Signaling at the crossroads of gonad development

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In mammals, the gonads arise as bipotential primordia that are capable of developing as either testes or ovaries. Expression of the Y-linked gene Sry in the XY gonad initiates testis differentiation; in the absence of Sry, ovarian pathways prevail. Although the molecular targets of SRY are not known, many of the early cellular and morphological events that occur downstream of Sry have been characterized. These include increased cell proliferation, Sertoli cell differentiation, testis cord formation, development of a testis-specific vasculature and differentiation of interstitial lineages. Recently, several of the molecular signals that direct these processes have been identified. In addition, the pathways that suppress features of testis development in the XX gonad are being elucidated.

Introduction

A question that is central to developmental biology is how undifferentiated organ primordia transform into complex, highly specialized tissues. During organogenesis, the processes of cell proliferation, differentiation, migration and death are precisely regulated by complex signaling networks. Differentiation of the mammalian gonads provides a particularly useful model with which to examine mechanisms of cell signaling during organ development. The gonadal anlage is unique among organ primordia in that it can follow one of two developmental paths: namely, it can differentiate as either a testis or an ovary. The bipotential nature of the gonad makes it an ideal system with which to study the mechanisms that direct cell fate decisions and also provides an opportunity to use comparative approaches that are not feasible in other systems.

Recently, significant effort has focused on identifying the signaling pathways that direct morphogenesis of the testis and ovary. Several signaling molecules, including members of the fibroblast growth factor (FGF), platelet-derived growth factor (PDGF), Wnt and transforming growth factor-β (TGF-β) families, have been shown to have crucial functions in gonad differentiation (Table 1). These discoveries have provided much insight into the molecular regulation of reproductive differentiation, as well as into the general mechanisms that control organ development. Here we review how these signaling pathways function and interact during differentiation of the mammalian gonad.

Overview of murine gonadogenesis

In the mouse, the gonad arises at about 10.0 days post coitum (dpc) as a thickening of the epithelium along the coelomic surface of the mesonephros (Table 1). Proliferation of these epithelial cells gives rise to somatic cells in the gonad. By contrast, the germ cell lineage arises outside the urogenital ridge before formation of the gonads. Mouse primordial germ cells (PGCs) are specified in the epiblast and are first detected at about 7.25 dpc in a region posterior to the primitive streak [1]. PGCs proliferate and migrate through the gut mesentery into the urogenital ridge and populate the gonads between 10.0 and 11.0 dpc. Sex-specific gonad development is initiated when Sry (sex-determining region of chromosome Y) is expressed in somatic cells of the XY gonad between 10.5 and 12.0 dpc [2,3]. Sry encodes a putative transcription factor that acts as the genetic switch for male development. Several experiments have shown that Sry is both

<table>
<thead>
<tr>
<th>Gene</th>
<th>Species</th>
<th>Phenotype</th>
<th>Refs</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dhh</td>
<td>Mouse</td>
<td>Disrupted differentiation of fetal Leydig cells; defects in spermatogenesis and Leydig and peritubular cell development in adult</td>
<td>[55,57,58]</td>
</tr>
<tr>
<td>DHH</td>
<td>Human</td>
<td>46,XY males with homozygous mutations in DHH show partial or complete pure gonadal dysgenesis</td>
<td>[59]</td>
</tr>
<tr>
<td>Fgf9</td>
<td>Mouse</td>
<td>Male-to-female sex reversal; reduced proliferation in XY mutants gonads</td>
<td>[18]</td>
</tr>
<tr>
<td>Fat</td>
<td>Mouse</td>
<td>Formation of male vascular pattern and extensive germ cell loss in Fat−/− XX gonads</td>
<td>[69]</td>
</tr>
<tr>
<td>lr, lgf1r, lrr</td>
<td>Mouse</td>
<td>Male-to-female sex reversal, disrupted Sertoli cell differentiation in triple mutants</td>
<td>[28]</td>
</tr>
<tr>
<td>Pdgfra</td>
<td>Mouse</td>
<td>Disrupted testis cord formation, vascular patterning and Leydig differentiation in XY mutants</td>
<td>[46]</td>
</tr>
<tr>
<td>Wnt4</td>
<td>Mouse</td>
<td>Formation of a male vascular pattern, germ cell loss and ectopic steroidogenic cells in XX gonads</td>
<td>[64,65,67]</td>
</tr>
<tr>
<td>WNT4</td>
<td>Human</td>
<td>46,XX female with loss-of-function mutation in WNT4 shows absence of Müllerian-derived structures and signs of androgen excess</td>
<td>[60]</td>
</tr>
</tbody>
</table>

