Mammals have an XX/XY sex chromosomal sex determination system in which males represent the heterogametic sex. The Y-linked gene, SRY, determines sex by inducing the undifferentiated, bipotential gonads to differentiate as testes, which produce androgens and promote in this way the development of a male phenotype. Thus, in mammals, sex determination can be equated to testis determination, which involves several important cell processes, including Sertoli cell differentiation, mesonephric cell migration, testis cord formation, testis-specific vascularization, and myoid and Leydig cell differentiation. Many genes are currently known to be involved in testis development. Some of them, including SF1, WT1, GATA4 and FOG2, are necessary for the formation of the bipotential, undifferentiated gonad but also have important roles in testis differentiation. Others can be considered testis-promoting, differentiation and/or maintenance genes: these include SRY, SOX9, FGF9, PTGDS, SOX8, SOX3, NR0B1, PDGFRα, DMRT1, AMH, NGF, NTF3 and NGFR as the most important examples. Finally, there is a smaller group of genes which are involved in ovarian development and which can cause aberrant testis development if mutated, including RSP01, WNT4, CTNNB1, FST, BMP2 and FOXL2. In this paper, we review our current knowledge on the function, spatio-temporal expression pattern and mutant sexual phenotypes associated with these genes, and discuss the various roles they play in gonad development.

Key words: SF1, WT1, GATA4, FOG2, SRY, SOX9, FGF9, SOX8, SOX10, SOX3, DAX1, PDGFRα, DMRT1, AMH, CTNNB1, FOXL2

Introduction

The concept of sex determination deals with the mechanism by which embryos are committed to develop as either males or females. In some reptiles the sex is environmentally determined, as it depends on the incubation temperature of the eggs, and there exists apparently no genetic difference between males and females (reviewed by Wilkins, 2002; Barske and Capel, 2008). In contrast, most species have a genetic mechanism of sex determination which implies the existence of heteromorphic sex chromosomes. Birds have a ZZ/ZW sex chromosomal system in which females are the heterogametic sex, and sex is probably determined according to a Z-chromosome dosage model in which the well conserved gene plays a key role (Smith et al., 2009). In contrast, mammals have a XX/XY sex chromosomal system with heterogametic males, in which the Y-linked, single master-regulatory gene SRY determines sex by inducing the undifferentiated, bipotential gonadal primordia of the embryo to differentiate as testes (Sinclair et al., 1990; Gubbay et al., 1990; Koopman et al., 1991). Once differentiated, testes produce androgens which induce the embryo to acquire a male somatic phenotype. The absence of testes, and thus of testosterone, allows XX mammalian embryos to develop as females (Jost, 1947).

Both testes and ovaries derive from undifferentiated, bipotential gonadal primordia which originate as elongated thickenings (the genital ridges) of the coelomic epithelium on the ventro-medial surface of the mesonephroi, the transient embryonic kidneys. In the mouse, gonadal primordia are formed between 10.5 and 11.5 days post coitum (dpc) by the conjunction of three cellular processes: 1) proliferation of somatic cells derived from the coelomic epithelium; 2) recruitment of cells from the underlying mesonephros; and 3) migration into the gonad of primordial germ cells (PGCs), which have an extra-embryonic origin. This initial gonadal
blastema contains epithelial, mesenchymal, endothelial and primordial germ cells and shows no definite supra-cellular structure (see Merchant-Larios and Taketo, 1975). Then, sex cords enclosing both somatic cells of the supporting lineage and PGCs are formed in both XX and XY gonadal primordia in most mammalian species, but not in the mouse (reviewed by Jiménez, 2009). The expression of the SRY gene in XY embryos triggers testis differentiation, a process which has been well studied in the mouse and includes the following cellular events: 1) specification of pre-Sertoli cells, where Sry is expressed between 10.5 and 12.5 dpc; 2) formation of the testis cords by aggregation and polarization of the pre-Sertoli cells, which differentiate this way as Sertoli cells, enclose all PGCs inside the cords and induce Müllerian ducts regression by producing anti-Müllerian hormone (AMH); 3) migration into the gonad of mesonephric endothelial cells, which rapidly form a profuse, testis-specific vascular system, including a large coelomic vessel; 4) formation of the testis cord envelope, constituted by a mono-layer of peritubular myoid cells which, together with Sertoli cells, contribute to deposit a basal lamina between these two cell types; 5) differentiation of interstitial Leydig cells, which produce the testosterone necessary for the somatic masculinization of the embryo; and 6) differentiation of the testis envelope, the tunica albuginea, by flattening of the cortical cells and accumulation of abundant collagen fibers. The process of testis organogenesis has been repeatedly reviewed in recent decades (e.g. McLaren, 1991; Tilmann and Capel, 2002; Wilhelm et al., 2007; Archambeault et al., 2009; Biaison-Lauber, 2010; Nel-Themaat et al., 2010; Stukenborg et al., 2010). Although the cellular processes involved in testis organogenesis are basically the same in all mammalian species analyzed, some differences, however, have been described in the spatio-temporal pattern in which these events occur in different mammals (Carmona et al., 2009a,b).

