ONE TISSUE, TWO FATES: MOLECULAR GENETIC EVENTS THAT UNDERLIE TESTIS VERSUS OVARY DEVELOPMENT

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The pivotal point of vertebrate sex determination is the development of the gonad into a testis or ovary, which governs phenotypic sex through the production of hormones. The identification of *Sry*, the genetic switch that controls testis development in mammals, fuelled the race for the discovery of downstream pathways. Comparative analyses of XY versus XX gonadogenesis in both mouse and human genetic mutants have uncovered a burgeoning network of intra- and extra-cellular pathways. Here, we review the old and new players that are involved in the initial steps of testis and ovary development.

PRIMORDIA
The initial founder group of cells that give rise to a tissue.

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The gonad is unique among all organ PRIMORDIA because of its bipotential nature. One of two organs, a testis or an ovary, can develop from a single primordium. For this reason, it is a particularly interesting model system in which to study organogenesis. Mutations or *in vitro* manipulations that block either male or female pathways often result in the development of the alternative organ. This feature of gonad development provides insights into the plasticity of developmental pathways and the processes by which cells choose between fates. From the point of view of experimental design, the development of two distinct organs from a single primordium affords opportunities for comparative analyses that are targeted at the point of divergence. These approaches have begun to identify genetic pathways that are specifically associated with the reorganization of cells into testis or ovary morphology.

In most mammals, sex determination is genetically controlled by the presence or absence of the Y chromosome. The development of secondary sex characteristics, including the external genitalia and the male or female ductal systems, depends on whether the gonad develops as a testis or an ovary. It is therefore the differentiation of the testis or ovary that determines the sex of the embryo. The identification of the sex-determining gene that initiates testis differentiation in mammals was a starting point for the analysis of the genetic pathways that control the rapid differentiation of testis-specific cell types and their organization into testis-specific structures. In mammals, the initiation of the male pathway depends on gonadal expression of the Y-linked gene, *Sry*. XY mice with no functional *Sry* develop ovaries; conversely, the addition of *Sry* to XX mice triggers the testis pathway[1–3]. Furthermore, a survey of human patients who show partial to complete sex reversal indicated that approximately 10% carry mutations in *SRY*[4].

Consistent with the prediction that the male sex-determining gene would act as a genetic switch to control multiple downstream pathways, *Sry* encodes a putative transcription factor that contains a high-mobility group (HMG)-box DNA-binding domain. However, the identification of specific downstream transcriptional targets has been difficult. For this reason, most of the progress in identifying the genetic pathways that are controlled by *Sry* has come from opportune mutations that cause disruptions to male development, and from analysing morphological changes that occur in the gonad immediately after *Sry* expression begins, to predict the involvement of candidate signalling pathways. It is now evident that many familiar signalling pathways are superimposed on transcriptional cascades in the decision to make a testis or an ovary (FIG. 1). It is an appropriate time to consolidate what we know and

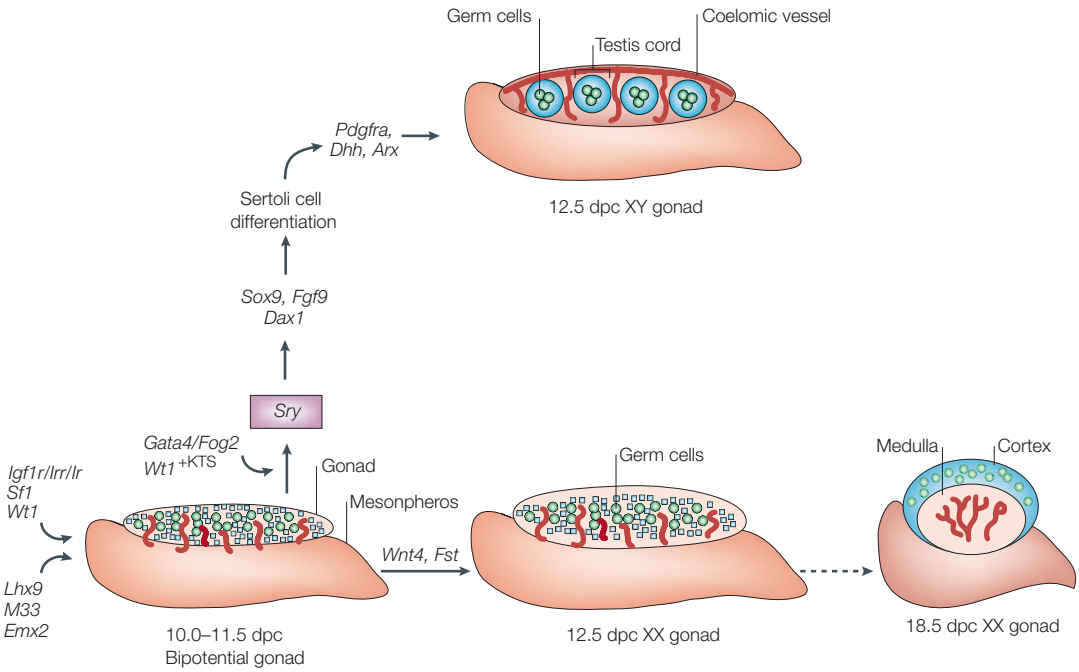

Figure 1 | **Genetic pathways with a characterized functional role in the divergent development of XX and XY gonads.** Several factors are required between 10.5–11.5 days post coitum (dpc) for the outgrowth of the early bipotential gonad by preventing apoptosis or promoting cell proliferation (*Sf1*, *Wt1*, *Lhx9*, *M33*, *Emx2*, *Igf1r/Ir/Irr*). Between 10.5–12.0 dpc, GATA4/FOG2 and WT1+KTS are implicated in the activation of *Sry* expression in the XY gonad. *Sry* expression diverts the XY gonad towards the testis fate. *Sox9*, *Fgf9* and *Dax1* are implicated in the early steps of the male pathway after the initiation of *Sry* expression. Downstream signalling pathways promote the rapid structural changes that characterize early testis development (*Pdgf*, *Dhh*, *Arx*). By contrast, few morphological changes are apparent in the XX gonad until near birth (18.5 dpc), when ovarian follicles begin to form in the ovarian cortex. *Wnt4* and *Fst* are the only two genes with characterized functions in early ovarian development. *Arx*, aristaless related homeobox; *Dax1*, nuclear receptor subfamily 0, B1 (Nr0b1); *Dhh*, desert hedgehog; *Emx2*, empty spiracles homologue 2; *Fgf9*, fibroblast growth factor 9; *Fog2*, zinc finger protein, multitype 2 (*Zfpm2*); *Fst*, follistatin; *Gata4*, GATA binding protein 4; *Igf1r*, insulin-like growth factor 1 receptor; *Ir*, insulin receptor; *Irr*, insulin receptor-related receptor; *Lhx9*, LIM homeobox protein 9; *M33*, chromobox homologue 2 (*Cbx2*); *Pdgf*, platelet-derived growth factor; *Sf1*, nuclear receptor subfamily 5, group A member 1 (*Nr5a1*); *Sox9*, *Sry*-like HMG-box protein 9; *Wnt4*, wingless-related MMTV integration site 4; *Wt1*, Wilms tumour homologue.

COELOMIC EPITHELIUM
An epithelial layer that lines the coelom, the body cavity, and covers the abdominal organs that arise beneath it.

MESONEPHROS
The mid-region of the embryonic kidney that arises within the intermediate mesoderm between the pronephros and the metanephros (the definitive kidney). The gonad develops on the medial surface of this transient tissue, which also houses the primordia for the male (mesonephric) and female (Müllerian) ducts.