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required for testis formation in XY embryos and sufficient to induce testis differentiation in XX embryos [4,5].

Although Sry was shown to be the testis-determining gene more than 10 years ago, we still have relatively little knowledge about how it functions and what its genetic targets are. By contrast, many of the cellular and morphological events that occur downstream of Sry have been well characterized (Figure 1). Shortly after the initiation of Sry expression, there is a marked increase in the proliferation of coelomic epithelial cells in XY gonads [6]. Between 11.2 and 12.5 dpc, Sertoli cells differentiate in the XY gonad and surround germ cells to form testis cords. Simultaneously, vascular endothelial cells migrate from the mesonephros into the XY gonad and are organized into a testis-specific vasculature [7]. Fetal Leydig cells, the steroidogenic cell type of the testis, differentiate between 12.5 and 13.5 dpc in the interstitial space between cords.

By contrast, few visible morphological changes occur in XX gonads during this time period. The first overt feature of ovarian development is entry of the germ cells into the prophase of meiosis, which occurs in an anterior-to-posterior wave between 13.5 and 14.5 dpc [8]. Other aspects of ovarian differentiation, including the differentiation of granulosa cells and the formation of primordial follicles, occur close to the time of birth.

In addition to Sry, several genes have been identified that either are involved in the initial formation of the bipotential gonads or are crucial for sex determination and early testis differentiation. However, most of these genes encode transcription factors, and much less is known about the signaling pathways that regulate gonad differentiation. The functions of factors including anti-Müllerian hormone, testosterone and insulin-like-3 in differentiation of the internal and external genitalia have been well characterized [9]. Recently, several molecular signals that induce the differentiation and organization of testis and ovarian lineages have been also identified, as we describe below.

**Regulation of Sertoli cell determination and differentiation**

Sertoli cells have many important functions in the adult testis, where they direct the proliferation and differentiation of germ cells during spermatogenesis. In addition, Sertoli cells have an essential role during testis differentiation in the embryo. In experiments examining XX/XY chimeras, Sertoli cells were found to be the only type of cell in the testis that showed a strong bias for presence of the Y chromosome [10]. This observation suggested that the Y-linked gene Sry is required only in the Sertoli cell lineage. More recently, it has been shown that the precursors of Sertoli cells (pre-Sertoli cells) express Sry, supporting this hypothesis [11].

SRY-box containing gene 9 (Sox9) encodes a close relative of SRY that is also expressed by pre-Sertoli cells. Sox9 is required for normal testis differentiation and can induce testis development in XX gonads in the absence of Sry [12,13]. Initially Sox9 is expressed in the urogenital ridge of both XX and XY embryos, but expression is upregulated in XY gonads at 11.5 dpc, whereas it is lost in XX gonads [14]. Sox9 is thought to be a direct target of Sry on the basis of its early expression in pre-Sertoli cells shortly after the onset of Sry expression [15]. Although it has been firmly established that Sry and Sox9 have essential roles in Sertoli cell differentiation and testis development, it remains unclear how they regulate these processes.