Like the testis, the ovary also contains three principal cell lineages: the germ cells, which in the developing ovary enter meiosis and become primary oocytes; the supporting cells, represented by the follicle (granulosa) cells, which are ontogenetically homologous to Sertoli cells (Albrecht and Eicher, 2001); and the steroidogenic cells, which in the ovary are the theca cells, presumably homologous to Leydig cells, although their origin remains unknown. There is no ovarian counterpart of the peritubular myoid cells of the testis. In the mouse, the main steps of ovarian differentiation are as follows: 1) proliferation of the PGCs to reach the critical germ cell mass of the ovary; 2) meiosis entry of the PGCs; 3) specification of the pre-follicle cells; 4) follicle formation which involves follicle cell differentiation; and 5) initiation at puberty of follicle maturation (growth) and theca-cell differentiation. Unlike the testis, the pattern of ovarian development is highly species-specific, and important developmental differences exist between particular mammalian taxa (Mossman and Duke, 1973; Jiménez, 2009).

In this paper we review most of the genes currently known to control mammalian testis organogenesis and also genes that can perturb testis development, either because they have an anti-testis role or because they promote the alternative developmental pathway, that is, ovarian development (Fig. 1; Table 1).

Genes that promote formation of the bipotential gonad

**SF1**

The Steroidogenic Factor 1 (SF1) is also known as the Fushi Tarazu Factor, *Drosophila*, homolog 1 (FTZF1
Table 1. Chromosomal location, spatio-temporal expression pattern and mutant sexual phenotypes of genes involved in mammalian gonad development.