PERITUBULAR MYOID CELLS
A muscle-lineage cell type that surrounds the Sertoli-cell cords and is believed to be responsible for peristaltic action that is involved in the expulsion of sperm from the seminiferous tubules.

LEYDIG CELLS
Interstitial cells in the testis that are responsible for the production of male steroid hormones, such as testosterone, and are important in male sexual differentiation.

to integrate extracellular and intracellular signals into our understanding of gonad development.

Overview of testis development

Gonadal primordia develop as paired thickenings of the epithelial layer that lines the body cavity (the COELOMIC EPITHELIUM), specifically in the region that overlays the ventral–medial surface of the MESONEPHROS. The signals that specify this regional thickening of the coelomic epithelium are unknown, but experiments in mice have identified several genes that have roles in the early growth and maintenance of the gonad in both sexes[5–10] (TABLE 1; FIG. 1).

Testes begin overt differentiation at an earlier stage than ovaries, an observation that has led to the idea that the initiation of the male pathway is an active process that intercedes to divert bipotential gonadal cells towards testis fates before ovarian development is initiated. Although *Sry* expression can be detected by 10.5 days post coitum (dpc) in mouse gonads, testis-cord formation — the earliest morphological sign of testis differentiation — occurs approximately 36 hours later, between 12.0 and 12.5 dpc. *Sry* expression in gonadal somatic cells initiates the differentiation of Sertoli cells, a key cell type of the testis that is essential in subsequent testis differentiation. Sertoli cells polarize, aggregate around germ cells and reorganize the gonad into two compartments: the tubular testis cords, composed of Sertoli and germ cells, and the interstitial space between the cords. PERITUBULAR MYOID CELLS surround Sertoli cells and cooperate to deposit the basal lamina at the periphery of tubule structures[11,12]. Other interstitial cell types include steroid-secreting LEYDIG CELLS, uncharacterized fibroblasts and the typical vasculature of the XY gonad (FIG. 2).

Analyses of testis organogenesis fall into two main lines of investigation: the characterization of the initial steps that establish the male pathway by promoting Sertoli-cell differentiation, with the goal of understanding how *Sry* directs gonadal somatic cells to the Sertoli fate (as opposed to the alternative ovarian follicular cell fate), and the comparison of the morphological development of the testis and the ovary, to determine how downstream signalling pathways induce the rapid and marked organization of cells into testis versus ovarian structure. Here, we focus on results from these approaches that have begun to be used to build a framework of pathways involved in testis and ovary organogenesis, and we briefly review prospects for the future of the field.

Table 1 | **Genes involved in early gonadal growth and testis differentiation**

Gene	Knockout phenotype	Proposed function
Early growth and survival of the gonad		
Emx2	*Emx2*$^{-/-}$ XX and XY gonads have a phenotype that is similar to *Wt1*, *Sf1* and *Lhx9* mutants. Gonads regress after 13.0 dpc[5].	Not known.
Igf1r/Irr/Ir	A triple knockout of the *Igf1r*, *Irr* and *Ir* genes results in male-to-female sex reversal. *Sry* is expressed, but downstream Sertoli markers are absent[33].	Insulin-receptor signalling promotes early growth of XX and XY gonads. Defects in proliferation could affect the XY gonad by decreasing the number of *Sry*-expressing cells.
Lhx9	*Lhx9*$^{-/-}$ XX and XY gonads fail to proliferate and regress by 13.0 dpc[8].	*Lhx9* might regulate *Sf1* or the early proliferation of SF1 positive cells at the coelomic epithelium.
M33	*M33*^{cterm} mutant gonads are developmentally delayed and show a high rate of male-to-female sex reversal. Gonads do not regress or undergo apoptosis[9].	Sex reversal could result from decreased proliferation in bipotential gonads or from proliferation and differentiation events at the level of or downstream of *Sry*.
Sf1	*Sf1*$^{-/-}$ XX and XY gonads undergo apoptosis and regress by 12.5 dpc[6].	SF1 regulates survival and/or proliferation of bipotential gonadal cells. SF1 might also be involved in the upregulation of *Amh* in the XY gonad[51].
Wt1	*Wt1*$^{-/-}$ and *Wt1–KTS*$^{-/-}$ XX and XY gonads begin to regress by 12.5 dpc[7,17].	The –KTS isoform is required for the survival/proliferation of bipotential gonadal cells.
	Wt1+KTS$^{-/-}$ gonads specifically exhibit male-to-female sex reversal[17].	The +KTS isoform contributes to the upregulation of *Sry* expression.
Pro-testis genes		
Arx	Fetal Leydig cells fails to differentiate in *Arx*$^{-/-}$ gonads[89]. No other defects are apparent in XY gonads.	*Arx* is not expressed in Leydig cells so it must function in a paracrine pathway to promote Leydig-cell differentiation. Additional experiments are necessary to test the interaction of ARX with the *Pdgf* and *Dhh* pathways.
Dax1	Deletion of *Dax1* results in severe defects in testis morphology. Full sex-reversal occurs on a Y^{Pos} background with normal *Sry* expression[36,37].	*Dax1* levels are crucial for testis development — too much or too little can prevent cord formation. *Dax1* acts downstream or in a parallel pathway with *Sry* to promote organization of testis cords and expression of male markers.
Dhh	Fetal Leydig-cell differentiation and cord formation are disrupted in *Dhh*$^{-/-}$ XY gonads[89].	The *Dhh* gonad phenotype is similar to *Pdgfrα*$^{-/-}$, although the two function in separate pathways. Unlike *Pdgfrα*, *Dhh* does not affect cell migration or proliferation. *Dhh* might be required to maintain high levels of SF1 in pre-Leydig cells.
Fgf9	Most *Fgf9*$^{-/-}$ XY gonads are fully sex-reversed with no expression of male markers downstream of *Sry*[34].	*Fgf9* promotes proliferation of Sertoli-cell precursors and could be involved in subsequent steps of Sertoli differentiation through nuclear localization of FGFR2 (REF. 35).
Gata4/Fog2	*Fog2*$^{-/-}$ and *Gata4*$^{ki-/-}$ XY do not develop testis cords or male-specific cell types, and *Sry* expression is reduced[18].	GATA4/FOG2 promotes expression of *Sry* and therefore the expansion or differentiation of the Sertoli-cell lineage. Later functions or roles in ovary development are unknown.
Pdgfrα	*Pdgfrα*$^{-/-}$ XY gonads have delayed, abnormal cord formation and defective Leydig-cell development. Cell migration and coelomic proliferation are disrupted[71].	*Pdgfrα* functions downstream of *Sry* in interstitial cells to promote cord formation and Leydig-cell differentiation. *Pdgfrα* signalling acts as an indirect migration signal. Leydig-cell differentiation could be influenced by effects on cell proliferation and signals that act in parallel with *Dhh* signalling.
Sox9	*Sox9*$^{-/-}$ embryos die before testis phenotype can be assessed. Haploinsufficiency can cause sex reversal in humans[124]. In mouse, transgenic expression (*Wt1*:*Sox9*) induces XX sex reversal[49].	*Sox9* is proposed as a direct downstream target of SRY. It can fully substitute for all functions of SRY. Downstream targets of SOX9 include *Amh* and *Sry*[47,50,51].