Sertoli cells derive from proliferating cells of the coelomic epithelium during a brief developmental window before 11.5 dpc [16]. As mentioned above, this increase in the proliferation of coelomic epithelial cells is a male-specific event and is one of the earliest detectable differences between XX and XY gonads [6]. This period

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**Figure 1.** Early murine gonad differentiation. The bipotential gonad (yellow) initially forms as a thickening of the coelomic epithelium of the mesonephros (gray). Between 9.5 and 11.5 dpc, primordial germ cells (green) migrate from the hindgut into the urogenital ridges and populate the gonads. Between 10.5 and 12.0 dpc, the Y-linked gene Sry is expressed specifically in the XY gonads, where it triggers the testis differentiation pathway. The earliest features of testis development include the differentiation of Sertoli cells (purple), which enclose germ cells to form testis cords, and the formation of a male-specific vasculature (red). These events are followed by the differentiation of steroidogenic Leydig cells (blue) in the testis interstitium, starting at about 12.5 dpc. By contrast, no marked morphological changes are observed in the XX gonads until 13.5 to 14.5 dpc, when germ cells enter the prophase of meiosis.
of proliferation is crucial for male development, because blocking cell proliferation during this window has been shown to prevent Sertoli cell differentiation and testis cord formation [17]. These findings have led to the hypothesis that one of the earliest functions of Sry is to induce cell proliferation to generate enough pre-Sertoli cells to initiate testis development. Recent experiments have shown that FGF signaling has a role in regulating cell proliferation and Sertoli differentiation in the XY gonad.

**Fgf9**

Studies of mice with a targeted mutation in Fgf9 have shown that most Fgf9 mutants appear phenotypically female at birth [18]. It has been reported that about 85% of Fgf9−/− XY embryos show male-to-female sex reversal on a mixed genetic background, whereas sex reversal occurs in 100% of Fgf9−/− XY embryos on a C57BL/6 background [19]. The sex-reversed Fgf9−/− XY gonads develop as ovaries and do not express early markers of Sertoli cell differentiation.

Because of the early and important role of cell proliferation in testis development, mitotic activity has been measured in these Fgf9 mutants. Proliferation of coelomic epithelial cells was found to be markedly reduced in mutant XY gonads as early as 11.2 dpc [19]. This suggests that FGF9 is involved in stimulating the proliferation of coelomic epithelial cells in XY gonads. In support of this idea, FGF9 can induce proliferation in primary cultures of Sertoli cells and in somatic cells of XX gonads in culture [19,20]. XX gonads treated with FGF9 do not express any Sertoli cell markers, which suggests that, although cell proliferation is essential for Sertoli cell differentiation in XY gonads, it is not sufficient to induce differentiation in the absence of Sry.

Although Fgf9 has a sex-specific function during the very early stages of gonad differentiation, it is expressed in both XX and XY gonads until about 11.5 dpc, after which it is downregulated in XX gonads [19]. Thus, the early sex-specific effects of FGF9 are not determined at the level of transcription. Of the four FGF receptors, FGFR1–FGFR4, only FGFR2 is localized to the cell surface of coelomic epithelial cells in XY gonads, making it a good candidate for mediating FGF9-induced proliferation in this cell population [19]. The receptor is expressed in the same cell population in XX gonads, however, suggesting that another mechanism must mediate the sex-specific effects of FGF9. Heparin sulfate proteoglycans form complexes with FGFs and FGFR receptors and are required for efficient signaling [21]. In vitro analyses indicate that male-specific FGF signaling could be mediated via the sex-specific production and/or modification of heparin sulfates [19].

Expression of FGFR2 has been also observed in the nuclei of scattered somatic cells in the XY (but not the XX) gonad as early as 11.2 dpc. By 12.5 dpc, strong expression of FGFR2 is seen in all Sertoli cells inside testis cords. Other reports have described the transport of growth factor receptors to cell nuclei, although the biological relevance of this localization is not clear [22,23]. Strikingly, the nuclear expression of FGFR2 overlaps with that of SOX9, raising the possibility that FGFR2 might regulate early Sertoli cell differentiation through interactions with SOX9 [19].

Another report has described expression of a different FGF receptor, FGFR3, in the nuclei of Sertoli cells and germ cells in embryonic gonads [20]. These expression patterns suggest that FGF signaling could have several roles during testis differentiation. Future work should provide insight into both the relationship between FGF signaling and SOX9, and the functional significance of FGF receptor localization in the nucleus.