<table>
<thead>
<tr>
<th>Gene Symbol1</th>
<th>Chromosomal location2</th>
<th>Expression in the mouse developing gonad</th>
<th>Mutant phenotypes</th>
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</thead>
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<tr>
<td><strong>SF1</strong></td>
<td>Human 9q33.3</td>
<td>Somatic cells undiff. gonad (9.5-11.5 dpc)</td>
<td>-Adult Sox8/- mice cause sterility (O'Bryan et al., 2008)</td>
</tr>
<tr>
<td><strong>NRA1</strong></td>
<td>Mouse 2B</td>
<td>Pre-Sertoli cells (11.5-12.5 dpc)</td>
<td>-Sox9-/- double mutant mice show either XY sex reversal (Chaboisssier et al., 2009)</td>
</tr>
<tr>
<td><strong>AD4BP</strong></td>
<td>Mouse 2E2</td>
<td>Leydig cells (12.5 dpc-)</td>
<td>-SoX10 duplication causes masculinization in XX patients (Alek, et al., 1999; Seherunvong et al., 2004)</td>
</tr>
<tr>
<td><strong>FTZF1</strong></td>
<td>Mouse 15B3.1</td>
<td>Somatic cells undiff. gonad (12.5-13.5 dpc)</td>
<td>-Ectopic expression of Sox3 in XX mouse gonads causes sex reversal (Polanco et al., 2010)</td>
</tr>
<tr>
<td><strong>WT1</strong></td>
<td>Human 11p3</td>
<td>Somatic cells undiff. gonad (9.5-11.5 dpc)</td>
<td>-XX Sry-transgenic mice are males (Koopman et al., 1991)</td>
</tr>
<tr>
<td></td>
<td>Mouse 2E3</td>
<td>Pre-Sertoli and Sertoli cells (11.5 dpc-)</td>
<td>-DAX1 duplication causes XY sex reversal in humans with DSS syndrome (Bardoni et al., 1994)</td>
</tr>
<tr>
<td><strong>GATA4</strong></td>
<td>Human 8p23.1</td>
<td>Somatic cells undiff. gonad (9.5-11.5 dpc)</td>
<td>-DAX1 over-expression causes sex reversal in humans with DSS syndrome (Bardoni et al., 1994)</td>
</tr>
<tr>
<td></td>
<td>Mouse 14D1</td>
<td>Leydig cells (12.5 dpc-)</td>
<td>-Transgenic mice over-expressing Dax1 show testicular dysgenesis (Swain et al., 1998)</td>
</tr>
<tr>
<td><strong>FOG2</strong></td>
<td>Human 8q23.1</td>
<td>Somatic cells undiff. gonad (9.5-11.5 dpc)</td>
<td>-XY Dax1 mutant mice show testicular dysgenesis (Yu et al., 1998)</td>
</tr>
<tr>
<td><strong>ZFP2M2</strong></td>
<td>Mouse 15B3.1</td>
<td>Somatic cells undiff. gonad (12.5-13.5 dpc)</td>
<td>-Fog2/- mice show testicular dysgenesis (Tevison et al., 2002)</td>
</tr>
<tr>
<td><strong>SRY</strong></td>
<td>Human Yp11.31</td>
<td>Pre-Sertoli and Sertoli cells (10.5-12.5 dpc)</td>
<td>-SRY knockout mice show XY sex reversal (Berta et al., 2010)</td>
</tr>
<tr>
<td><strong>TDF</strong></td>
<td>Mouse YA1</td>
<td>Somatic cells (9.5-11.5 dpc)</td>
<td>-SRY-/- mice show XY sex reversal (Berta et al., 2010)</td>
</tr>
<tr>
<td><strong>TDY</strong></td>
<td>Mouse 11E2</td>
<td>Somatic cells undiff. gonad (11.5 dpc-)</td>
<td>-AMH-/- and AMHR2-/- mice show XX sex reversal (Bertoni et al., 2009)</td>
</tr>
<tr>
<td><strong>SOX9</strong></td>
<td>Human 17q24.3</td>
<td>Somatic cells undiff. gonad (9.5-11.5 dpc)</td>
<td>-AMH-/- and AMHR2-/- mice show XX sex reversal (Zhao et al., 2009)</td>
</tr>
<tr>
<td></td>
<td>Mouse 11E2</td>
<td>Sertoli cells (11.5 dpc-)</td>
<td>-Sertoli and Sertoli cells (11.5 dpc-)</td>
</tr>
<tr>
<td><strong>SOX10</strong></td>
<td>Human 22q13.1</td>
<td>Somatic cells undiff. gonad (9.5-11.5 dpc)</td>
<td>-Sertoli and Sertoli cells (11.5 dpc-)</td>
</tr>
<tr>
<td></td>
<td>Mouse 15E1</td>
<td>Sertoli cells (11.5 dpc-)</td>
<td>-Sertoli and Sertoli cells (11.5 dpc-)</td>
</tr>
<tr>
<td><strong>SOX3</strong></td>
<td>Human Xq27.1</td>
<td>Somatic cells of the testis (11.5dpc-)</td>
<td>-XX male patients have rearrangements within the SOX3 regulatory region (Sutton et al., 2011)</td>
</tr>
<tr>
<td></td>
<td>Mouse XA6</td>
<td>Undifferentiated spermatogonia (6dp)</td>
<td>-Ectopic expression of Sox3 in XX mouse gonads causes sex reversal (Sutton et al., 2011)</td>
</tr>
<tr>
<td><strong>DAX1</strong></td>
<td>Human Xq21.2</td>
<td>Somatic cells undiff. gonad (9.5-11.5 dpc)</td>
<td>-DAX1 over-expression causes sex reversal in males with DSS syndrome (Bardoni et al., 1994)</td>
</tr>
<tr>
<td></td>
<td>Mouse XC1</td>
<td>Sertoli cells (12.5-13.5 dpc)</td>
<td>-DAX1 over-expression causes sex reversal in males with DSS syndrome (Bardoni et al., 1994)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Interstitial cells (13.5-14.5 dpc)</td>