Arx, aristaless related homeobox; *Dax1*, nuclear receptor subfamily 0, B1 (Nr0b1); *Dhh*, desert hedgehog; dpc, days post coitum; *Emx2*, empty spiracles homolog 2; *Fgf9*, fibroblast growth factor 9; *Fog2*, zinc finger protein, multitype 2 (*Zfpm2*); *Gata4*, GATA binding protein 4; *Igf1r*, insulin-like growth factor 1 receptor; *Ir*, insulin receptor; *Irr*, insulin receptor-related receptor; *Lhx9*, LIM homeobox protein 9; *M33*, chromobox homologue 2 (*Cbx2*); *Pdgfrα*, platelet-derived growth factor receptor-α; *Sf1*, nuclear receptor subfamily 5, group A, member 1 (*Nr5a1*); *Sox9*, *Sry*-like HMG-box protein 9; *Wt1*, Wilms tumour homologue.

Establishing the male pathway

***Sry*: the genetic switch.** *Sry* expression in the XY gonad at 10.5 dpc is the first difference between XX and XY gonads. In the mouse gonad, *Sry* RNA expression is restricted to a brief period of time during the commitment to the testis pathway (10.5–12.5 dpc)[13–16]. There is little molecular information about the regulation of *Sry*; however, three transcription factors/co-factors, WT1+KTS, GATA4 and FOG2, are implicated in the transcriptional or post-transcriptional regulation of the gene (TABLE 1)[17,18].

SRY can alter chromatin structure and regulate transcription through its HMG domain[19,20] (see REFS 21,22 for reviews of SRY structure and function). Potential SRY-binding sites have been identified in the promoters of several genes, including *Sry*-like HMG-box protein 9 (*Sox9*)[23]; however, these consensus binding sites occur frequently in the genome, and the features that characterize active sites *in vivo* are not yet known. There is still no direct evidence for SRY regulation of any specific downstream gene, although it has been suggested that

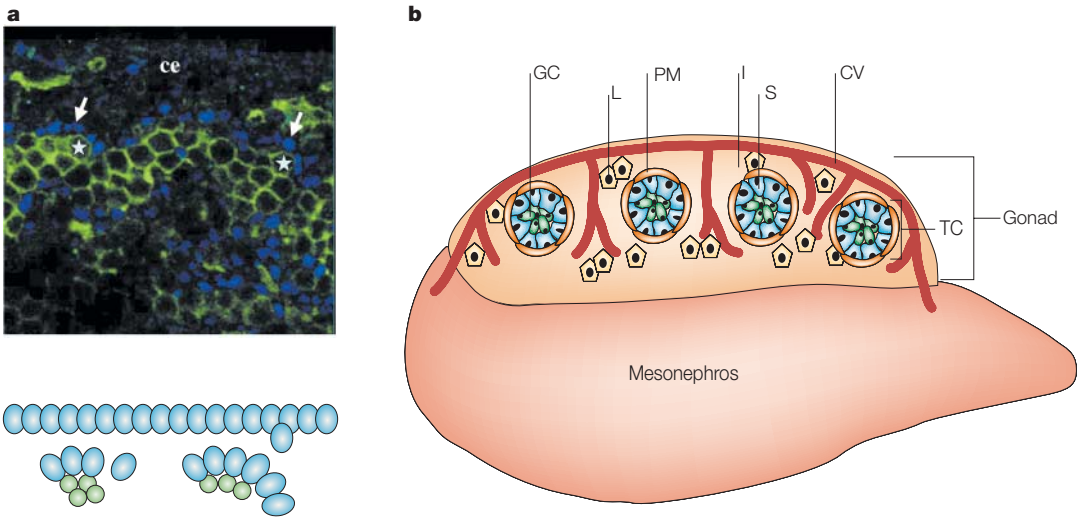

Figure 2 | **Compartmentalization of the testis. a** | At the earliest stages of testis organogenesis (11.75–12.0 days post coitum; dpc), Sertoli cells (stained with SF1 antibody; blue) polarize and begin to aggregate around clusters of primordial germ cells (stained with PECAM antibody; asterisk) to initiate development of testis cords. ce, coelomic epithelium. **b** | Between 11.5–12.5 dpc, the cells of the testis are organized into two functional compartments: testis cords (TC) and the interstitial space (I) outside the cords. Within testis cords, Sertoli cells (S; blue) surround germ cells (GC; green). A basal lamina is deposited between Sertoli cells and peritubular myoid cells (PM). The interstitial compartment contains Leydig cells (L; yellow) and the coelomic vessel (CV; red), with branches that extend between cords.

SRY has one or few direct targets and that the pathway branches downstream of those targets[24].

Rather than acting as a classic transcription factor, SRY might be involved in altering chromatin-domain organization. Mutations in two other genes that are involved in chromatin modification and remodelling, *M33* (*Cbx2*) and *ATRX*, have been associated with gonadal abnormalities[25,26], which supports the idea that chromatin regulation has an important role in sex determination. Mouse *M33*$^{-/-}$ XY gonads fail to organize as testes and resemble ovaries at birth. Human patients with mutations in *ATRX* have DYSGENIC testes, but the role of ATRX in early gonadal development has not yet been addressed. By analogy to other systems, either of these genes could be required together with SRY to alter chromatin configuration, thereby activating testis-determining genes or repressing pro-ovarian genes.

The origin of Sertoli cells: proliferation. Sertoli cells are known as the supporting cell lineage of the testis because they support the growth and maturation of germ cells. They are also crucial for the early stages of testis development. Analysis of cells from the testes of chimeric XX↔XY embryos indicated that Sertoli cells showed a strong bias for the Y chromosome (~90% of Sertoli cells were XY, whereas other cell types of the testis were XX or XY with equal frequency)[27]. This finding indicated that the Y chromosome (and therefore *Sry*) is required only in the Sertoli-cell lineage. On the basis of this genetic evidence, it is believed that the primary role of *Sry* is to initiate Sertoli-cell differentiation. But, surprisingly, even in Sertoli cells, the requirement for *Sry* was not absolute as ~10% of Sertoli cells in these chimeras were XX (that is, they had no Y chromosome or *Sry* gene), which indicates that a downstream PARACRINE factor recruited non-*Sry*-expressing cells to the Sertoli pathway. Chimeric gonads that are composed of less than 30% XY cells developed as ovaries, which suggests that a critical threshold number of *Sry*-expressing cells is needed to recruit the gonad to the testis pathway[27].

Other evidence also indicates that a threshold number of Sertoli cells is essential for the initiation of the male pathway. It has long been recognized that during the early stages of gonad differentiation, the size of the male gonad increases relative to the female gonad in mammals and in other vertebrates that depend on non-*Sry*-based mechanisms of sex determination, such as birds and reptiles[28,29]. In the mouse, the size increase of the XY gonad results from increased cell proliferation[30]. Proliferation increases in XY gonads by 11.2 dpc and is concentrated in the coelomic epithelium, which is a zone of multipotent precursors for somatic cells in the gonads of both sexes (FIG. 3)[30,31]. During the early phase of proliferation (11.0–11.5 dpc), both Sertoli and interstitial cells derive from the division of SF1-expressing cells in this layer. SF1 expression is subsequently lost from coelomic epithelial cells. Proliferation continues, although only interstitial cells are produced. Injection of proliferation inhibitors during an 8-hour time window centred around 11.0 dpc (the early phase of proliferation) inhibits proliferation of coelomic epithelial cells, blocks expression of *Sox9* (a marker of Sertoli-cell differentiation) and prevents testis-cord formation[32]. This is consistent with the chimera experiments described earlier, which indicate that a critical threshold number of Sertoli precursors is required to initiate the testis pathway[27]. These results indicate a community effect in the gonad, in which a sufficient number of Sertoli cells

DYSGENIC
Showing failed or disrupted morphology.

PARACRINE
A form of cell–cell communication that depends on a secreted substance that acts over a short distance and does not enter the circulation.

that produce testis-determining signals are required to counterbalance competing ovarian signals in this bipotential field of cells. Another interpretation of these results is that cell division is somehow required to initiate Sertoli differentiation. This might be the case if cell division were required to asymmetrically distribute molecular components to the two sister cells that are produced by the division. Alternatively, cell division might be necessary to reorganize chromatin domains. These possibilities are attractive because they could link the proliferation that is required to generate an adequate number of pre-Sertoli cells with their differentiation.

Mutations that cause general proliferation defects early in gonadogenesis can result in a reduction in the number of Sertoli cells (TABLE 1). For this reason, results that show reduced expression of *Sry* (usually measured by reverse transcriptase-PCR), as in *Gata4*/*Fog2* mutants, must be interpreted with caution. A reduction in *Sry* expression could result from proliferation defects or failures in transcriptional regulation of *Sry*.

The insulin-like growth factor (*Igf*) is another recently reported example. A triple knockout of the three insulin-receptor genes, *Ir*, *Igf1r* and *Irr*[33], results in complete male-to-female sex reversal, indicating a role for insulin signalling at early stages of gonadogenesis. Insulin signalling is required for the establishment of the male pathway, based on the fact that expression of *Sry* and *Sox9* were reduced, and later male markers were absent in the triple mutants. In this mutant, both XY and XX gonads were significantly reduced in size and both later expressed female markers. So, rather than there being a direct mechanism that regulates *Sry* expression, reduced *Sry* expression could be a secondary effect of the general proliferation defect and subsequent reduction of *Sry*-expressing pre-Sertoli cells. These results indicate that although insulin-receptor signalling is not specific to the testis pathway, because proliferation is specifically required at this stage in the XY but not in the XX gonad, mutations in these receptors differentially affect testis development.

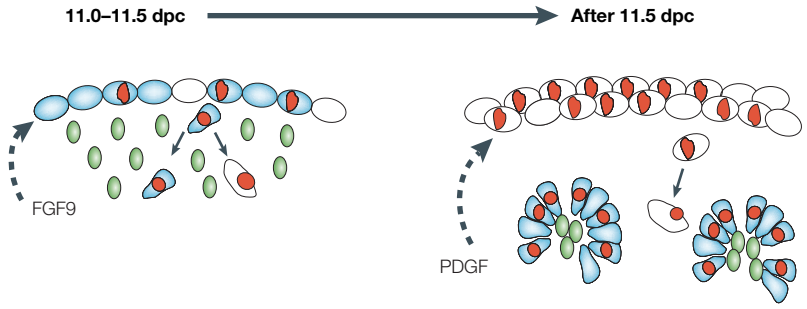

Figure 3 | **XY-specific proliferation of coelomic epithelial cells.** Proliferation of coelomic epithelial cells in XY gonads occurs in two stages. FGF9 promotes male-specific proliferation (red nuclei) of Sertoli-cell precursors between 11.0–11.5 days post coitum (dpc). DiI lineage-tracing experiments show that daughters of single proliferating cells then move into the gonad and contribute to the Sertoli (blue) and other cell lineages (white). After 11.5 dpc, the rate of proliferation of cells in the coelomic epithelium increases, promoted by PDGF signalling. Although proliferation at this stage continues to give rise to other interstitial-cell lineages in the gonad, it no longer gives rise to Sertoli cells. Sertoli precursors within the gonad (blue) cease proliferation after 11.5 dpc and begin to organize around clusters of germ cells (green). FGF9, fibroblast growth factor 9; PDGF, platelet-derived growth factor.

Other factors control male-specific proliferation and do not disrupt the growth of the female gonad. An essential male-specific role for *Fgf9* (fibroblast growth factor 9) was revealed by a targeted deletion in mice that resulted in a high percentage of male-to-female sex reversals[34]. On a C57BL/6 genetic background, 100% of *Fgf9*$^{-/-}$ XY gonads developed into ovaries, and there was no evidence of *Sox9* or other markers downstream of *Sry* in 12.5-dpc gonads. FGF9 regulates the early proliferation of Sertoli precursor cells as evidenced by the marked reduction in coelomic proliferation in XY gonads. *Fgf9*$^{-/-}$ XX gonads are unaffected, which indicates that *Fgf9* activity is specific to the male pathway. Importantly, although the addition of recombinant FGF9 to the culture medium can induce coelomic proliferation in XX gonads, Sertoli cells fail to differentiate[35]. This finding indicates that simply increasing proliferation is not sufficient to induce Sertoli fate and that the concerted action of other signals downstream of *Sry* is required.

The origin of Sertoli cells: differentiation. Much recent evidence indicates that there is a transient pulse of *Sry* expression in each pre-Sertoli cell as it initiates differentiation. We initially postulated that *Sry* would be expressed in coelomic epithelial cells during the early stages of gonad development, as Sertoli cells originate from this population[30,31]. But *Sry* is not expressed in coelomic epithelial cells[14–16]: instead, it is expressed in scattered cells in the interior of the gonad and in some cells just beneath the coelomic epithelium. No antibody against mouse SRY has yet been reported. On the basis of a transgene expressing GFP from the *Sry* promoter (*Sry:GFP*)[15], or a transgene expressing an SRY fusion protein (*Sry*^{Myc})[16], *Sry* is not expressed in all pre-Sertoli cells simultaneously. However, by 12.5 dpc, all Sertoli cells are labelled in gonads from mice that are transgenic for a persistent *Sry* lineage tracer (*Sry:alkaline phosphatase*). The detection of alkaline phosphatase in every Sertoli cell by 12.5 dpc indicates that each of these cells activated the *Sry* promoter at some earlier stage[16]. This result supports a cell-autonomous role for SRY in normal Sertoli-cell development, but does not rule out a paracrine effect as seen in the chimera studies mentioned earlier.

Recently, the X-linked orphan nuclear hormone receptor *Dax1* (*Nr0b1*) was reported to function as an early mediator of testis development downstream of *Sry*. Initial observations on a mixed genetic background showed that *Dax*$^{-/Y}$ mice did not form distinct testis cords. However, Sertoli-cell numbers and early male markers, such as *Sox9*, *Amh*, *Dhh* and *Gata4*, were normally expressed[36]. Subsequent manipulation of the genetic background showed that *Dax1* mutants carrying a Y^{POS} chromosome, which encodes a hypomorphic allele of *Sry*[37], or on a pure C57BL/6 genetic background (E. Eicher, personal communication), were completely sex-reversed with no evidence of testis morphology or expression of early male markers such as *Sox9*. The finding that *Sry* levels were not further reduced in C57BL/6-XY^{POS} *Dax1*$^{-/-}$ mutants implies that *Dax1* functions at an early step downstream of

Sry, or possibly in a parallel pathway together with *Sry*, to establish Sertoli-cell differentiation.

These data are surprising in light of original experiments that indicated that *DAX1* functions to oppose the male pathway. *DAX1* was initially isolated from the dosage-sensitive sex-reversal region of the human X chromosome, which, when translocated as an extra active copy, leads to male-to-female sex reversal in human XY patients[38]. In accordance with the original data in humans, overexpression of *Dax1* in the mouse resulted in male-to-female sex reversal on vulnerable genetic backgrounds in which *Sry* expression was delayed or weakened[39]. Explanations for this paradoxical data include the possibilities that precise dosage of *Dax1* is required to establish the male pathway, as suggested in a recent review[40], or that *Dax1* has several roles in gonad development, perhaps in different lineages as indicated by the stage-specific expression pattern in different cell types[41]. The generation of lineage and stage-specific rescues of *Dax1* in *Dax1*$^{-/-}$ mice might help to unravel several roles for this gene during gonad development[42].