**Insulin and insulin-like growth factor family signaling**

In the mouse, the insulin and insulin growth factor (IGF) signaling family includes at least nine genes (Ins1, Ins2, Igf1, Igf2, Relaxin1, Relaxin2, Insl3, Insl5, and Insl6), which encode ligands for three tyrosine kinase receptors: the insulin receptor (INSR), the IGF1 receptor (IGF1R), and the IINSR-related receptor (IRR) [24]. Genetic analyses of mice deficient in ligands and receptors of this family have revealed numerous roles for these signaling pathways during embryonic development and postnatal growth and homeostasis. In addition, several members of the family have been implicated in reproductive development. For example, loss of Igf1 results in infertility owing to reduced testosterone levels, decreased testis and epididymides size in males and hypoplastic uteri in females [25]. Furthermore, mice with a null mutation in Insl3 develop bilateral cryptorchidism [26].

A recent analysis of mice with null mutations in three of the receptor genes (Insr, Igf1r, and Irr) has identified an additional requirement for insulin family signaling in the very early stages of testis differentiation. Mice with null mutations in either Insr or Igf1r die shortly after birth and show several developmental defects, whereas mice lacking Irr are viable [24,27]. Strikingly, mice with targeted mutations in all three genes show male-to-female sex reversal [28]. Examination of the early stages of gonad development in the triple mutants has shown that Sry is expressed initially in XY gonads, whereas other markers of Sertoli differentiation are markedly reduced or absent. Both XX and XY triple mutant gonads are smaller than controls, and the proliferation of somatic cells is reduced in the triple mutants [28].

Taken together, these studies suggest that insulin signaling plays a role in increasing the population of pre-Sertoli cells or in influencing Sertoli cell differentiation in the gonads. Little is known, however, about the specific mechanisms of insulin and IGF signaling in the developing testes, and further investigation is required to understand the roles of this family of growth factors in testis development.

**Embryonic germ cell development**

A number of signaling molecules have been identified that are required for the initial specification of primordial germ cells (bone morphogenetic protein 2, -4, and -8b) and/or for the survival and migration of PGCs as they home to the urogenital ridge (Kit ligand, stromal cell derived factor 1) [29–31]. By contrast, much less is known about the factors that regulate the development of germ cells after they have colonized the gonads. Once in the
gonads, PGCs undergo several rounds of mitotic division in both sexes. Several factors can regulate PGC proliferation or survival in vitro, including FGFs, Kit ligand, leukemia inhibitory factor (LIF), tumor-necrosis factor (TNF) and interleukin-4 (IL4). [32–34]. Little is known, however, about the signals that regulate the early rounds of PGC proliferation in the embryonic gonads in vivo.

By 13.5 dpc, germ cells in XX and XY gonads have taken different developmental paths. In XY gonads germ cells arrest in mitosis as spermatogonia, whereas in XX gonads the germ cells enter prophase of the first meiotic division (Figure 1). The fate of germ cells is dependent on the somatic environment, and not on the chromosomal sex of the germ cells [35]. Thus, signals from adjacent somatic cells must direct the differentiation of germ cells in the embryonic gonads, although these signals have not been identified.

In addition, evidence indicates that signals from germ cells can affect somatic cell differentiation and/or function. Prostaglandin D synthase is expressed by germ cells in XY gonads, but not XX gonads, between 11.5 and 12.5 dpc. Addition of the signaling molecule prostaglandin D2 to cultures of XX gonads partially masculinizes the gonads, suggesting that signals from germ cells might induce aspects of testis differentiation [35]. By contrast, it has been shown that meiotic germ cells can block testis cord formation when aggregated with XY somatic cells, providing evidence that signals from XX germ cells committed to the ovarian pathway can antagonize male development [36].