<td>-Transgenic mice over-expressing Dax1 show testicular dysgenesis (Swain et al., 1998)</td>
</tr>
<tr>
<td><strong>PDGFRA</strong></td>
<td>Human 4q12</td>
<td>Somatic cells undiff. gonad (9.5-11.5 dpc)</td>
<td>-XY Dax1 mutant mice show testicular dysgenesis (Yu et al., 1998)</td>
</tr>
<tr>
<td></td>
<td>Mouse 5C3.3</td>
<td>Sertoli cells (12.5 dpc-)</td>
<td>-PDGFα/- mice show testicular dysgenesis (Gnassi et al., 2000)</td>
</tr>
<tr>
<td><strong>DMRT1</strong></td>
<td>Human 1p24.3</td>
<td>Somatic cells undiff. gonad (9.5-11.5 dpc)</td>
<td>-Pdgfα/- mice show testicular dysgenesis (Gnassi et al., 2000)</td>
</tr>
<tr>
<td></td>
<td>Mouse 19B1-C</td>
<td>Sertoli cells (12.5 dpc-)</td>
<td>-Pdgfα/- mice show testicular dysgenesis (Gnassi et al., 2000)</td>
</tr>
<tr>
<td><strong>AMH</strong></td>
<td>Human 19p13.3</td>
<td>Sertoli cells (12.5-dpc-puberty)</td>
<td>-XX freemartin cattle exposed to AMH show masculinized gonads (Vigier et al., 1987)</td>
</tr>
<tr>
<td><strong>MIS</strong></td>
<td>Mouse 10C1</td>
<td>Adult follicle cells</td>
<td>-Humans with mutated AMH show persistent Müllerian duct syndrome (Krebbein et al., 2010)</td>
</tr>
<tr>
<td><strong>MIF</strong></td>
<td>Human 19p13.3</td>
<td>Sertoli cells (12.5-dpc-puberty)</td>
<td>-Transgenic mice over-expressing Amh show freemartim phenotype (Behringer et al., 1990)</td>
</tr>
<tr>
<td><strong>NGF</strong></td>
<td>Human 1p13.2</td>
<td>Embryonic Sertoli and interstitial cells</td>
<td>-Both Amh-/- and Amhr2-/- mice show male pseudohermaphroditism (Mishina et al., 1996)</td>
</tr>
<tr>
<td><strong>NFGB</strong></td>
<td>Mouse 3F2.2</td>
<td>N.A.</td>
<td>-N.A.</td>
</tr>
<tr>
<td><strong>NT3</strong></td>
<td>Human 12p13.31</td>
<td>Embryonic Sertoli and interstitial cells</td>
<td>-N.A.</td>
</tr>
<tr>
<td><strong>NTF3</strong></td>
<td>Mouse 6F3</td>
<td>N.A.</td>
<td>-N.A.</td>
</tr>
<tr>
<td><strong>P53NTR</strong></td>
<td>Human 17q21.33</td>
<td>Mesonephros prior testis differentiation</td>
<td>-N.A.</td>
</tr>
<tr>
<td><strong>NGFR</strong></td>
<td>Mouse 11D</td>
<td>Peritubular myoidcells until birth</td>
<td>-N.A.</td>
</tr>
<tr>
<td><strong>CD271</strong></td>
<td>Human 1p34.3</td>
<td>Embryonic somatic ovarian cells</td>
<td>-N.A.</td>
</tr>
<tr>
<td><strong>Rspo1</strong></td>
<td>Mouse 4D2.2</td>
<td>Embryonic somatic ovarian cells</td>
<td>-N.A.</td>
</tr>
<tr>
<td><strong>Rspo</strong></td>
<td>Human 1p34.3</td>
<td>Embryonic somatic ovarian cells</td>
<td>-N.A.</td>
</tr>
<tr>
<td><strong>CRISTIN3</strong></td>
<td>FLJ49006</td>
<td>Embryonic somatic ovarian cells</td>
<td>-N.A.</td>
</tr>
<tr>
<td></td>
<td>Mouse 4D3</td>
<td>Somatic cells undiff. gonad (9.5-11.5 dpc)</td>
<td>-Patients with duplicated WNT4 show XY sex reversal (Espalda et al., 1984; Jordan et al., 2001)</td>
</tr>
<tr>
<td><strong>WNT4</strong></td>
<td>Human 1p36.12</td>
<td>Somatic cells undiff. gonad (9.5-11.5 dpc)</td>
<td>-XX Wnt4-/- mice show masculinized ovaries (Vainio et al., 1999)</td>
</tr>
<tr>
<td></td>
<td>Mouse 4D3</td>
<td>Somatic ovarian cells (11.5 dpc-)</td>
<td>-Wnt4 over-expression in XY testes causes abnormal vasculature (Jeays-Ward et al., 2003)</td>
</tr>
<tr>
<td><strong>CTNNB1</strong></td>
<td>Human 3p22.1</td>
<td>Somatic ovarian cells (11.5 dpc-)</td>
<td>-Premature WNT4 down-regulation is associated with ovotestis formation in female moles (Carmona et al., 2009a,b)</td>
</tr>
<tr>
<td><strong>CTNNB2</strong></td>
<td>Mouse 9F4</td>
<td>Somatic ovarian cells (11.5 dpc-)</td>
<td>-N.A.</td>
</tr>
<tr>
<td><strong>FST</strong></td>
<td>Human 5g1.12</td>
<td>Somatic ovarian cells (11.5-14.5 dpc)</td>
<td>-XX Fst-/- mice show masculinized ovaries with reduced germ cell number (Yao et al., 2009)</td>
</tr>
<tr>
<td><strong>BMP2</strong></td>
<td>Human 20p12.3</td>
<td>Somatic ovarian cells (11.5-14.5 dpc)</td>
<td>-N.A.</td>
</tr>
<tr>
<td><strong>BMP2A</strong></td>
<td>Mouse 2F2</td>
<td>Somatic ovarian cells (11.5-14.5 dpc)</td>
<td>-N.A.</td>
</tr>
<tr>
<td><strong>FOXL2</strong></td>
<td>Human 9E3.3</td>
<td>Somatic ovarian cells (12.5 dpc-)</td>
<td>-N.A.</td>
</tr>
<tr>
<td><strong>PFFK</strong></td>
<td>Human 3q22.3</td>
<td>Somatic ovarian cells (12.5 dpc-)</td>
<td>-N.A.</td>
</tr>
</tbody>
</table>

