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Females Battle to Suppress Their Inner Male

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Conventional wisdom holds that the ovary and testis are terminally differentiated organs in adult mammals. However, Uhlenhaut et al. (2009) now report that deletion of a single gene, *Foxl2*, is sufficient to induce transdifferentiation of ovary into testis in adult mice, suggesting that testicular development is actively repressed throughout the life of females.

The mammalian embryonic gonad has long been viewed as the ideal model system for the study of organogenesis because it provides a choice between ovary or testis development. The way in which this choice is made can tell us a great deal about the molecular regulation of cell fate and pattern formation during development. In all mammalian embryos, the gonad is “bipotential,” that is, it can form either an ovary or a testis. In genetic males (XY), the testis pathway is set in motion by the *Sry* gene on the Y chromosome (Sinclair et al., 1990), which activates the related *Sox9* gene (Sekido and Lovell-Badge, 2008). In female (XX) embryos, ovarian development occurs in the absence of *Sry*, but the molecular mechanisms have remained obscure. One factor that plays a role in embryonic ovarian development is the forkhead/winged-helix transcription factor encoded by the *Foxl2* gene. In this issue, Uhlenhaut et al. (2009) now show that, in adult mice, conditional deletion of *Foxl2* induces transdifferentiation of the ovary into a functional testis. This finding reveals

an important role for *Foxl2* in the ovary that extends beyond embryogenesis, and reveals the remarkable plasticity of the adult gonad.

The undifferentiated gonad of mammalian embryos has a population of precursor cell types that can follow either the female or male pathway. These are the so-called supporting cells, the steroidogenic cells and the primordial germ cells (presumptive spermatozoa or oocytes) (Figure 1). In the genetic male, activation of *Sox9* by *Sry* leads to the differentiation of the supporting cell lineage into pre-Sertoli cells. These cells organize into seminiferous cords and enclose the germ cells, which are directed to enter mitotic arrest. Signals are also sent to the steroidogenic precursors, which give rise to fetal Leydig cells that synthesize testosterone (Figure 1). By contrast, in females, the embryonic supporting cells become granulosa cells of the primordial follicle, the steroidogenic precursors become the theca cells, and the germ cells enter the first stages of meiosis (Figure 1). It has recently

been shown that β -catenin, activated by the signaling molecules R-spondin1 and *Wnt4*, is required for ovarian differentiation in the mouse embryo (Liu et al., 2008). The forkhead transcription factor, *Foxl2*, also plays a role in ovarian differentiation (Garcia-Ortiz et al., 2009). In the mouse embryo, genetic ablation of *Foxl2* leads to defects in ovarian development that manifest late in embryogenesis as a failure of granulosa cell development. In humans, heterozygous *FOXL2* loss-of-function mutations result in ovarian dysfunction and the autosomal dominant disease blepharophimosis-ptosis-epicanthus inversus.

Which of these factors, *Foxl2*, *Wnt4*, or R-Spondin1, is the key embryonic trigger for ovarian determination, akin to *Sry* in males? Targeted deletion of each of these genes alone results in masculinization of the XX embryonic gonads but does not cause complete testis development. However, testis development can be induced in XX mouse embryos lacking both *Wnt4* and *Foxl2* (Ottolenghi et al., 2007). Male