A few genes have been identified that affect germ cell development in vivo, but our knowledge of their function is limited. Mice with mutations in Il6st, which encodes a shared receptor for the interleukin-6 cytokine family, show a small male-specific reduction in germ cell number by 13.5 dpc [37]. Mice with null mutations in the genes Ntrk2 or Ntrk3 (commonly referred to as trkA and trKC, respectively), which encode two neurotrophin receptors, have reduced numbers of germ cells in the testis shortly before birth [38]. Little is known, however, about how these receptors affect germ cell development at the molecular level. An understanding of the pathways that control sexually dimorphic germ cell differentiation during embryogenesis remains elusive and should be the focus of intensive efforts in the future.

Sex-specific vascular patterning in the gonads

An early and well-characterized event in testis differentiation is the migration of mesonephric cells into the XY gonad [39–41]. As demonstrated by numerous tissue recombination experiments, XY gonads induce mesonephric cell migration, whereas XX gonads do not. This migration is essential for normal development of the testis, because blocking migration has been shown to inhibit testis cord formation [39,42]. Most of these migrating mesonephric cells are vascular endothelial cells and are crucial for establishing the testis-specific vasculature (Figure 1) [7,41].

Between 11.5 dpc and 12.0 dpc, migrating endothelial cells coalesce to form a large vessel that runs just beneath the coelomic epithelium. This vessel branches, and the branches intercalate between forming testis cords. Blood flow is rerouted through the XY gonad shortly after formation of this vascular system [7]. Formation of a testis-specific vasculature might be important either for supplying oxygen to this rapidly growing tissue or for the efficient export of testosterone produced by fetal Leydig cells. In addition, recent experiments in the pancreas and liver have shown that vascular endothelial cells can produce regulatory signals that are important for organ development [43,44]. Thus, vascular development in the embryonic testis could potentially influence the patterning and differentiation of this organ in numerous ways.

Extensive work has been done to identify the signals produced by XY gonads that induce the migration of endothelial cells. Many growth factors can induce mesonephric cell migration into XX gonads in culture; however, several of these are not good candidates for regulating this process in vivo [18,45–47]. One class of factors that might be involved in regulating mesonephric cell migration are the neurotropins. Neurotropin 3 (NT3) is expressed by Sertoli cells, and one of its receptors, NTRK3, is expressed by migrating mesonephric cells [48,49]. In addition, a pharmacological inhibitor of NTRK3 can block mesonephric cell migration and testis cord formation in vitro [50]. Ntrk3-null mutant mice show only very mild defects in interstitial development and testis cord formation, but there could be functional redundancy among the multiple neurotropin receptors expressed in the mesonephros [38].

Platelet-derived growth factors are another family of signaling molecules that have been implicated in mesonephric cell migration. The PDGF family consists of four ligands, termed PDGFA to PDGFD, that form different combinations of homo- and heterodimers that bind to one of the two receptors, PDGFRα or PDGFRβ. Homodimers of PDGFA and PDGFB, as well as heterodimers of PDGFA and PDGFB, induce mesonephric cell migration in XX gonads [46,51]. Null mutations of the Pdgfa or Pdgfb genes do not, however, cause defects in early gonad differentiation. By contrast, mice with a mutation in the gene encoding the PDGFRα receptor (Pdgfra) do show defects in cell migration and vascular formation.

Pdgfra is expressed in the gonads of both sexes at 11.5 dpc but is strongly upregulated in the interstitium of XY gonads at 12.5 dpc [46,51]. Pdgfra mutants die midgestation, but survive long enough to enable an examination of early testis development on some genetic backgrounds. XY gonads from Pdgfra mutants contain a highly disorganized vasculature. The coelomic vessel is irregular and does not branch as it does in wild-type XY gonads. Recombination experiments have shown that Pdgfra is necessary for endothelial migration; however, the receptor is required in cells of the gonad and not in mesonephric cells [46]. This finding suggests that PDGF signaling does not directly induce migration, but that it might activate a secondary signal. Testis cord formation is also disrupted in Pdgfra mutants, with mutant testes containing large and irregularly shaped cords [46]. It is not known whether PDGFRα mediates both vascular development and cord formation, or whether defects in the development of one structure influence the other.
Leydig cell differentiation