1: The HGNC approved gene symbol is marked as bold font; 2: Data taken from the ENSEMBL web-site; N.A.: Not available.
or FTZ1) or as the Adrenal 4 Binding Protein (AD4BP); its approved gene symbol is NR5A1 (Nuclear Receptor Subfamily 5, group A, Member 1).

A mutation in human SF1 was first described in a patient with complete XY sex reversal in combination with primary adrenal failure (Achermann et al., 1999). Subsequent studies revealed that heterozygous loss-of-function mutations in SF1 can also be found in patients with 46,XY disorders of sex development with normal adrenal function, where the phenotypic spectrum ranges from complete testicular dysgenesis with Müllerian derivatives, to genital ambiguity, to severe hypospadias or even anorchia (reviewed in Lin and Achermann, 2008). Mutations in SF1 have also been linked to premature ovarian failure (Lourenço et al., 2009) and to spermatogenic failure (Bashamboo et al., 2010).

In the male mouse, Sf1 is expressed in the adrenogonadal primordium starting at 9.5 dpc, and by 12.5 dpc expression in the testis persists in both Sertoli and Leydig cells, whereas it is soon downregulated in the ovary (Ikeda et al., 1994). Here, expression is maintained at low levels until adulthood, when strong expression in theca cells is detected. In Leydig cells, SF1 activates the expression of steroidogenic enzymes, producing androgens. SF1 is also expressed in the ventromedial pituitary and hypothalamus (Ikeda et al., 1996, 2001). Consistent with this expression pattern, mice homozygously mutant for Sf1 showed complete gonadal and adrenal agenesis as well as abnormalities in gonadotropin hormone production, revealing an essential role of this gene in the formation of the bipotential gonad for both adrenal steroidogenesis and the hypothalamic-pituitary control of reproduction (Luo et al., 1994).

In addition to its function in promoting steroidogenesis, SF1 has been shown to directly activate the expression of two important testicular genes. One is Amh, where SF1 binds to the promoter together with the KTS isoform of WT1 (Shen et al., 1994; Nachtigal et al., 1998). The other is Sox9, where SF1 binds to an upstream testis-specific enhancer of Sox9 core sequence (TESCO), first alone to ensure low-level Sox9 expression, then together with SRY to up-regulate Sox9 expression, and subsequently together with SOX9 itself to maintain Sox9 expression (Sekido and Lovell-Badge, 2008; see also below). In conclusion, SF1 has key roles in several important developmental processes, including steroidogenesis, gonadal primordium formation and testis differentiation.

WT1

The Wilms’ tumor 1 gene (WT1) encodes a zinc finger protein first isolated by Call et al. (1990) and Gessler et al. (1990). The WT1 gene consists of 10 exons and is transcribed from a complex transcription unit that, in addition to WT1, encodes for several proteins (Huang et al., 1990; Campbell et al., 1994; Eccles et al., 1994; Dallosso et al., 2004). WT1 has four major isoforms due to the insertion or exclusion of three amino acids (lysine, threonine, serine: KTS) between the zinc fingers 3 and 4 (KTS+/KTS- isoform), and the alternative splicing of a fragment encoding a stretch of 17 amino acids in the middle of exon 5 (Hossain and Saunders, 2001), but up to 32 different isoforms have been described (Scharnhorst et al., 1999). Englert et al. (1995) reported important changes in the sub-nuclear localization of the different WT1 isoforms and of WT1 truncation mutants. WT1 controls cellular proliferation and differentiation involving different targets and functions, and may act as a transcriptional repressor or activator, depending on the cellular or chromosomal context. The KTS isoforms bind more stably to DNA as the KTS+ isoforms increase the flexibility between the third and fourth zinc fingers and thus abrogate the binding of this fourth zinc finger to its cognate site (Laity et al., 2000).

Wilms’ tumor, or nephroblastoma, is a pediatric kidney cancer that affects 1 in 10000 children (Matsunaga, 1981) and that can occur in combination with genitourinary system abnormalities in the complex disorder WAGR syndrome (Wilms tumor – Aniridia – Genitourinary tract abnormalities – mental Retardation), (Pendergrass, 1976). Cytogenetic analyses revealed that most patients with Wilms’ tumor had a deletion on chromosome 11 (Riccardi et al., 1978), and the WT1 gene on 11p13 was shown to be located in the deleted fragment (Gessler et al., 1990). Germinal and somatically acquired mutations in WT1 have been identified in tumors from WAGR patients (Baird et al., 1992) and in patients with isolated Wilms’ tumor (Huff et al., 1991), defining WT1 as a tumor suppressor gene. In addition, two disorders with overt XY sex reversal have been shown to result from WT1 mutations: Denys-Drash syndrome (DDS), characterized by ambiguous or female external genitalia and dysgenetic gonads together with Wilms’ tumor (Denys et al., 1967; Drash et al., 1970), and Frasier syndrome, characterized by normal female external genitalia, streak gonads with gonadoblastoma and progressive glomerulopathy (Moorthy et al., 1987), which result from various mutations in the WT1 coding region (Pelletier et al., 1991; Jaubert et al., 2003) and from donor splice site mutations causing loss of the KTS+ isoforms, respectively (Barbaux et al., 1997; Jaubert et al., 2003).

WT1 is expressed in several tissues of the developing kidney, including the condensed mesenchym, the renal vesicle and the glomerular epithelium, as well as in the genital ridge, fetal gonad and mesothelium (Pritchard-Jones et al., 1990). It is first expressed in the mesonephros and in the genital ridge at the seventh week of gestation in the human (Mundlos et al., 1993; Pritchard-Jones et al., 1990). In the mouse, Wt1 is first expressed in the intermediate mesoderm at 9 dpc in the area developing into the genital ridge. Subsequently, it is expressed in the coelomic epithelium, the gonadal primordia, and in the adjacent mesonephros (Armstrong et al., 1993; Rackley et al., 1993). In adulthood, its expression is maintained in Sertoli cells in males, while
in females it is expressed in ovarian follicular cells, in both the embryonic and adult uterus, in the oviducts and in the endometrium (Pelletier et al., 1991).