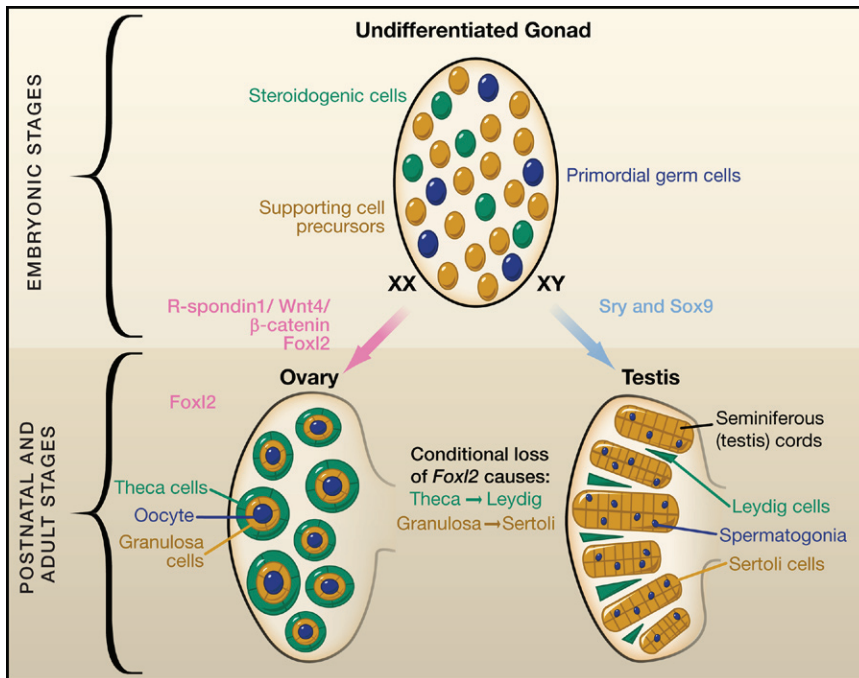


Figure 1. Gonad Differentiation in Mammals

Shown is the effect on the gonads of deleting *Foxl2* in adult mice. The undifferentiated embryonic gonad comprises supporting and steroidogenic cell precursors, together with primordial germ cells. In the presence of *Sry* and *Sox9*, differentiation into testis occurs. In the presence of *Foxl2* and *R-spondin1/Wnt4* acting via β -catenin, the embryonic gonad differentiates into ovary. When *Foxl2* is genetically ablated in adult female mice (XX), the ovary transdifferentiates into testis, with granulosa and theca cells becoming Sertoli and Leydig cells, respectively, and the germ cells are lost.

mice lacking *Wnt4* and *Foxl2* show activation of *Sox9*, development of seminiferous testis cords, and spermatogenesis. *Wnt4* and *Foxl2* appear to operate independently to orchestrate normal ovarian development in XX embryos. Hence, unlike the situation in males, in which a single Y-linked testis gene (*Sry*) triggers the male pathway, at least two different genes operating in apparently separate pathways are involved in ovarian development in the mouse embryo (Nef and Vassalli, 2008). Uhlenhaut and colleagues now reveal a role for *Foxl2* in the ovary in adults, and in doing so have demonstrated something quite extraordinary: the somatic reprogramming of an adult ovary into a testis. This concept runs counter to most of our ideas about terminal differentiation of adult tissues. Using a cre-recombinase system induced by treatment with the estrogen receptor antagonist, tamoxifen, they show that conditional deletion of *Foxl2* in the adult ovary is sufficient to induce transdifferentiation of the ovary into a testis.

The ramifications of this finding are many. First, the new work adds to the previous finding that *Foxl2* is required for maintenance of the ovarian phenotype throughout development in mammals (Ottolenghi et al., 2005). The results of Uhlenhaut and colleagues indicate that the ovarian pathway is active throughout adult life, requiring constant suppression of the key testis gene *Sox9* by *Foxl2*. The ovarian development pathway has been considered a default or passive pathway, and this is clearly not the case. Second, the new findings point to the strength of *Sox9* in triggering the testis development pathway. All of the existing evidence suggests that *Sox9* must be suppressed in females both during embryonic development by *R-spondin1* and *Wnt4* acting via β -catenin, and then later in adult life through the suppressive effect of *Foxl2*. These mechanisms in females indicate how critical it is to suppress the key testis gene, *Sox9*. If *Sox9* escapes active suppression in the ovary then, as demonstrated in this study, ovarian

granulosa and theca cells transdifferentiate into testicular Sertoli and Leydig cells, respectively (Figure 1). Most of the germ cells in these sex-reversed gonads were lost. In addition, the whole suite of testis genes is upregulated with concomitant downregulation of ovarian genes. The testis formed in such an XX mouse is capable of producing testosterone at levels comparable to that of a normal XY animal. This is remarkable, and the data presented by Uhlenhaut and coworkers confirm that true adult lineage reprogramming has occurred in vivo. Importantly, they also demonstrated that the reprogramming was cell autonomous (that is, operating directly within cells rather than by an indirect mechanism). Third, this study addresses a key hypothesis in the field. It has long been held that oocytes are required to maintain granulosa cell fate and that, in their absence, granulosa cells would transdifferentiate into Sertoli-like cells. Uhlenhaut et al. clearly demonstrate that the oocytes are not required to maintain somatic cell identity of the ovary and that the transdifferentiation process can be oocyte independent. Fourth, *Foxl2* is shown to be capable of synergistically interacting with estrogen receptors 1 and 2 (ESR1/2) and is most likely to be acting directly upon the *cis* gonad-specific regulatory element of *Sox9* known as TESCO. This finding has implications for human patients suffering from premature ovarian failure and disorders of sex development.

The data of Uhlenhaut et al. have implications for other species. The loss of *Foxl2* is likely to be the sole underlying cause of female-to-male sex reversal observed in goats with polled intersex syndrome, which have a large chromosomal deletion of the region including the *Foxl2* gene. Furthermore, the phenomenon of adult stage sex conversion seen in many fish may be explained by interaction between *Foxl2/estrogen* and *Sox9*. Interestingly, the *Dmrt1* gene (Doublesex, Mab-3-related transcription factor 1) is upregulated strongly in mice lacking *Foxl2*. We have recently demonstrated that *DMRT1* is required for testis development in the chicken and probably all birds, and that knockdown of *DMRT1* in

male chicken embryos leads to feminization that involves activation of *Foxl2* expression (Smith et al., 2009).

It appears that two independent antitestis pathways are at work in the ovary: R-spondin1/Wnt4 via β -catenin and *Foxl2* in the embryo, and at least *Foxl2* in the adult. Is *Foxl2* the only gene required to constitutively repress the male pathway in the adult ovary? Given the importance of R-spondin1 and Wnt4 acting via β -catenin in establishing the embryonic ovary, it would be of interest to conditionally delete this pathway in adult ovaries to see whether the gonads also show ovary to testis trans-differentiation. In other words, is this effect solely mediated by *Foxl2*, or are other factors of equal importance also involved?

Many disorders of sex development in humans remain unexplained. Similarly, the molecular mechanisms underlying premature ovarian failure in women are not fully understood. If the same phenomenon observed in this study applies to humans, then it may at least partly explain the etiology of these conditions.

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A Tethering Complex Recruits SNAREs and Grabs Vesicles

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Protein tethers can bridge gaps between membranes. Ren et al. (2009) now provide evidence that the yeast Dsl1 complex tethers vesicles to the endoplasmic reticulum (ER) by binding ER SNARE proteins at its base and capturing vesicles using a loop region that extends 20 nm from the ER membrane.

In eukaryotic cells, membranes are often interconnected by tethers. For instance, tethering of the endoplasmic reticulum to mitochondria, vacuoles/lysosomes, or the plasma membrane is involved in calcium homeostasis or lipid exchange (Levine and Loewen, 2006). Tethers that connect the stacks of the Golgi apparatus at a predefined distance from each other provide another example (Gillingham and Munro, 2003). In comparison, tethers involved in membrane fusion have a more challenging task. They first need to bring the two membranes in close apposition and then need to step

aside so that SNAREs can interact and mediate their merger. One group of tethers is formed by large multiprotein complexes, and it remains largely enigmatic how these bulky complexes complete these carefully orchestrated steps. To understand these events, it is essential to ascertain the architecture of the tethers and the relative positioning of their interacting partners, in particular the SNARE proteins and the membranes destined to fuse.

In this issue of *Cell*, Ren et al. (2009) describe a complete structural model of Dsl1, a multisubunit vesicle tether-

ing complex in yeast. The Dsl1 complex as well as its mammalian counterpart, the syntaxin 18 complex (Hirose et al., 2004), mediate the fusion of COPI-coated vesicles with the endoplasmic reticulum (ER) as part of retrograde trafficking between the Golgi and ER. The Dsl1 structure is particularly interesting in comparison to the previously reported structure of the tethering complex TRAPP I (trafficking protein particle complex I) (Kim et al., 2006; Cai et al., 2008), which facilitates the fusion of COPII-coated vesicles involved in ER-Golgi anterograde transport. The mod-