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Short Conceptual Overview

Origin and function of embryonic Sertoli cells

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Abstract

In the adult testis, Sertoli cells (SCs) are the epithelial supporting cells of the seminiferous tubules that provide germ cells (GCs) with the required nutrients and structural and regulatory support to complete spermatogenesis. SCs also form the blood-testis barrier, phagocytose apoptotic spermatocytes and cell debris derived from spermiogenesis, and produce and secrete numerous paracrine and endocrine signals involved in different regulatory processes. In addition to their essential functions in the adult testis, SCs play a pivotal role during testis development. They are the first cells to differentiate in the embryonic XY gonadal primordium and are involved in the regulation of testis-specific differentiation processes, such as prevention of GC entry into meiosis, Leydig and peritubular myoid cell differentiation, and regression of the Müllerian duct, the anlagen of the uterus, oviducts, and the upper part of the vagina. Expression of the Y-linked gene *SRY* in pre-SCs initiates a genetic cascade that leads to SC differentiation and subsequently to testis development. Since the identification of the *SRY* gene, many Sertoli-specific transcription factors and signals underlying the molecular mechanisms of early testis differentiation have been identified. Here, we review the state of the art of the molecular interactions that commit the supporting cell lineage of the gonadal primordium to differentiate as SCs and the subsequent Sertoli-specific signaling pathways involved in early testis differentiation.

Keywords: embryonic Sertoli cell; embryonic testis function; SOX9; SRY; testis differentiation.

Introduction

Sertoli cells (SCs) represent the somatic component of the germinative epithelium of the testis (1), and their presence is crucial for both testicular development and function. The other component of the germinative epithelium, located inside the seminiferous tubules, is the germ cells (GCs), which, in the adult testis, are spermatogonia, meiotic, and postmeiotic cells. SCs are epithelial in nature, with the basal pole oriented toward the peritubular myoid cells (PMCs) that surround the seminiferous tubule and the apical pole oriented toward the tubular lumen. Their cytological structure is quite complex, with a generally basal nucleus and a very large and morphologically changing cytoplasm that accommodates the spaces between the neighboring GCs. In the adult testis, SCs establish specialized inter-Sertolian junctions near the basal lamina of the seminiferous tubules, which form a blood-testis barrier [see (2) for a review on SC biology].

According to the classical view, SCs are sustentacular and nurse cells that provide GCs with the required nutrients and structural support. Now, it is known that these cells have many other important functions, both structural and regulatory. Structurally, the blood-testis barrier defines two compartments in the germinative epithelium: adluminal and basal. Spermatogonia are peripherally located in the basal compartment, and once they enter meiosis, SCs actively transports the newly formed primary spermatocytes toward the adluminal compartment, from which the haploid meiotic products are finally released in the tubular lumen. This implies that the junctional system established between SCs and GCs must be very complex and dynamic to permit the basal-to-apical migration of the GCs through the interstitial spaces between adjacent SCs (3). SCs also participate, together with PMCs, in the formation of the basal lamina of the seminiferous tubules. Furthermore, the blood-testis barrier maintains meiotic and postmeiotic GCs isolated from the vascular environment, thus precluding the formation of antisperm antibodies. Another relevant function of SCs is to phagocytose apoptotic spermatocytes and cellular debris derived from spermiogenesis, the differentiation of the meiotic products into sperm (4, 5).

SCs are the main targets of the hormones regulating the spermatogenic function in the seminiferous tubules. They receive hormonal signals that regulate reproduction, including follicle-stimulating hormone (FSH), testosterone, and triiodothyronine (T_3) (6), establishing regulatory cross-talk with GCs, thus controlling the timing of spermatogenesis. The regulatory action of SCs is exerted mainly through the production and secretion of numerous paracrine and endocrine signals, including inhibins and activins, which contribute to control the secretion of FSH by the hypophysis [reviewed by (7)]; androgen-binding protein (ABP), thought to be required for maintaining high levels of testosterone inside the seminiferous tubules (8); glial cell line-derived neurotrophic factor, which controls the proliferation of the spermatogonial stem cells, thus regulating their renewal to maintain the pool

of testicular GCs [reviewed by (9)]; 17β-estradiol, which regulates spermatogenesis (10); a variety of growth factors, including nerve growth factors, fibroblast growth factors (FGF), insulin-like growth factors, and transforming growth factors (TGF), which establish profuse SC-GC communication [reviewed in (11)]; Ets-related molecule (ERM), required for transcriptional control of the spermatogonial stem cell niche (12), among others.

It is thus evident that SCs play several pivotal roles in the adult testis, but their functions are not less important in the developing testis. The present article seeks to review the ontogenic origin, differentiation, and function of the early embryonic SCs, thus showing that SCs direct crucial events of testis organogenesis.