WT1 is essential for gonadogenesis, as the gonadal primordium is formed in WT1 null mouse embryos, but degenerates by apoptosis after 11 dpc, and for nephrogenesis, as the mutants also show renal agenesis (Kreidberg et al., 1993). As described above, the KTS’ isoform collaborates with SF1 to activate the AMH gene, and failure of the WT1-SF1 interaction is associated with male pseudohermaphroditism in Denys-Drash syndrome (Nachtigal et al., 1998). Also, KTS’ isoforms may act as activators of other genes such as SF1 (Wilhelm and Englert, 2002), WNT4 (Sim et al., 2002), DAX1 (Kim et al., 1999) and SOX9 (Gao et al., 2006), and as repressors of AR (Shimamura et al., 1997; Zaia et al., 2001). It has also been shown that the KTS’ isoform can transactivate Sry expression (Hossain y Saunders, 2001) whereas the KTS’ isoform is involved in its post-transcriptional regulation, thus playing an essential role in sex determination (Hammes et al., 2001).

WT1 is thus involved in several steps of testis development: 1) the formation and survival of the gonadal primordium (Kreidberg et al., 1993; Hammes et al., 2001), 2) the maintenance of the tubular structure of the testis (Gao et al., 2006), 3) the differentiation of Sertoli cells, 4) the survival and proliferation of embryonic germ cells, and 5) the development and function of fetal Leydig cells (Natoli et al., 2004).

GATA4

The GATA-binding proteins are a family of transcription factors with zinc fingers that recognize the promoter consensus sequence (A/T)GATA(A/G) (Patient and McGhee, 2002). These proteins were first identified as key modulators of hematopoiesis (Weiss and Orkin, 1995), but they are also involved in the embryonic development of various organs (Viger et al., 2004). GATA4 contains two zinc finger domains (Huang et al., 1995) and its gene is located on human chromosome 8p23 (White et al., 1995; Huang et al., 1996), and on mouse chromosome 14 (White et al., 1995).

The mouse Gata4 gene was isolated by Arceci et al. (1993), and the human orthologue by (Huang et al., 1995). Its expression in human adult heart and, at a lower level, in fetal heart led Huang et al. (1995) to conclude that GATA4 may regulate a set of cardiac-specific genes and play a crucial role in cardiogenesis. In fact, mutations in GATA4 have been associated with atrial septal defect 2 (Garg et al., 2003; Hirayama-Yamada et al., 2005).

GATA4 is abundantly expressed in the somatic cells of the genital ridge of both sexes in mice, but at 13.5 dpc, the expression becomes dimorphic, remaining at high levels in Sertoli and Leydig cells and in the tunica albuginea in the XY gonad, while it is down-regulated in the XX gonad. However, expression of Gata4 in the somatic cells of the developing ovary from 12.5 dpc until birth has also been reported (Anttonen et al., 2003). After birth, its expression is maintained in the somatic cells of the testes and it is reactivated in the follicle cells of the adult ovaries (Heikinheimo et al., 1997; Viger et al., 1998; Ketola et al., 1999, 2002). In human gonads, GATA4 is highly expressed in Sertoli cells at 19-22 weeks of gestation. In Leydig cells it is expressed during the fetal period and after puberty, coinciding with the periods of active androgen synthesis, suggesting a role of GATA4 in steroidogenesis.

Data on the regulation of GATA4 expression are scarce, but the association of mutations in the FSH receptor with very low levels of GATA4 indicate that FSH could indirectly activate GATA4, at least in the human ovary (Vaskivuo et al. 2002).

Cantor and Orkin (2001) and Crispino et al. (2001) showed that GATA factors interact with proteins of the FOG family. Transgenic mice homozygous for a defective GATA4 protein unable to bind to FOG2 (Crispino et al., 2001) showed a reduced expression of Sry and severe deficiencies in testicular development, suggesting that an interaction between GATA4 and FOG2 is essential for Sry activation (Tevosian et al., 2002). However, in mice haploinsufficient for Gata4 or Fog2, gonadal XY sex reversal has been shown to depend on the genetic background, and that Sry is expressed in these sex-reversed XY gonads, but Sox9 up-regulation does not occur (Bouma et al., 2007). Also, in XX mice that would develop ovaries due to ectopic expression of Sox9 carrying the Ods mutation or the Wt1-Sox9 transgene (see below), Sox9 expression is lost if they are made homozygously mutant for either Gata4 or Fog2, indicating that Sox9 expression, at least in this context, is under the control of the GATA4/FOG2 transcriptional complex (Manuylov et al., 2008).

GATA4 has also been shown to be a potent activator of the Amh promoter (Viger et al., 1998), and to cooperate with SF1 in this process (Tremblay and Viger, 1999), while DAX1 represses this cooperation (Tremblay and Viger, 2001). Tremblay and Viger (2003) reported evidence that the inactivation of the GATA4-SF1 cooperation may be responsible for abnormalities in male sex differentiation caused by insufficient levels of AMH. It has also been shown that FOG2 represses the GATA4-mediated activation of Amh (Tremblay et al., 2001; Anttonen et al., 2003). GATA4 recognition sequences have also been described in the promoter of SF1 and Dmrt1, both of them normally expressed in Sertoli cells (Lei and Heckert, 2004; see below)

GATA4 is thus a master gene regulating other genes involved in the differentiation of Sertoli and Leydig cells, playing an important role in the early development of the testis, in male sexual differentiation and in steroidogenesis.

FOG2

The FOG (friend of GATA) family of proteins includes a number of transcription factors which are
characterized by multiple zinc finger domains and which are apparently not able to bind to DNA, but act as co-factors of GATA proteins by increasing or decreasing their activity depending on the cellular context (Fox et al., 1999; Tsang et al., 1997). FOG2 was shown to be involved in the differentiation of the heart, neurons and gonads (Tevosian et al., 2000) and has been associated with congenital abnormalities of the diaphragm (Ackerman et al., 2005; Temple et al., 1994). FOG2, also known as ZFPM2 (zinc finger protein multitype 2), is located on human 8q23.1 (Pizzuti et al., 2003). The mouse Fog2 gene encodes a nuclear factor with eight zinc fingers and a transcriptional repression domain and associates physically with the N-terminal zinc finger of GATA4 (Lin et al., 2004; Svensson et al., 2000).

In the mouse, Fog2 is expressed at 10.5dpc in the genital ridge of both sexes. During ovarian development, it is expressed at 12.5dpc in somatic cells according to a pattern coinciding with that of GATA4 (Anttonen et al., 2003), and expression continues in follicle cells of the adult ovary. In the XY gonad, Fog2 expression is detected at 12.5dpc in Sertoli cells and decreases as testicular development proceeds. At 13.5 dpc the expression of Fog2 in Sertoli cells is very low, but expression of Gata4 and Amh increases. Two days later, FOG2 protein is only detectable in the Tunica albuginea and in some interstitial cells, but not in cells expressing Amh. After birth, Fog2 is abundantly expressed in Sertoli cells until the third week of life, and it cyclically reactivates in the seminiferous tubules at stages VII-XII of the spermatogenic cycle (Ketola et al., 2002; Anttonen et al., 2003).

Fog2-null mutant mice die at 13.5dpc due to defective development of the cardiovascular system. They also show anomalies in testicular development similar to those observed in Gata4-defective mice (Tevosian et al., 2002) due to misregulation of Sry by the GATA4/FOG2 complex during the early stages of gonadogenesis. It has also been suggested that FOG2 may play a role in the down-regulation of Amh in post-natal Sertoli cells because high levels of FOG2 cause a noticeable decrease in the promoter activity of this gene in primary cultures of Sertoli cells (Tremblay et al., 2001). Furthermore, the expression of Fog2 during ovarian development suggests that this protein may have a role in the repression of Amh in females (Anttonen et al., 2003).

Other genes (LHX9, EMX2, CBX2, IR)

In addition to those mentioned above, there are other genes which have been found to have some roles in early gonad development.

The LIM homeobox gene 9 (LHX9) is expressed in the interstitial cells of the gonadal primordium from 9.5 dpc and has been shown to be necessary for somatic cell proliferation in the developing gonad, causing gonadal agenesis and the subsequent formation of XY phenotypic females when mutated (Birk et al., 2000). The function of LHX9 in gonad development is probably exerted by participating in the activation of Sfi1, as Wilhelm and Englert (2002) showed that, together with the -KTS isoform of WT1, it can bind directly to the Sfi1 promoter.

The EMX2 gene (homolog of Drosophila empty spiracles gene 2) is expressed in epithelial cells of the gonadal primordium and is necessary for the early development of kidneys, ureters and gonads (Miyamoto et al., 1997).

M33, a mouse homologue of the Drosophila chromobox-containing polycomb genes, also known as Cbx2, causes male-to-female sex reversal and juvenile death when mutated in homozygosis (Kato-Fukui et al., 1998). Both XX and XY mutant embryos showed retarded formation of the urogenital systems, suggesting that this gene is necessary for the formation of the gonadal primordium. Also, since gonadal growth defects appeared near the time of Sry expression, the authors suggested that sex reversal in M33-/- mutant mice could be a consequence of Sry expression disturbance. Biaso-Lauber et al. (2009) described an XY sex-reversed girl with normal ovaries, uterus and external female genitalia who was a compound heterozygote for CBX2 loss-of-function mutations, and showed that the mutant CBX2 did not properly regulate expression of Sfi1.

XY mice mutant for all three insulin receptor tyrosine kinase genes Ir, Igf1r and Ir are female phenotype and reduced expression of both testis-determining genes Sry and Sox9 (Nef et al., 2003). These data evidence that insulin receptor signalling is required for normal Sry expression and thus for the development of male gonads and male sexual differentiation.

Genes that promote testis formation and differentiation

SRY

The gene located in the sex-determining region of the Y chromosome on Yp11.31, SRY, was the founding member of the SOX (SRY-related HMG box) family of transcription factors, which are characterized by a conserved DNA-binding domain similar to the chromatin high mobility group (HMG) proteins. In 1990, Goodfellow’s lab identified the SRY gene (Sinclair et al., 1990) by analyzing a 35-kb region on the human Y chromosome translocated to the X chromosome in the genomes of XX males. Subsequent studies confirmed that SRY was the testis determining gene: a) analysis of XX sex reversed mice containing the smallest part of the Y chromosome known to be sex-determining (Sxr) showed the presence of the mouse homolog Sry in the Sxr region (Gubbay et al., 1990), b) mutational analysis of XX sex reversed patients revealed that SRY was mutated (Berta et al., 1990; Jäger et al., 1990), and c) XX mice transgenic for Sry developed as males (Koopman et al., 1991).

Human SRY is an intronless gene which encodes a
204-amino-acid protein which contains, like all SOX proteins, two nuclear localization signals (NLSs) within the HMG box (Südbeck and Scherer, 1997). The HMG box is the only conserved domain, and comparison of the amino acid sequence of SRY among several species revealed around 70% identity. In contrast, there is no sequence conservation outside the HMG domain, supporting the idea that the SRY gene has evolved rapidly. The SRY HMG box is a conserved motif for binding and bending of DNA that recognizes the DNA consensus sequence A/TAACAA/T. A SRY gene has three exons and is, other than the mammalian-specific SRY gene, highly conserved throughout the vertebrate phylum and through other phyla. The human SOX9 protein of 509 amino acids contains several conserved regions, including, besides the HMG box with its two NLSs, a C-terminal transcriptional activation domain (Südbeck et al., 1996), a dimerization domain preceding the HMG box (Sock et al., 2003; Bernard et al., 2003), and a nuclear export signal (NES) in the center of the HMG domain (Gasca et al., 2002). The SOX9 HMG box shares 70% amino acid homology to the HMG box of SRY and acts by binding to specific DNA sites to activate the transcription of target genes (Ng et al., 1997; McDowall et al., 1999). The SOX9 HMG box, which has 50% amino acid sequence identity to that of SRY, binds to the consensus sequence AACAATA/S (Südbeck et al., 1996; McDowall et al., 1999), and SOX9 has been shown to up-regulate the expression of COL2A1 and AMH by binding to sites sharing some homology with this consensus sequence (Bell et al., 1997; Ng et al., 1997; De Santa Barbara et al., 1998). SOX9 is a transcription factor with multiple roles during organogenesis, being essential for chondrocyte differentiation and cartilage formation (Bi et al., 1999), glial cell fate choice in the developing spinal cord (Stolt et al., 2003), notochord development (Barrionuevo et al., 2006a) and maintenance of the pancreatic progenitor cell pool (Seymour et al., 2007), among several other developmental processes.

Two thirds of XY patients with a mutation in and around SOX9 showed sex reversal (Foster et al., 1994; Wagner et al., 1994), indicating an essential role for the gene in testis determination. Consistent with this notion, murine Sox9, initially expressed in the genital ridge of both sexes, becomes upregulated in Sertoli cells coincident with the peak of Sry expression at 11.5 dpc, whereas it is downregulated in the ovary (Moraes da Silva et al., 1996; De Santa Barbara et al., 2000). Ectopic expression of Sox9 in undifferentiated XX gonads caused by the Ods mutation (Bishop et al., 2000) or by a Wt1-Sox9 transgene (Vidal et al., 2001) induces testis formation and subsequent development of sterile XX males lacking Sry. Conversely, inactivation of Sox9 before the sex determination stage results in XY sex reversal (Chaboissier et al., 2004; Barrionuevo et al., 2006b). Thus, SOX9 is necessary and sufficient to induce testis formation and can substitute for SRY function in testis determination.

As mentioned above, SRY has been shown to bind to
a testis-specific enhancer of Sox9 core sequence (TESCO) within the murine Sox9 promoter, and, together with SF1, to activate the expression of Sox9 through this element, providing the long-sought link between SRY and SOX9 (Sekido and Lovell-Badge, 2008). It thus seems that the only role for SRY is to activate SOX9, which, once active, will promote testis differentiation. In addition to SRY and SF1, other factors are involved in SOX9 up-regulation, including a) SOX9 which autoregulates itself (Sekido and Lovell-Badge, 2008), b) FGF9, which forms a positive feedback loop with SOX9 necessary to down-regulate the female-promoting WNT signaling pathway (Kim et al., 2006) and c) PTGDS which also forms a positive feedback loop to promote Sertoli cell function (Malki et al., 2005, 2010; Wilhelm et al., 2007). Despite the fact that SOX9 seems to be the pivotal factor in Sertoli cell differentiation and testis cord formation, only AMH, VANIN1 and CBLN4 are currently known as SOX9 molecular targets (de Santa Barbara et al., 1998; Arango et al., 1999; Wilson et al., 2005; Bradford et al., 2009).

As no mutation in the human TESCO homolog has been identified in SRY-positive females with XY gonadal dysgenesis (Georg et al., 2010), and as none of the breakpoints in CD translocation cases with XY sex reversal removes TESCO from SOX9 (Leipoldt et al., 2007), the functional human equivalent to mouse TESCO may be located elsewhere. Recent evidence indicates that such an element is located within a 78 kb region 517-595 kb from SOX9, as this common region is spanned by duplications or deletions in XX or XY sex reversal cases, respectively (Benko et al., 2011; Cox et al., 2011; