. At this bipotential stage, genes that are later associated with testis fate (i.e. *Sox9* (Sry (sex determining region of the Y)-box 9) and *Fgf9* (fibroblast growth factor 9)) and ovary fate (i.e. *Wnt4* (wingless-type MMTV integration site family, member 4) and *Rspo1* (R-spondin homolog 1)) are expressed at similar levels in XX and XY gonads [11••]. This is also true if different cell types in the XX and XY gonad are isolated by flow cytometry and analyzed separately at E11.5 [13]. These results suggest that the bipotential plasticity of the mammalian gonad results from a transient balanced transcriptional state in which many genes later associated with male or female fate are expressed at similar levels in supporting cell precursors of both XX and XY gonads. Although the gonad is poised to follow either pathway at this bipotential stage, the supporting cell lineage expresses more genes later associated with the female than the male pathway, suggesting a female bias in the underlying program [13].

The first steps of male or female fate commitment

Sex determination initiates by tilting the balance in the transcription network toward the male or female fate. The switch to initiate the male pathway in the poised supporting cell progenitors is the Y-linked gene, *Sry*. An *Sry* transgene, driven in the XX gonad from its own promoter, caused differentiation of a testis [14]. This experiment showed that first, *Sry* is the only gene from the Y chromosome that is required for male sex determination, and second, the molecular environment of the XX gonad is fully competent to activate *Sry* and initiate testis development (for a recent excellent review focused on the regulation of *Sry* itself, see [15]).

Sry gene expression initiates just after E10.5 (10 tail somites (ts)) based on an RNase protection study [16]. Using *in situ* hybridization, expression is detectable in the middle of the gonad at ts14 (~E11.0) and expands toward the anterior, then posterior poles [17]. The timing and level of expression of *Sry* are critical. XY mice carrying a

weak allele of *Sry* that shows a decrease/delay in expression, are susceptible to male-to-female sex reversal [18–20]. Experiments that drive *Sry* expression in XX gonads using a heat shock promoter, revealed a requirement for *Sry* in the 6-h time window between E11.0 and E11.25 [21]. If expression is delayed, the testis pathway is aborted and ovarian development ensues. Exactly why the window of opportunity to initiate the male pathway closes at E11.25 remains unclear. Downstream of *Sry* expression, *Sox9* is the earliest gene to be upregulated in the male pathway at E11.2, closely followed by *Cited4* (*Cbp*/p300-interacting transactivator-4, with Glu/Asp-rich carboxy-terminal domain, and *Sox13* (SRY-box 13) at E11.4, and a larger group at E11.6 [11••]. Many of these genes are critical to establish male fate [22–24].

Genes associated with the female pathway become dimorphic slightly later, between E11.4 and E11.6, including *Wnt4*, *Rspo1*, *Irx3* (Iroquois related homeobox 3), *Lhx9*, *Fst* (follistatin), and *Lef1* (lymphoid enhancer binding factor 1) [11••,13]. The downstream effect of WNT4/RSPO1 signaling is the stabilization of β-catenin [25,26]. β-Catenin accumulates in the nucleus [27,28] where it may interact with the transcription factor LEF1 leading to the activation of downstream genes, as in other systems [29]. Stabilization of β-catenin in the XY gonad leads to down-regulation of SOX9 and male to female sex-reversal [30]. Antagonism between SOX9 and β-catenin may underlie the molecular decision in individual cells. However, loss of *Wnt4* and/or *Rspo1* and/or *β-catenin* does not lead to complete female to male sex reversal until perinatal stages [27,31–33].

Female fate is also regulated by the transcription factor *Foxl2* (forkhead box L2). FOXL2 co-operates with BMP2 (bone morphogenetic protein 2) to up-regulate the expression of *Fst* [34]. In goats, loss of function of *Foxl2* leads to female to male sex reversal in fetal life [35••]. However, in mice and humans, disruption of the female pathway does not occur until neonatal stages. Although loss of *Foxl2* in combination with *Rspo1* or *Wnt4* slightly accelerates the sex reversal phenotype in mice [36,37], no gene has been discovered whose loss leads to female to male sex reversal at early fetal stages. Since the bipotential gonad is initially biased toward the ovarian fate, a gene with a comparable role to *Sry* may not be required to initiate the female pathway. It may be sufficient *not* to initiate the male pathway.

Supporting cell precursors enter a quiescent state by E12.5 in XX gonads [38], consistent with the upregulation of negative regulators of the cell cycle observed in transcriptome studies [13,39]. The quiescent state of progenitor cells in the ovary may protect them from switching fate until proliferation resumes around the time of birth [38,40•]. In contrast, supporting cell progenitors in the XY gonad upregulate proliferation immediately downstream of *Sry*, and blocking proliferation disrupts the male pathway [2,41]. Whether proliferation is important for intracellular fate commitment or is required to establish a threshold population of Sertoli progenitors is still unclear.

Sexually dimorphic expression can result from activation in one sex, repression in the other sex, or a combination of both mechanisms. All of these patterns were evident in a study in which the gene expression profile for each gene was compared in XX and XY gonads at fine time points between E11.0 and E12.0 [11••]. Enrichment of male pathway genes occurred primarily through activation in the XY gonad with a minor contribution from repression of male genes in the XX gonad. In contrast, about half of the genes that became enriched in the female gonad did so due to repression in the XY gonad (Figure 1). This is a critical feature of the counterbalanced system that controls sex determination and gonadal fate: to establish a fate decision in the gonad, it is not sufficient to activate one of the alternative pathways — it is also necessary to repress the other.

Propagation of fate commitment across the gonad field

Subsequent to the primary fate decision in both differentiation pathways, feedback mechanisms are activated that canalize the chosen sexual fate. This occurs within individual cells and across the gonad field.

Although XX ⇔ XY chimeras typically have a similar number of XX and XY cells in the fetal gonad (as in other organs), more than half develop as males. This result suggests that a threshold number of XY cells can establish the testis pathway throughout the organ, but in cases where this cell threshold is not met, the gonad develops as an ovary. In the adult testes of chimeras, most of the Sertoli cells are XY, however, a small number of XX Sertoli cells are reproducibly found, indicating that XY supporting cells in the gonad can recruit XX cells (that do not have a Y chromosome or *Sry* gene) to Sertoli fate, presumably through paracrine signals [42,43]. These results indicate that in addition to the fate determination step that occurs in each supporting cell (likely based on antagonism between SOX9 and β-catenin), there is a community decision that takes place across the field of the gonad.

Two paracrine signaling molecules downstream of SOX9, FGF9 and PGD2 (prostaglandin D2 synthase), are known to contribute to propagation and maintenance of the male fate decision [44–50]. *Fgf9* is required to signal from the central part of the gonad and establish *Sox9* expression in the two ends [51]. Fgf signaling functions to antagonize Wnt signaling [45]. Elimination of the expression of *Fgf9* or its receptor *Fgfr2* (fibroblast growth factor receptor 2), leads to upregulation of the Wnt pathway and male-to-female