Ontogenic origin and differentiation of SCs

The differentiation of SCs is the key event in testis development, a process that has been well studied in a few mammalian species. In the sexually undifferentiated mouse embryo, genital ridges appear in the ventromedial surface of the mesonephroi, between 10.5 and 11.5 days postcoitum (dpc), as a consequence of proliferation of the coelomic epithelium. These genital ridges give rise to the bipotential gonads, which are equally formed on both males and females. In addition to the somatic cells derived from the coelomic epithelium, the bipotential gonad also contains primordial germ cells (PGCs), which have an extragonadal origin, as they migrate into the gonad from a reduced population of epiblast cells in the extraembryonic mesoderm through the dorsal mesentery (13, 14). Somatic cells and PGCs in the bipotential gonad form a gonadal blastema, where gonadal (sexual) cords are formed in both sexes in most species, including the rat (15), but not in the mouse [reviewed in (16)] (Figure 1).

Testis development begins with three main events in most mammals studied: differentiation of pre-SCs, in which the sex-determining genes are expressed (see below), into SCs; migration of endothelial cells from the adjacent mesonephros; formation of the testicular cords composed of SCs and PGCs (Figure 1). Using transgenic mice expressing the reporter gene *EGFP* (enhanced green fluorescent protein) under the control of the *Sry* promoter, Albrecht and Eicher (17) showed that pre-SCs are mesenchymal in nature, implying that they must undergo a mesenchymal-to-epithelial cell-type transition to differentiate as SCs. For this, they have to polarize by accumulating extracellular matrix proteins, such as collagen type IV, laminin, fibronectin, and heparin sulfate proteoglycan in the basal pole [reviewed in (18)]. As they differentiate, SCs and PGCs aggregate to form the testis cords, which involves a major remodeling of their cell junction system, a process in which integrin subunits as well as lectins are known to be involved (19, 20).

It is well known that cell migration from the mesonephros to the gonad is a crucial event in gonad differentiation, as it is exclusive of the XY gonad at early stages of gonad development (21). It depends on the presence of the *Sry* gene (22) and is needed for the formation of both the testis cords (23) and

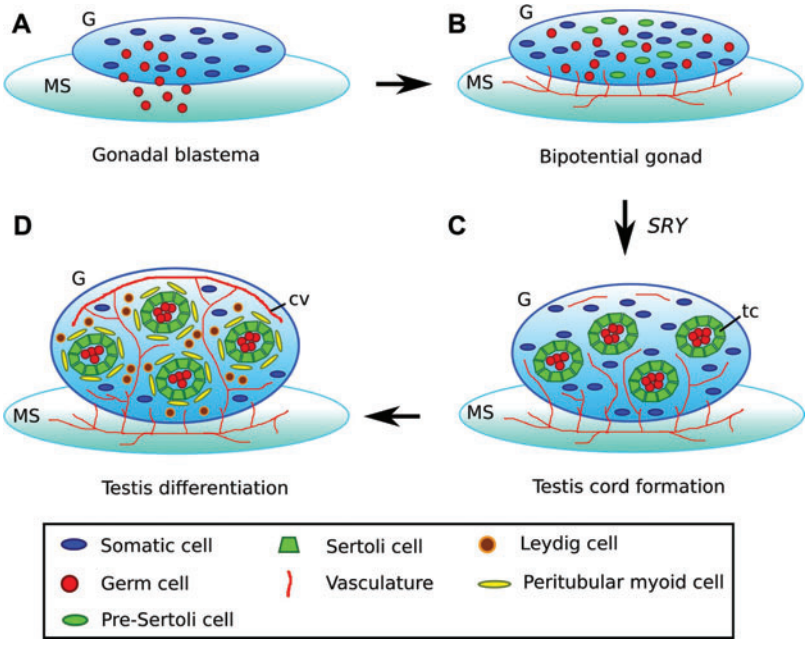

Figure 1 An overview of early SC and testis differentiation.
Shortly after the gonadal ridge arises as a thickening of the coelomic epithelium of the mesonephros (A), migrating PGCs colonize the gonad and form, together with the somatic cells, the gonadal blastema (B). Shortly later, a group of somatic cells, the pre-SCs express *SRY* in the bipotential gonad and undergo a mesenchyme-to-epithelial transition, aggregate, and form the testis cords, which include the GCs (C). *SRY* expression also induces endothelial cell migration from the mesonephros and the development of an extensive vasculature including the coelomic vessel (CV). After testis cord formation, other testicular cell types differentiate, such as LCs and PMCs (D).