Leydig cells are the steroidogenic lineage of the testis, being responsible for the production of testosterone in both the embryonic and the adult testis. Embryonic and adult Leydig cells are, however, two distinct populations. During embryogenesis, Leydig cells differentiate in the interstitial region between 12.5 and 13.5 dpc (Figure 1) and produce testosterone, which masculinizes the internal and external genitalia. This population of Leydig cells is gradually lost after birth, whereas the adult population forms during puberty and is maintained throughout adult life [52,53]. A significant body of work has described the molecular signals that regulate the proliferation, differentiation and function of adult Leydig cells [54]. By contrast, much less is known about the origin and differentiation of the embryonic Leydig population, despite the essential role of these cells in male reproductive development.

A factor that is known to be required for the differentiation and expansion of fetal Leydig cells is Desert hedgehog (DHH), one of three mammalian homologs of Drosophila Hedgehog. The original analysis of mice deficient for Dhh on a 129/Sv genetic background identified defects in spermatogenesis [55]; however, Dhh mutants on a mixed genetic background (129/Sv, C57BL/6 and Swiss Webster) have been subsequently shown to have defects in the development of peritubular myoid cells and Leydig cells in the adult testis [56,57]. Further examination of Dhh mutants on the mixed background has indicated that Dhh also is required during the development of fetal Leydig cells. Dhh is first expressed by Sertoli cells of XY gonads at 11.5 dpc, whereas the gene encoding its receptor Patched1 (Ptc1) is initially expressed in the interstitial space between testis cords at 12.5 dpc [58]. Dhh XY mutant gonads contain no Leydig cells at 13.5 dpc; at later stages, some Leydig cells are observed in these mutant gonads, but the number of cells is markedly fewer than in controls [58]. The defects in Leydig cell differentiation explain the observation that Dhh XY mutants on this background have feminized external genitalia. Additional evidence that Dhh has a crucial role in testis development has come from the recent identification of mutations in human DHH in 46,XY individuals affected with partial or complete gonadal dysgenesis [59,60].

In addition, PDGF signaling is required for the normal differentiation of fetal Leydig cells. As discussed above, XY embryos with mutations in the Pdgfra gene show defects in vascular development and testis cord formation. In addition, early Leydig cell differentiation is disrupted in these mutants, as evidenced by the absence of the steroidogenic marker Scc, which encodes the p450 side chain cleavage enzyme, in mutant embryos at 12.5 dpc. It is possible that the loss of Leydig cell differentiation is simply a downstream result of the other defects that occur in these mutants; however, the idea that PDGF signaling is specifically required for the differentiation of steroidogenic lineages is supported by the finding that Pdgfra mutants also have defects in the development of adult Leydig cells [61]. Although the differentiation of embryonic Leydig cells has not been examined in Pdgfra mutants, development of the external genitalia is normal in XY Pdgfra mutants, suggesting that embryonic Leydig cell formation is not disrupted. Because several PDGF ligands can bind to each receptor, however, there might be functional redundancy among the different PDGF ligands.

Pathways that repress testis development in XX gonads

In this review, we have mainly focused on signaling pathways that regulate testis differentiation, with little discussion of ovarian development. This is partly because obvious morphological differentiation of the ovary occurs at later stages, but it is also due to the limited knowledge that currently exists regarding the molecular regulation of early female gonad differentiation. Several genes are known to be required for follicle formation in the ovary near the time of birth, including Figla and Foxl2, both of which encode transcription factors [62,63]. Additional studies have identified two secreted factors, Wnt4 and follistatin, which are required during early gonadal development to repress aspects of testis differentiation in XX gonads. For some time, ovarian development was referred to as the ‘default’ pathway that occurs in the absence of Sry and was often considered to be a passive process. Recent discoveries have now made it clear that early ovarian development is an active process that involves the interaction and competition of multiple signaling pathways that specify male or female development.