The effect of genetic background is apparent in several mutants in which it has been studied, including *Fgf9* (REF. 34), *Dax1* (REF. 37), *Sf1* and *Wt1* (E. Eicher, personal communication). In these cases, carrying the mutation on some genetic backgrounds leads to relatively minor defects in testis morphogenesis, in contrast to a complete sex reversal on a C57BL/6 background. The genetic explanation for this pronounced effect is not yet clear; however, several autosomal regions have been mapped that affect sex determination[43]. The primary decision between male and female pathways seems to be finely balanced, so that allelic differences that result in minor differences in the expression of key genes might have a significant impact at the pivotal point of sex determination. Once the balance is tipped one way or the other, male or female development is strongly CANALIZED by secondary feedback regulation.

Following *Sry* expression, *Sox9* upregulation is the earliest marker of pre-Sertoli cells[44,45]. *Sry* and *Sox9* share a highly conserved HMG box, such that in domain-swapping experiments, the *Sox9* HMG box can functionally substitute the *Sry* HMG box in XX transgenic mice[46]. SOX9 co-localizes with SRY in the nucleus of pre-Sertoli cells as early as 11.5 dpc[16]. As the SOX9-expressing population expands between 11.5–12.5 dpc, the number of cells that co-express SRY decreases until 12.5 dpc, when SRY expression is extinguished and SOX9 expression is confined to Sertoli cells inside cords. This pattern is consistent with the hypothesis that SRY activates the expression of *Sox9*; however, it is not yet clear whether this regulation is direct or even cell-autonomous. SOX9 acts as a downstream effector protein, which might also be involved in feedback regulation to extinguish expression of *Sry* itself[15,16,47]. An important question is whether the sole role of SRY is the activation of *Sox9* or if there are other targets for SRY (for a discussion, see REF. 24). The most parsimonious explanation is that SRY is required only to elevate *Sox9* expression. This idea is bolstered by recent experiments that indicate that *Sox9* can functionally substitute *Sry*. Ectopic activation of *Sox9* in XX *Odsex* (*Ods*) mice or expression of a *Sox9* transgene (*Wt1*:*Sox9*) in XX embryos produce fully sex-reversed (but sterile) male mice[48,49]. A targeted replacement of the *Sry* coding region with *Sox9* would provide one further test of this hypothesis. Although targeting the Y chromosome is not routine, this experiment would test the ability of *Sox9* to replace *Sry* and restore every aspect of male development, including male fertility.

As in the case of SRY, SOX9 targets have been elusive. *Amh* is the only target of SOX9 that has been identified by both *in vivo* and *in vitro* methods[50,51]. Other *Sox* family members, such as *Sox3* and *Sox8*, are also expressed in embryonic gonads; however, neither of these family members has a unique role in primary sex determination[52–54]. *Sox8* belongs to the same subgroup as *Sox9*, and could have redundant functions during gonad development. This is supported by the overlapping expression patterns of *Sox8* and *Sox9*, and similar target specificity in the activation of the *Amh* promoter[55].

Nuclear localization of both SRY and SOX9 is required for their function. Important roles for importin β1 and calmodulin in the nuclear localization of SRY and SOX9 were initially indicated by *in vitro* experiments[56,57]. In addition, sex-reversed patients were identified with defective importin β1 (REF. 21) and calmodulin-dependent recognition sequences[58], confirming the important role of these nuclear import signals in sex determination. Further experiments indicated that nuclear export might also be a regulatory mechanism for SOX9 function. Blocking nuclear export in cultured XX gonads results in nuclear localization of SOX9 and activation of AMH expression[59], implying that the male pathway is initiated. Although further experiments will be required to sort out the interplay of import and export pathways, these experiments indicate that the steady-state level of SOX9 in the nucleus might be crucial for the regulation of the commitment to the male pathway.

FGF9 signalling is required to establish *Sox9* expression[34,35]. Current evidence indicates that FGF9 signalling works through FGFR2, which is localized to the cell membrane of proliferating coelomic epithelial cells that give rise to Sertoli cells. Just beneath this layer of cells, FGFR2 relocates to the nucleus in cells that show nuclear SRY and SOX9 expression[35].

The biological relevance of the nuclear localization of growth-factor receptors is not known (reviewed in REFS 60,61), although there is growing evidence that growth-factor peptide ligands and their receptors might have several roles in cells through receptor-associated cytoplasmic signalling networks (sometimes referred to as 'non-genomic action') and direct intranuclear activities (referred to as 'genomic action')[62]. In the XY gonad, FGFR2 is at the plasma membrane of coelomic epithelial cells that are undergoing rapid cell division. By contrast, gonadal cells with nuclear FGFR2 do not divide at this stage of development[30]. Instead, these cells are initiating differentiation under the influence of SRY, which indicates that the nuclear localization of FGFR2 in Sertoli precursor cells might have a role in cell differentiation that is distinct from its role in cell proliferation. In *Fgf9*

CANALIZE
Guided down a narrowly constrained pathway.