the testis-specific vascular system (24). Although, it is clear that the chemoattractant signal that induces mesonephric cell migration is secreted by cells located within the gonad (21, 25, 26) and that platelet-derived growth factor (PDGF) signaling is involved in the process (27), there is, however, some controversy concerning the nature of the mesonephric cells that migrate into the gonad. Endothelial cells and precursors of both PMCs and Leydig cells (LCs) have been proposed to comprise the population of mesonephric migrating cells (23, 28, 29). Some studies have shown that the mesonephros contribute with LC precursors to the developing testis (28, 29), but others support the idea that LC precursors are already present in the gonad by 11.5 dpc, when cell migration takes place, and that they are not derived from the mesonephros after this stage (27). Similarly, several experiments have strongly suggested that PMCs could derive from precursor cells migrating from the mesonephros (21, 23, 25, 30). However, more recent studies (31) have shown that cells expressing αSma-EYFP (in these cells, the fluorescent reporter gene *EYFP* is expressed under the control of the promoter of α*Sma*, a typical molecular marker for PMCs) do not migrate into the gonad during the critical window of sex determination, which strongly suggests that PMC precursors do not derive from mesonephric cells. Hence, endothelial cells are the only cell type known to date to migrate from the mesonephros to the gonad (31).

In the mouse, the three main events of testis differentiation (SC differentiation, testis cord formation, and mesonephric cell migration) occur almost simultaneously between 11.5 and 12.5 dpc (21, 23, 25, 26). Also, both the morphological and the functional differentiation of mouse SCs are simultaneous [the latter evidenced mainly by the early production of anti-Müllerian hormone (AMH)], which makes it difficult to distinguish between these two aspects of SC differentiation. However, studies performed in the Iberian mole, *Talpa occidentalis* (32), have shown a very early expression of both *SOX9* and *AMH* genes (the two most evident SCs markers), coinciding with the onset of the formation of the genital ridge at 15 dpc (stage s4b), 3 days before the formation of the testis cords at 18 dpc (s5b stage), when mesonephric cell migration occurs in this species (33). Hence, currently available data indicate that the functional differentiation of SCs, as a consequence of the activation of the gene cascade initiated by *SRY* that includes *SOX9* and *AMH*, is a prerequisite for the occurrence of further events of testis differentiation, including their morphological differentiation and the mesonephric cell migration. Testis cord formation appears to require the presence both of SCs and of some unknown cell precursors migrating from the mesonephros.

Long-standing controversy surrounds the ontogenic origin of SCs. The hypothesis that the mesonephric tubule cells dedifferentiate from the epithelial structure at the mesonephros-gonad border, migrate into the gonad, and contribute to the SC population has been supported by several authors (34–36). On the other hand, the fact that AMH can be detected in the medium when 11.5-dpc isolated gonads are cultured (30), just when migration starts, clearly suggests that SCs are present in the gonad prior to mesonephric cell migration [reviewed in (37)]. Using the lipophilic dye, *DiI*, as a living cell marker for coelomic epithelium cells in cell tracing experiments, Karl and Capel (38) showed that SCs derive from a portion of coelomic epithelium cells that proliferate and migrate into the gonad during the early stages of gonadal development, whereas other coelomic epithelium cells colonizing the gonad at later stages become testis interstitial cells. Albrecht and Eicher's (17) experiment with transgenic mice showed that *Sry* expression takes place in the SCs and that both SCs and granulosa (follicle) cells share a common ontogenic origin.

Genetic control of SC differentiation: the basis of mammalian sex determination

Upstream of SRY

Several genes are known to be expressed in pre-SCs, including *WT1*, *SF1*, *GATA4*, *FOG2*, and *FGF9*, before sex differentiation, which is induced by the activation of the mammalian-specific, master regulatory testis-determining gene, *SRY* (sex determining region of chromosome Y) in pre-SCs. *SRY* initiates a cell-autonomous cascade of gene regulation leading to SC differentiation and subsequent testis development (39–41). Little is known about the molecular mechanisms underlying *SRY* activation in the XY bipotential gonad, but several transcription factors are known to be involved in the regulation of this gene. One of them is *WT1* (Wilms tumor suppressor gene 1), which is expressed in the genital ridge, in the coelomic epithelium, and in the sex cords of the gonadal primordium in both sexes, as well as in the adjacent mesonephros (42). Several human syndromes reveal the importance of *WT1* in early gonadal development, such as the Wilms tumor-aniridia-mental retardation-genitourinary tract abnormalities syndrome (43), in which patients show anomalies in the genitourinary tract; the Denys-Drash syndrome, a condition associated to male pseudohermaphroditism (44); and the Frasier syndrome, which involves XY sex reversal and glomerulonephropathy (45). Accordingly, $Wt1^{-/-}$ embryos initially form a gonadal primordium, but subsequently, it degenerates by increased apoptosis (46). *WT1* possesses an alternative splice site that results in the insertion (+KTS) or exclusion (-KTS) of the three amino acids Lys-Thr-Ser (47). Mice of any sex with a null mutation of the -KTS isoform had poorly differentiated small gonads, indicating that this isoform is necessary for cell survival in the gonadal primordium. In contrast, those with a null mutation of the +KTS isoform showed XY sex reversal with reduced levels of *Sry* expression, indicating that this splice variant is involved in *Sry* regulation (47). However, the *in vitro* data are controversial, as one study showed that only the -KTS variant of WT1 can activate the promoter of the human *SRY* (48), whereas other results revealed that both WT1 isoforms may interact with GATA4 and synergistically activate the *SRY* promoter (49). Thus, although *WT1* is a good candidate gene for regulating *SRY*, it is not yet clear whether *SRY* is a direct or an indirect transcriptional target of WT1. In addition to WT1, other factors could participate in the regulation of *SRY* expression, including the GATA zinc finger transcription factor 4 (GATA4) and its cofactor FOG2

(friend of GATA 2), which are expressed in somatic cells of both XX and XY genital ridges (50, 51). *Fog2*$^{-/-}$ embryos as well as embryos homozygous for a *Gata4* mutation that abrogates the interaction of GATA4 with FOG cofactors exhibit a blockage in SC differentiation. In addition, *Sry* transcripts levels are reduced in *Fog2*$^{-/-}$ testes (51). Also, the insulin receptor family is expressed in the urogenital ridge and is necessary for *Sry* expression (52). Furthermore, studies made with knockout mice have shown that other genes, such as *Sf1* (*Nr5a1* gene) (53), *Emx2* (54), and *Lhx9* (55), contribute to the proliferation of the genital ridge and the formation of the indifferent gonad (Figure 2). In conclusion, despite that many *in vitro* studies have been performed to identify new molecular interactions among these factors that may be involved in the subsequent activation or repression of *SRY*, the molecular mechanisms underlying the regulation of this gene are still poorly understood.

Downstream of SRY

Once *SRY* is activated, pre-SCs are committed to differentiate into SCs. Although many studies have found a number of molecules acting downstream of *SRY*, *SOX9* is currently its only known direct target. *SOX9* is expressed in the gonadal ridge of both sexes, becoming upregulated in the male but not in the female gonad after the critical stage of sex determination (56). Cases of XY sex reversal associated with *SOX9* mutations have been described both in humans (57, 58) and in mice (59, 60). In addition, ectopic activation of *Sox9* in the bipotential gonad causes XX sex reversal (61, 62). More recently, it has been shown that SRY binds to a gonad-specific enhancer of the murine *Sox9*, as does SF1, and a model in which SF1 and SRY cooperatively upregulate *SOX9* has been proposed (63). According to this model, SOX9 also binds to the same enhancer and cooperates to maintain its own expression in an autoregulatory loop. Another protein necessary for SC differentiation is the FGF9, as *Fgf9*$^{-/-}$ mice exhibit XY reversal (64). This gene is initially expressed in the gonads of both sexes, but becomes male-specific once *SRY* is activated (65). By establishing a feed-forward loop with *SOX9*, *FGF9* antagonizes ovary-promoting processes, such as the WNT signaling. *WNT4* (wingless-related MMTV integration site 4 gene) and RSPO1 (R-spondin 1) are two molecules involved in the canonical WNT signaling pathway, its expression in the urogenital ridge becoming female-specific after the sex-determination stage (66, 67). Mutations in any of these two molecules cause XY sex reversal (67–70). Furthermore, ectopic activation of the WNT signaling pathway in the XY gonad leads to ovary development (71), indicating that the WNT signaling pathway actively promotes female gonadal differentiation. Thus, FGF9 and WNT act as opposing signals during sex determination, so that during testis differentiation SRY activates *SOX9*, which initiates a feed-forward loop together with FGF9, which in turn upregulates *FGF9* and represses WNT signals, leading to SC differentiation (66).

Embryonic SC functions

Once SCs differentiate, they orchestrate the development of the bipotential gonad into the testis. SC function is necessary for the correct development of the testis cords (structures that transform into the seminiferous tubules after puberty), the differentiation of GCs into sperm, the differentiation of the somatic LCs and PMCs, and the proper regression of the Müllerian ducts (the embryonic structures that differentiate into the female secondary sex organs).

Induction of testis cord formation

After the time of sex determination, SCs actively proliferate until shortly after birth, resulting in an expansion of the number of testis cords. Later, SCs proliferation and testis cord expansion progressively declines, and at puberty, SCs proliferation is not detectable (72). The cell-autonomous action of *Sry* seems to be the only mechanism of SC differentiation (73), and mitotic proliferation appears to be the only mechanism underlying SCs expansion (65, 74). However, a re-evaluation of the XX-XY chimeric mice initially studied by Burgoyne et al. (73) showed the proportion of XX/XY cells to be about

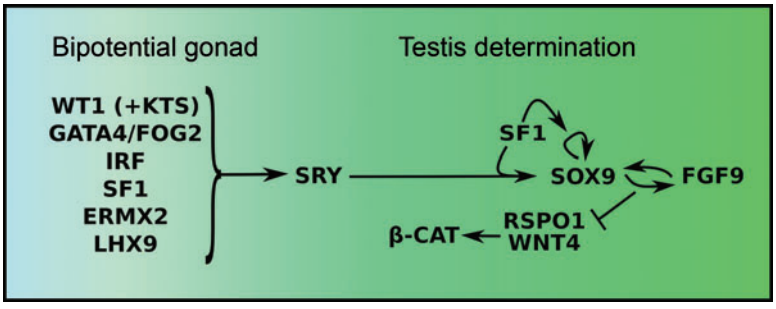

Figure 2 Genetic control of SC differentiation.
Several genes that contribute to the proliferation and formation of the indifferent gonad are expressed in the bipotential gonad, including *WT1* (+KTS), *GATA*/*FOG2*, *SF1*, *IRF* family, *ERMX2*, and *LXH9*; some are involved in the activation of *SRY*. At the testis determination stage, SRY and SF1 initially upregulate *Sox9* expression. Then SOX9 autoregulates *Sox9* together with SF1. At the same time, SOX9 establishes a positive feedback loop *via* FGF9 signaling leading to SC differentiation. The SOX9/FGF9 loop leads to downregulation of the WNT signaling pathway, thus inhibiting the female pathway.

1:1 in all tissues and for all cell types, except in the testes where SCs were 90% XY, indicating that the presence of the Y chromosome, and therefore, the cell-autonomous expression of *Sry*, is required for the differentiation of most but not all SCs (75). Hence, a molecular mechanism, independent of SRY, must exist that permits the recruitment of undifferentiated cells into the SC fate. Recently, a paracrine signal molecule produced and secreted by SCs, prostaglandin D2 (PGD2), has been shown to be necessary and sufficient to recruit XX cells to express *Sox9* and differentiate as SCs (76). *Sox9* activates and maintains the paracrine PGD2 pathway, which in turn establishes another feed-forward loop with SOX9 to promote SC function (77, 78). As indicated above, a key function of embryonic SCs is to trigger the formation of the testis cords and promote its further expansion. To date, only a reduced number of genes are known to play a role during this process. Besides its essential role in the formation of the bipotential gonad, *WT1* also has additional functions at later stages of SC development. Inactivation of the murine *Wt1* in SCs after the time of sex determination results into a progressive loss of the seminiferous tubule architecture and the cessation of SC proliferation, the mutant testes being devoid of SCs and GCs at birth (79). Therefore, *WT1* is essential for SC survival and testis cord maintenance. *SOX9* expression in SCs continues during fetal and postnatal stages of testis development, indicating that it may have further functions in addition to the essential role in SC differentiation (testis determination). In fact, SOX9 expression shows seasonal variations in the seasonal breeder mole species *T. occidentalis*, suggesting a possible role in the seasonal regulation of adult SC function (80). However, this appears not to be the case in the embryonic SCs after sex determination, as mice in which *Sox9* was deleted after SC differentiation showed no alterations of the fetal and postnatal testis phenotype and were initially fertile, but they became sterile at around 5 months (81). Moreover, *SOX8* and *SOX10* constitute, together with *SOX9*, the group E of SOX transcription factors. Like *SOX9*, *SOX8* is also expressed during embryonic and postnatal development of SCs, and *Sox8*$^{-/-}$ mice develop a similar testis phenotype, being initially fertile and becoming sterile at around 5 months (82). By contrast, the inactivation of both *SoxE* genes leads to a progressive testis cord disruption and primary infertility, indicating a redundant role for both genes during SC differentiation after the sex determination stage (81). *Wt1* expression is necessary for both *Sox8* and *Sox9* expression, whereas *Wt1* is expressed in the absence of the two *SoxE* genes, suggesting that *Wt1* acts upstream of them in the mouse testis. Stabilization of β-catenin in SCs, which implies ectopic activation of the canonical WNT signaling pathway, after the sex determination period also causes testis cord disruption, where the expression of *Sox9*, but not that of *Wt1*, is inhibited (83). In the absence of either *Wt1* or *Sox9-Sox8*, the WNT signaling pathway becomes upregulated (79, 81). Overall, available data suggest that the WNT signaling, which is silenced at the moment of sex determination in SCs, must be permanently repressed during further SC development. This repression is brought about by *Wt1* through partial or full mediation by *Sox9* and *Sox8* (Figure 3).

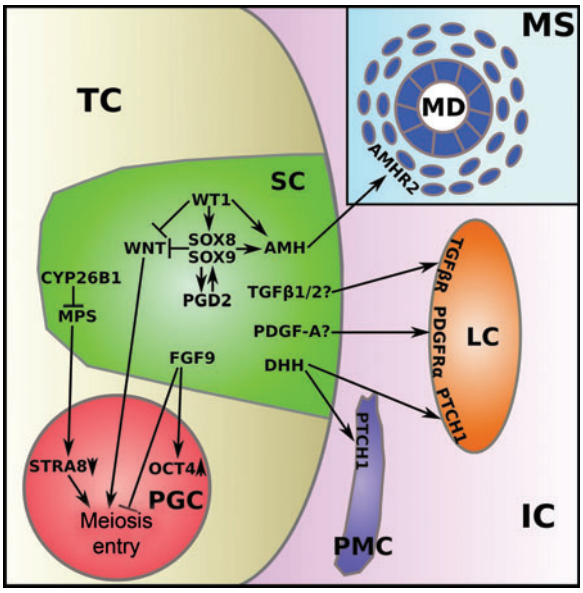

Figure 3 Central role of SCs in testis differentiation.
After the sex determination stage, *Wt1* expression is necessary for SC survival and maintenance of both *Sox8* and *Sox9* expression. These three factors repress the WNT signaling pathway, which in the ovary causes testis cord disruption and promote meiosis entry of GCs. Also, SOX9 establishes a feed-forward loop with the PGD2 signaling to promote SC function. FGF9 signaling from SCs also prevents the meiosis entry of GCs and the maintenance of pluripotent cell markers as OCT4. In addition, CYP26B1 seems to antagonize a meiotic-promoting substance (MPS) leading to *STRA8* downregulation and meiosis entry prevention. Three Sertoli signaling pathways are involved in LCs differentiation: the Hedgehog signaling, which includes DHH as the ligand and PCTH1 as the receptor; the PDGF signaling, with PDGFA as a possible ligand and PDGFRα as a receptor; and the TGFβ signaling, in which TGFβ1 and TGFβ2 are expressed in SCs and type I and type II TGFβ receptors, as well as the coreceptor TGFβR3, are present in LCs. DHH and PCTH1 are also necessary for PMCs differentiation. SCs also secrete AMH, involved in Müllerian duct regression through the action of its receptor AMHR2. The expression of *AMH* is regulated by WT1, SOX9, and SOX8. IC, interstitial compartment; LC, Leydig cell; MD, Müllerian duct; MS, mesonephros; PGC, primordial germ cell; PMC, peritubular myoid cell; SC, Sertoli cell; TC, testis cord.

Regulation of GC differentiation

One of the major events of testis development is the differentiation of PGCs into spermatogonia instead of oocytes. Meiosis onset exhibits a distinct sex-specific difference in timing, with female meiosis starting in the embryo, shortly after the sex determination stage in the case of the mouse, whereas male meiosis initiates postnatally, at puberty. In the male, as testis cords are formed, PGCs are enclosed within them and thus exposed to the intracordonal environment, separated from the interstitial compartment by an epithelial layer of SCs. This situation implies that any further PGC communication signals must be either established with SCs or filtered by them. As a result, PGCs enter the male pathway of differentiation, implying the avoidance of meiosis initiation and the maintenance of