Wnt4 encodes a secreted growth factor that was one of the first known markers of ovarian differentiation. Wnt4 is initially expressed in the gonads of both sexes, but it becomes specific to XX gonads after 11.5 dpc. Analysis of mice that are homozygous null for a mutation in Wnt4 have shown that this factor has an important role during female reproductive development. Wnt4 mutants do not form a Müllerian duct, the progenitor of the female reproductive tract, and XX Wnt4−/− gonads show extensive germ cell loss and contain many ectopic steroidogenic cells [64].

The presence of steroidogenic cells in Wnt4 mutants is unusual, because steroid production does not occur in wild-type ovaries until after birth. These cells were initially thought to be ectopic Leydig cells; however, further studies have shown that the steroidogenic cells in the Wnt4 mutant gonads are likely to be adrenal precursor cells misapportioned to the gonad [65]. A recent discovery suggests that WNT4 has similar functions during human development. A female individual lacking Müllerian-derived structures and showing features characteristic of androgen excess has been shown to carry a missense mutation in the WNT4 gene [66].

Further studies have shown that Wnt4 has an additional role in the suppression of testis-specific vascular development. XX Wnt4 mutant gonads have a large vessel underneath the coelomic epithelium that is formed by migrating mesonephric endothelial cells, similar to the vessel that forms in XY gonads [67]. Thus, it seems that Wnt4 is required to block mesonephric cell migration in XX gonads very early in ovarian development. This idea is supported by the finding that the testis vascular pattern is disrupted in XY mice that have been genetically engineered to overexpress mouse or human Wnt4 [67,68].
Two genes encoding secreted signaling molecules that function downstream of Wnt4 have been identified. Bmp2 is expressed specifically in XX gonads starting at 11.5 dpc, and its expression is lost in Wnt4 mutants [69]. The early embryonic lethality of the Bmp2 mutants precludes, however, an analysis of its function in ovarian development in vivo. Fst encodes follistatin, a secreted protein that binds to activins and some BMPs to inhibit their function [70]. Like Bmp2, Fst is expressed specifically in XX gonads and its expression is lost in Wnt4 mutants. Examination of Fst mutants has confirmed the idea that Fst functions in a pathway downstream of Wnt4. Although Wnt4 is expressed in XX mutant gonads, the Fst mutants show a phenotype similar to that of Wnt4 mutants. A coelomic vessel forms in gonads from XX Wnt4 and Fst homozygous mutants, and the mutants also show extensive germ cell loss [69]. Fst mutant gonads do not contain ectopic steroidogenic cells, however, which suggests that Wnt4 mediates other pathways that do not involve Fst.

Because of the similarities of the Wnt4 and Fst mutant phenotypes, it was thought that follistatin acts downstream of Wnt4 to block mesonephric cell migration in XX gonads. Follistatin functions by inhibiting members of the TGF-β family, which could suggest that activin, or possibly a BMP, might regulate endothelial cell migration in XY gonads. Thus, these studies are particularly useful because they have provided insight into the mechanisms directing ovarian development and have implicated candidates that might be involved in aspects of testis differentiation.

Concluding remarks
Our understanding of the signaling events that regulate testis and ovary development has greatly increased in recent years. How Sry tips the balance towards Sertoli cell differentiation in the XY gonad, however, remains unclear. Downstream of this event, numerous signaling molecules including FGF9, DHH and PDGF promote Sertoli differentiation and recruit other cells in the XY gonad to the testis pathway. But direct molecular links between SRY and these signaling pathways have not been established. Although some of the cellular mechanisms regulated by these signals, including proliferation, cell migration and vascularization, have been described, we know relatively little about the molecular changes that lead to these morphological events.

Our understanding of the mechanisms that regulate germ cell differentiation is particularly limited, and the relationship between somatic lineages of the gonads and germ cells will be an important area of study in coming years. Finally, we are only just beginning to understand the signals that mediate many of the early events of ovarian differentiation. Gaining a better understanding of these processes will provide further insight into the mechanisms that regulate the bipotential fate of the gonad.

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