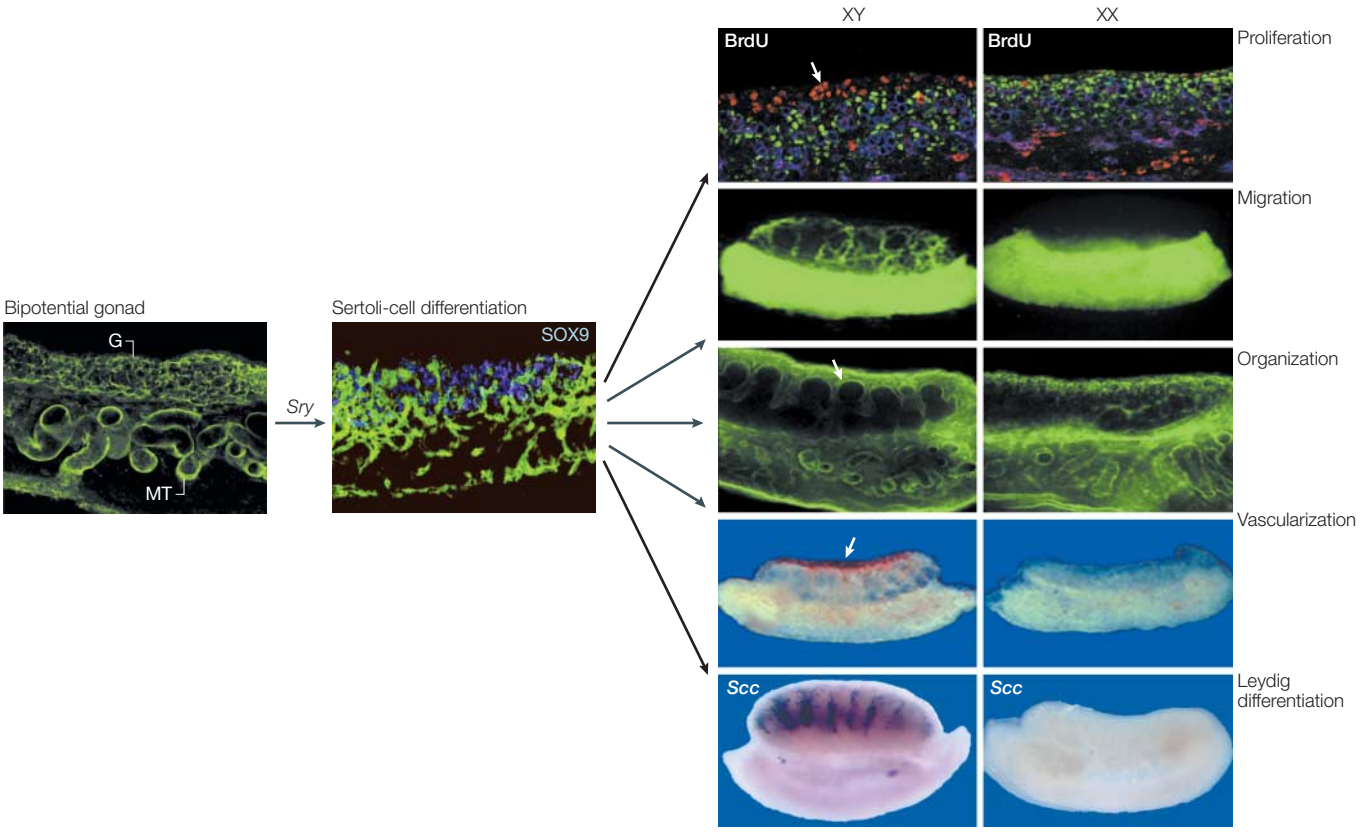

Figure 4 | **Cellular events downstream of *Sry* rapidly organize testis structure.** At the bipotential stage (10.5–11.5 days post coitum; dpc), no obvious morphological features distinguish XX and XY gonads. Antibodies against laminin (green) outline all cells in the gonad (G) and also label the basal lamina of mesonephric tubules (MT) in XX and XY samples. In XY gonads, *Sry* upregulates nuclear SOX9 (blue) in pre-Sertoli cells, and initiates Sertoli-cell differentiation by 11.5 dpc (germ cells and vasculature are labelled with platelet endothelial cell adhesion molecule (PECAM); green). Between 11.5–12.5 dpc, male-specific pathways activate marked morphological and cellular changes in the XY gonad (left column) that do not occur in the XX gonad (right column). These include an upregulation of proliferation in coelomic epithelial cells (measured by BrdU incorporation; red, arrow); migration of cells from the mesonephros (detected in recombinant cultures between a wild-type gonad and a mesonephros in which all cells express GFP; green); structural organization of testis cords (detected by laminin deposition; green); male-specific vascularization (red; blood cells are visible in the light microscope; arrow); and Leydig-cell differentiation (detected by RNA *in situ* hybridization for the steroid enzyme, *Scc*). BrdU image pair repoduced with permission from REF. 29 © (2000) The Company of Biologists Ltd. XY migration image and vascular image pairs reproduced with permission from REF. 77 © (2002) Elsevier Science.

mutants, nuclear localization of both FGFR2 and SOX9 are coordinately lost, although *Sry* is expressed (REF. 35; B.C. and Y. Kim, unpublished observations). Analysis of *Sox9* and *Fgfr2* mutants will be required to determine whether the upregulation and nuclear localization of SOX9 is upstream or downstream of FGFR2 nuclear activity.

Cellular events downstream of SRY and SOX9

Several sex-specific cellular events have been characterized that contribute to the morphogenesis of the XY gonad following Sertoli-cell differentiation. These include increased proliferation of interstitial cells, migration of cells from the mesonephros, vascularization and Leydig-cell differentiation (FIG. 4).

Cells of the XY gonad undergo a marked reorganization into two compartments that fulfill the basic functions of the testis: to provide an environment for maturation of the germ line, and to produce and export hormones that masculinize the embryo. The first function is met by the development of testis cords, where Sertoli cells enclose germ cells and mediate their growth and maturation throughout reproductive life. The second function is accomplished by the development of steroidogenic Leydig cells and a functional vasculature in the interstitium of the testis to produce and export hormones.

Cell migration and testis-cord formation. A key sex-specific event in testis development and cord formation is the migration of mesonephric cells into the XY gonad[63–66]. Recombinant organ-culture experiments demonstrated that the recruitment of cells from the mesonephros is a male-specific event. Migrating cells consist mainly of endothelial cells. Cells that resemble perivascular and peritubular myoid cells also seem to migrate from the mesonephros; however, these cell types have not been directly identified by molecular lineage markers[65]. *In vitro* culture experiments using gonads that normally form ovotestes have shown that migration is absent from ovarian regions and is restricted to regions where testis cords develop[67].

The importance of migration for cord formation has been directly tested by blocking migration at 11.5 dpc, either by culturing the gonad separately from the mesonephros or by placing a barrier between the two tissues in culture. In both cases, cord formation was impaired[63,68]. By culturing a piece of an XY gonad on the surface of the XX gonad, migration of cells from the mesonephros was induced in an XX gonad between 11.5 and 13.5 dpc, resulting in the formation of cord-like structures and expression of male markers in some samples[68]. Culturing XX and XY gonad halves side-by-side on top of a mesonephros does not induce migration or induction of markers in the XX portion of the graft, indicating that the migrating cells, and not just diffusible factors from the XY tissue, are required for this effect. It remains possible that a combination of migrating cells and diffusible factors is required. These experiments also determined that between 12.5 and 13.5 dpc, the XX gonad becomes refractory to induction by male factors, defining a bipotential window in gonad development that was indicated by earlier experiments (for a review, see REF. 69).

The addition of single recombinant growth factors expressed by Sertoli cells, including nerve growth factors (NGFs), hepatocyte growth factor (HGF), platelet-derived growth factors (PDGFs), FGFs and Anti-Müllerian Hormone (AMH), can induce migration from the mesonephros into XX gonads in culture[34,70–74]. Although this method can identify potential candidate migration factors, it does not provide definitive evidence for a role *in vivo*. For example, AMH can induce migration *in vitro*, yet in gonads from *Amh*$^{-/-}$ mice, migration occurs normally and testis development and function is not impaired. So, if AMH has a role in mesonephric cell migration *in vivo*, it is redundant with other TGF-β family members[72]. Similarly, deletion of two NGF receptors, *TrkA* (*Ntrk1*) and *TrkC* (*Ntrk3*) results in only minor disruptions in testis-cord morphology and interstitial development[75]. It is possible that redundant pathways mediate migration *in vivo* to ensure the morphological development of testis cords. Alternatively, under *in vivo* conditions, many of these factors might not have a role in the induction of cell migration.

Sex-specific cell migration is disrupted in genetic mutants for two candidate migration factors, *Fgf9* and *Pdgfrα*. Although cell migration does not occur in *Fgf9* mutants, early defects in proliferation and Sertoli-cell-fate commitment obscure any direct role of FGF9 because all downstream male events are absent[34,35]. *Pdgfrα* is required for migration, but surprisingly, this receptor is required in the cells of the gonad rather than in the migrating mesonephric cells, which indicates that PDGF signalling in the gonad activates a secondary migration signal. In *Pdgfrα* mutants, Sertoli differentiation occurs, but testis-cord formation is delayed until 13.5 dpc when a few abnormally shaped cords develop. These defects might result from a failure of the interstitial population to form or the testis-specific vasculature to develop[71].