the expression of pluripotent cell markers, such as *OCT4* (84). Several observations lead to the hypothesis that PGCs are programmed to enter meiosis and initiate oogenesis through the action of a meiosis-promoting substance, whereas other studies suggest the existence of a male meiosis-preventing factor [reviewed in (85)]. Retinoic acid (RA) has been proposed as a meiotic-promoting substance (86, 87). The *STRA8* gene (stimulated by retinoic acid gene 8) encodes a factor required for PGCs to enter meiosis in both sexes (88). *Stra8* is expressed in PGCs in the mouse embryonic ovaries around the time of meiosis onset, but not in embryonic testes, as male meiosis does not initiate until puberty (89). *CYP26B1* encodes a RA-metabolizing cytochrome P450 enzyme that is expressed in SCs. PGCs of mouse testes lacking *Cyp26b1* enter meiosis prematurely, although they arrest in pachytene and undergo apoptosis. In contrast, ovarian GCs appear unaffected by the lack of *Cyp26b1* (90). These two molecules interact with RA signaling, as RA has been shown to activate the expression of *Stra8* in embryonic carcinoma cells and in stem cells in culture (91, 92), and CYP26B1 catabolizes RA to more polar metabolites for excretion, so that those gonadal regions expressing *Cyp26b1* lack detectable levels of RA (93, 94). Also, it was shown that during gonadal development, RA is synthesized in the mesonephros of both males and females, and it apparently diffuses into the adjacent gonad. According to these findings, a consistent hypothesis has been proposed on the control of meiosis onset in male and female gonads: in ovaries, diffused RA from the mesonephros would activate *Stra8*, which in turns would induce PGCs to enter meiosis thus initiating oocyte differentiation. In contrast, in the testes, RA would be catabolized by CYP26B1, thus preventing *Stra8* upregulation and meiosis entry (86, 87). However, a recent study has questioned this model (95). Analysis of mice ablated for retinaldehyde dehydrogenase 2 (*Raldh2*), a gene that controls RA synthesis, revealed that activation of *Stra8* expression in the fetal ovary does not require RA signaling, as this gene can be expressed in the absence of physiologically detectable levels of RA. It was also shown that when *Cyp26b1* was inactivated in *Raldh2* mutant testes, *Stra8* was induced in a RA-independent manner, but only when the mesonephros remained attached. The authors propose that CYP26B1 prevents the onset of meiosis by metabolizing a mesonephric substrate, other than RA, that controls *Stra8* expression. A second SC factor involved in this process is *FGF9*. In XY but not XX *Fgf9* null mouse embryos, most GCs die shortly after the sex determination stage, and some of the surviving XY GCs express meiotic markers (64). Further analysis of the *Fgf9* null testes showed that FGF9 acts directly on PGCs to prevent their entry into meiosis, maintain pluripotency, and actively promote the male fate (96). These authors have proposed that FGF9 signaling antagonizes RA signaling in SCs to determine male GC fate commitment. However, according to the new data reported by Kumar et al. (95), rather than RA, FGF9 would antagonize a different meiosis-promoting substance. Two additional molecules involved in the WNT signaling pathway, which are necessary for XX but not XY GC survival, are WNT4 and RSPO1. Ovaries of both *Wnt4*$^{-/-}$ and *Rspo1*$^{-/-}$ mutant mice contain only a few degenerating oocytes at birth (68, 70), and the expression of C*yp26b1* in these embryonic mutant ovaries is upregulated (69, 97). Accordingly, if it is considered that the stabilization of β-catenin, the intracellular mediator of the WNT signaling, in somatic cells of XY gonads results in the activation of the ovarian pathway in GCs (71) and when SC function is compromised during testis development, WNT signaling is upregulated (79, 81), then it can be concluded that WNT signaling may promote female GC fate, whereas SC function would antagonize this process (Figure 3).

Induction of LCs and PMCs differentiation

SCs are also involved in promoting the differentiation of other somatic cell types of the testis, including LCs and PMCs. At least two LC populations, fetal Leydig cells (FLCs) and adult Leydig cells (ALCs), arise sequentially during testis development and, although all LCs have androgen biosynthetic capacity, FLC and ALC populations are different in morphology and gene expression profiles (98). In mice, FLCs are first visible in the interstitial compartment of the testis, the space between the testis cords, at around 24 h after SC differentiation (99). The FLC population expands enormously in the following two days, although this increase in number is not a result of cell proliferation during this period, but rather is a consequence of differentiation of interstitial mesenchymal cells migrated either from the coelomic epithelium domain or from the mesonephros. It is assumed that the differentiation of FLCs depends on SC function, although little is known about the specific signals involved in this process. One of these signaling molecules is desert hedgehog (DHH), one of the three mammalian hedgehog family ligands, which is expressed in SCs. FLCs express the gene encoding the hedgehog receptor patched1 (PTCH1), and analysis of *Dhh* knockout testes revealed that differentiation of FLCs was severely impaired (100). This aberrant FLC cell differentiation was not the result of either a failure of the mesonephric cell migration or defective cell proliferation or survival. Rather, it seems to be the consequence of a defective upregulation of *Sf1* expression in the precursor cells expressing *Ptch1*, indicating that *Sf1* could be an intracellular target of the *Dhh/Ptch1* pathway. However, in *Dhh*$^{-/-}$; *Sf1*$^{+/-}$ compound mutant mice, FLCs failed to differentiate, with the subsequent absence of fetal testosterone, which results in defective masculinization (secondary sex reversal) (101). These data indicate that the combined function of *Dhh* and *Sf1* is required for LC development. Another molecule known to be involved in FLC differentiation is the PDGF receptor α (PDGFRα), as testes in *Pdgfrα*$^{-/-}$ mutant mice have reduced or absent FLCs (27). There are three known ligands for *PDGFRα*, PDGF-A, PDGF-B, and PDGF-C, but only the *Pdgf-A* gene is expressed in SCs (102), suggesting that SCs could modulate FLC differentiation through the action of this ligand. Indeed, deletion of *Pdgf-A* induces defects in the development of ALCs, but the number of FLCs was apparently normal (103). Thus, it is not clear whether *Pdgf-A* has an important role in the differentiation of FLCs. There are also other molecular signaling pathways affecting the differentiation of FLCs, the receptors of which are

expressed in this cell type, but it is not known whether SCs are the source of the ligand activating these pathways. This is the case for the TGFβ superfamily. Expression of type I and type II TGFβ receptors, as well as of betaglycan (T*gf*β*r3*), a coreceptor for TGFβ superfamily ligands, has been detected in LCs (104, 105). The loss of *Tgf*β*r3* expression in fetal mouse testes induces alterations of testis cord development and compromised FLC function. By contrast, SC function seems to be normal until two days after FLC differentiation, suggesting that disruption in TGFβ signaling in FLCs, rather than defective SC function, is the origin of the anomalies seen in the FLCs of testes from *Tgf*β*r3*$^{-/-}$ mutant mice (106). Two of the three mammalian TGFβ ligands, TGFβ1 and TGFβ2, are expressed in SCs (107), and knockout mice for every factor exhibited defective testes (108). However, none of these studies evidenced any association between the defective production of such ligands by SCs and the abnormal development of FLCs.

PMCs form a single layer of flattened cells surrounding the testis cords. Little is known about the molecular mechanisms underlying the initial differentiation of this cell type, although there is evidence that signals from SCs have an important role in this process. The best known example is again DHH signaling: the hedgehog receptor, *Ptch1*, is expressed in PMCs, and the lack of *Dhh* in murine SCs leads to testes with PMC defects (109). FLC and/or PMC differentiation in the absence of SCs have been observed in some normal and pathologic cases of XX sex reversal [(110, 111), among others], but this is a quite rare trait. A convincing explanation for these cases is the ectopic activation of a Sertoli-specific signaling pathway in XX gonadal cells. Accordingly, when the hedgehog signaling is ectopically activated in the mouse fetal ovary, FLCs differentiate in the absence of SCs (112) (Figure 3).

Induction of Müllerian duct regression during male development

A hormone secreted by early SCs is responsible for inducing the degeneration in the male mesonephroi of the Müllerian ducts, the precursors of the female secondary sexual organs (113). This factor is the Anti-Müllerian Hormone (AMH), a signal molecule belonging to the TGFβ superfamily. AMH binds to its type II receptor (AMHR2) in the Müllerian duct mesenchyme, leading to the regression of the Müllerian duct mesoepithelium (113–115). Male mice lacking either *Amh* or *Amhr2* genes develop as pseudohermaphrodites, exhibiting internally a complete male reproductive tract as well as a uterus and oviducts. Both *Amh*$^{-/-}$ and *Amhr2*$^{-/-}$ mutant male mice produce sperm, but most are infertile because their female reproductive organs block the sperm transfer into females (114, 115). Regulation of *AMH* expression depends on several Sertoli transcription factors, including *SOX9*, *SOX8*, *SF1*, and *WT1*. When *Sox9* is inactivated before the sex determination stage, *Amh* expression is not initiated, and Müllerian duct regression does not take place (59, 60). *In vivo* and *in vitro* studies revealed that SOX9 acts through a SOX binding site within the *AMH* promoter (116, 117). *SOX8* also activates the *AMH* promoter in the same manner, but less efficiently (118). Null mutant mice for *Sox8* or mice in which *Sox9* was ablated shortly after *Amh* expression is initiated produce sufficient levels of *Amh* transcripts to induce the regression of the Müllerian ducts (81, 119). By contrast, when both genes are deleted simultaneously shortly after *Amh* expression begins, lower levels of *Amh* expression and partial persistence of Müllerian duct derivatives result (81). These findings indicate that *Sox9* is necessary for the initiation of *Amh* expression, but redundancy between *Sox9* and *Sox8* is necessary for the maintenance of *Amh* expression. There is also an SF1 binding site in the *AMH* promoter, a factor that interacts together with SOX9 to synergistically activate *AMH* expression (Figure 3) (116, 117). *Wt1* ablation after the sex determination stage results in the complete absence of SOX9, SOX8, and AMH proteins and adult *Wt1* mutant males maintain Müllerian duct derivatives, such as a residual uterus (79), indicating that *Wt1* regulates the maintenance of *Amh* expression partly through the control of *Sox9* and *Sox8* expression and partly *via* additional unknown routes.

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