Development of XY-specific vasculature. The primary growth of the vasculature from the mesonephros into the gonad is similar in XX and XY gonads at early bipotential stages of gonad development[76,77]. However, following *Sry* expression, extra endothelial cells are recruited to the XY, but not the XX, gonad. Migrating cells associate to establish the coelomic vessel, an arterial network that is identified by the arterial marker ephrin-B2 and elements of the Notch signalling pathway[77]. The arterial system in the early testis sets up an alternative pattern of blood flow that is diverted from the mesonephros through the coelomic vessel. It has been proposed that elaboration of the arterial system increases blood flow through the testis to promote the efficient export of testosterone from the early testis, a process that is crucially important for the masculinization of the rest of the embryo (secondary sex determination)[77].

The reorganization of the vasculature is coincident with cord formation between 11.5 and 12.5 dpc. An intriguing hypothesis is that endothelial branches that extend from the coelomic vessel help to partition the field of cells into cord-forming units, and participate in reciprocal signalling interactions with other somatic cells in the testis to coordinate vascular and organ structure. As in the liver and the pancreas, where the vasculature is known to have an active signalling role in organ development and differentiation of other cell lineages[78,79], close coordination between vessels, testis cords and endocrine cells might be required to form a physiologically functional testis. In the *Pdgfrα*$^{-/-}$ testis, disruptions in cord formation are coincident with disruptions in endothelial migration and the assembly and branching of the male-specific coelomic artery[71]. The differentiation of fetal Leydig cells, the early endocrine cells of the testis, is also disrupted in *Pdgfrα*$^{-/-}$ testes, which indicates that these three events might be closely integrated to establish a functional organ. Because vascular development, testis-cord formation and Leydig-cell differentiation occur so rapidly in the mouse, it is difficult to determine the relative timing and potential interactions of these three events. Comparative analyses in other systems, such as the marsupial, in which testis development occurs over a more extended period of time, might provide more insights.

Leydig-cell development

Leydig cells are responsible for steroidogenesis in the testis and develop as distinct fetal and adult lineages[80]. Fetal Leydig cells are present in the testis from 12.5 dpc until their numbers decline shortly after birth. Their primary function is the production of testosterone, which virilizes the internal and external genitalia. Although the details of the origin and differentiation of fetal Leydig cells have not been clearly determined, it is known that a common steroidogenic population (SF1 positive) is shared and divided between the adrenal cortex and the gonad. This cell population expands along the anterior of the mesonephros and into the gonad before 11.5 dpc[81]. In XX *Wnt4* mutants, a proportion of the adrenal steroid-cell population is misallocated to the gonad, which supports the idea of a shared progenitor pool of steroidogenic precursors and a role for *Wnt4*

in segregating the two populations[82–84]. At 11.5 dpc, expression of *Sf1* marks both the pre-Sertoli and pre-Leydig-cell populations. Subsequently, *Sf1* expression decreases in Sertoli cells and increases in differentiating Leydig cells at the time when they begin to express the enzymes that are necessary for testosterone production[85].

Dhh and *Pdgfrα* are XY-specific factors that are crucial for the early differentiation and expansion of the fetal Leydig-cell lineage. *Dhh* is expressed by Sertoli cells, and its receptor, *Ptch1*, is reciprocally expressed by the cells of the interstitium[86]. Similarly, *Pdgfrα* is expressed in the interstitium and *PdgfA* in Sertoli cells. Mutants in both *Dhh* and *Pdgfrα* have early defects in the partitioning of the testis-cord and interstitial compartments, and severely impaired differentiation of fetal Leydig cells[71,87,88]. The data from these experiments support a model in which PDGF signalling is required to promote proliferation and/or differentiation of Leydig-cell precursors, and in which DHH works through a separate pathway to induce or maintain high levels of SF1 in the precursor population.

The homeobox gene aristaless-related (*ARX*) is associated with abnormal genitalia in human patients. Targeted deletion of *Arx* in the mouse recapitulates many of the features in human patients and indicates that the genital abnormalities result from defects in fetal Leydig-cell differentiation[89]. In $Arx^{-/-}$ mice, initial testis-cord development and Sertoli and peritubular myoid-cell differentiation occurred normally, indicating that ARX acts predominantly on Leydig cells. Because *Arx* is expressed in the testis interstitium (peritubular myoid cells, endothelial cells and fibroblasts) but not in Leydig cells, it probably acts in a paracrine manner. A model that coordinates the nonlinear signalling relationships between *Dhh, Pdgfrα* and *Arx* requires further experimentation. However, the data are so far consistent with the idea that Leydig-cell differentiation depends on the superimposition of several signalling gradients on multipotent progenitor cells in the testis interstitium.

The role of germ cells in gonadogenesis

Germ cells migrate through the GUT MESENTERY and arrive in the gonad at the bipotential stage (10.0–11.5 dpc). Little is known about specific adhesion and signalling interactions between somatic and germ cells in the early mammalian gonad. Germ-cell markers that are expressed during the migratory phase, such as tissue nonspecific alkaline phosphatase (*Tnap*; *Akp2*)[90] and stage-specific embryonic antigen-1 (*Ssea1*)[91], are gradually downregulated once germ cells reach the gonad. Genes that are upregulated include mouse vasa homologue (*Mvh*; *Ddx4*)[92], germ cell nuclear antigen-1 (*Gcna1*)[93] and germ cell-less (*Gcl*)[94]. E-cadherin[95], ERb2 and ERb3 (REF. 96) are also expressed on the germ-cell surface in both sexes, but are downregulated in XX gonads after 13.5 dpc. Signals between *Kit* (expressed by germ cells) and its ligand, *Kitl* (expressed by somatic cells in the gonad) are believed to regulate germ-cell survival at early stages in both XX and XY gonads[97], but whether there are male- and female-specific signals at early stages is not known.

Although there is evidence to indicate that germ cells in XY gonads produce factors that have a supporting paracrine role in inducing Sertoli differentiation[98], germ cells are dispensable for testis development, as testis cords form in agametic gonads[99], albeit with a slight delay (B.C., unpublished observations). By contrast, germ cells are essential for the formation and maintenance of follicles in the ovary. In their absence, follicles degenerate into cord-like structures[100,101] and XX cells express male markers such as *Sox9* and *Amh* (see, for example, REF. 102).

In mammals, germ cells differentiate according to their somatic-cell environment[103]. XX germ cells that reside in a testis (for example, in XX embryos that are transgenic for *Sry*) follow the male pattern of differentiation, but do not survive male meiosis[2,104]. By contrast, XY germ cells that reside in an ovary (for example, in XY embryos that have a deletion of *Sry*) can complete oogenesis and form Y-bearing oocytes[1]. The control of the cell cycle in germ cells is crucially important in XX and XY gonads, and reflects the underlying antagonism between male and female developmental pathways. In the XY gonad, germ cells undergo mitotic arrest until after birth, when they move to the periphery of testis cords and constitute a renewing population throughout adult male life. By contrast, in the XX gonad, germ cells enter meiosis at 13.5 dpc and arrest in meiotic prophase as follicles form near birth. Germ cells in ectopic locations outside the gonad also enter meiosis with similar timing, implying that germ-cell entry into meiosis is a cell-autonomous programme that is based on an intrinsic clock[105]. These findings also indicate that entry into meiosis is actively blocked in XY gonads by signals that impose mitotic arrest. Aggregation experiments indicate that these signals occur between 11.5 and 12.5 dpc, after which both XX and XY germ cells seem to be programmed in their respective pathways[98].

It has been suggested that meiotic XX germ cells antagonize testis-cord formation[105]. This hypothesis was tested *in vitro* by culturing aggregates of male somatic cells with female germ cells from various stages[106]. In these aggregates, cord formation was inhibited in cultures that contained XX germ cells from meiotic stages, but not in cultures that contained pre-meiotic germ cells. Further experiments showed that XX gonads with meiotic germ cells were resistant to the induction of cell migration, whereas gonads without germ cells or with pre-meiotic germ cells were not resistant to migration. These experiments indicate that the permissive window for induction of the male pathway in the XX gonad terminates as germ cells progress towards meiosis and produce signals that antagonize testis-cord development[69,106]. In cases in which the male pathway is initiated late in the bipotential gonad (for example, in C57BL/6 XY^{POS} gonads, in which *Sry* is expressed later and at lower levels[107–109]), the advanced developmental stage of the germ cells could interfere with male development.

Interestingly, meiotic markers, such as γH2AX and Syn/Cor, appear in the XX gonad in a rapid wave of expression from anterior to posterior[106]. A similar pattern of expression of the female germ-cell-specific gene,

GUT MESENTERY
The tissue that is connected to the dorsal wall that supports the gut in the body cavity.

Stra8, presages entry of germ cells into meiosis[110]. These findings are at odds with the idea that germ-cell entry into meiosis is based on an intrinsic clock. McLaren has suggested that minor differences in the timing of entry into meiosis could be related to the time from commitment to the germ-cell lineage or departure from the initial cluster of germ cells[111]. This could translate into a wave of meiosis if germ cells that leave the initial cluster populate the gonad from anterior to posterior, but further experiments will be required to reconcile these findings.

Ovarian signals that antagonize the male pathway

The discovery that gonads develop as ovaries in the absence of the Y-chromosome (or, more specifically, the *Sry* gene) supported the prevailing view that the testis pathway is the active pathway in gonad development. However, as Eicher and others have emphasized, the ovarian pathway must also be an active genetic pathway[112]. Cases of sex reversal when *Sry* expression is delayed or expressed below a threshold level indicate that *Sry* timing and levels are crucial for overcoming active ovarian signals[107–109]. The existence of XX individuals that develop as males in the absence of the *Sry* gene led to the proposal that *Sry* normally represses a factor (Z) that functions at the top of a genetic cascade as a repressor of male development. XX individuals that have a mutation in Z might derepress the entire male pathway in the absence of the *Sry* gene[112–115].

So far, no overriding Z factor has been identified; however, *Wnt4* acts as a partial anti-testis gene by repressing aspects of male development in the female gonad. In *Wnt4*$^{-/-}$ XX mice, gonads trans-differentiate, but not until near birth when germ cells are lost, cord-like structures develop and male markers such as *Sox9* and *Dhh* are expressed[82]. Recently, it was shown that XX *Wnt4*$^{-/-}$ mutants also develop the XY-specific coelomic vessel[84], and that overexpression of *Wnt4* leads to disruption of the male vascular pattern[84,116].

Expression of two female-specific genes, *Bmp2* (REF. 117) and *Fst*[118], is absent in *Wnt4*$^{-/-}$ XX gonads, indicating that either of these genes could regulate pathways downstream of *Wnt4*. In *Fst* mutants, *Wnt4* is still expressed, but the mice recapitulate some aspects of the *Wnt4* mutant phenotype, including the formation of the male-specific vessel and the marked loss of germ cells. However, there is no evidence of Leydig-cell differentiation in *Fst*$^{-/-}$ XX gonads[117], indicating that *Wnt4* controls at least two independent pathways in ovarian development. One pathway is required to prevent misappropriation of steroidogenic cells to the ovary; the other to activate *Fst*, thereby repressing the formation of the coelomic vessel in the ovary, and regulating germ-cell survival. Surprisingly, development of this vessel (and migration of endothelial cells that contribute to its formation) occurs in the absence of *Sry* or Sertoli differentiation. Rather than being activated by *Sry* in the testis, this aspect of the male pathway is apparently negatively regulated in the ovary[117].

Other genes in the ovarian pathway have been identified by studies in goats with polled intersex syndrome (PIS), which is characterized by polledness (absence of horns) and female-to-male sex reversal[119]. Sex reversal and the development of sex cords are coincident with the upregulation of male markers slightly later than in normal testis differentiation. Two transcripts, *Pisrt1* and *Foxl2*, are downregulated in sex-reversed PIS XX gonads, similar to the levels in normal XY gonads. It is postulated that the PIS region normally enhances transcription of *Foxl2*/*Pisrt1* in XX gonads, and that an XY factor binds the PIS region in the XY gonad, bending it into a repressive conformation[120]. *Pisrt1* is a non-coding RNA, and *Foxl2* encodes a winged helix-loop-helix transcription factor. *Foxl2* is expressed sex-specifically in the ovary in mice, and mutations in *FOXL2* are associated with ovarian failure in humans[121]. A targeted deletion in mice indicates that *Foxl2* is required for ovarian follicle-cell differentiation around the time of birth; however, because there is no XX sex reversal in the mutant, a role for FOXL2 in the early stages of sex determination in mice can be ruled out[122].

Identifying new players in sex determination

Testis and ovary organogenesis require the integration of many signals, most of which remain unidentified. Key genes in sex-determination pathways were identified by mapping sex-reversing mutations in the human population (see, for example, REFS 123–125) and by fortuitous discoveries in targeted mouse mutants (see, for example, REFS 33,34,82). Characterization of the morphological events in testis organogenesis led to the prediction of several signalling pathways by analogy to similar developmental systems (see, for example, REF. 71). Other new players in the pathway were discovered using evolutionary approaches and various genetic screens.

A comparison of the genetic and cellular pathways of sex determination in other vertebrates that do not rely on *Sry* as the sex-determination switch will probably reveal new genes and unique mechanisms that underlie sex determination. The DM-domain proteins, originally identified in *Drosophila melanogaster* and *Caenorhabditis elegans,* are evolutionarily conserved in sex-determining processes in many species[126–128]. The single member of this family that has been subjected to deletion analysis in mouse, *Dmrt1*, has a later role in testis development[129]; however, it seems probable that other members of the family will have earlier roles in mammalian sex determination.

Because of the rapid bifurcation of the male and female developmental pathways, the gonad is an ideal candidate for subtractive screening approaches. Several expression screens that compare XY and XX gonads at stages soon after *Sry* expression is initiated have been undertaken[118,130–134], and many genes that are dimorphically expressed have been identified. Functional analysis of many of these genes is underway. Other approaches that will probably expand the network of genes that function in the gonad include large-scale mutagenesis screens based on the identification of dominant or recessive mutations that result in the disruption of sex determination.

The *Odsex* mutant was identified using a transgenic mutagenesis approach[48], and several other mutational screens are currently in progress that should contribute more players in both male and female pathways.

In addition to its importance for reproductive biology, the study of sex determination and gonadogenesis has yielded tremendous insight into basic cellular and molecular aspects of organogenesis, and holds promise for an exciting future. Current molecular and genetic approaches, such as chromatin immunoprecipitation, proteomic techniques and functional experiments in animal systems and organ culture, can help to assemble the pathways that control testis and ovary organogenesis. In the next few years, it will be important to work closely with the structural and functional cell biology of the system to integrate extracellular and intracellular pathways. In addition, as more and more genes are discovered, it will be necessary to develop bioinformatics methods for the analysis of transcriptional cascades and three-dimensional modelling systems to accommodate the complexity of the intricate signalling pathways that make us male or female.

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Competing interests statement

The authors declare that they have no competing financial interests.

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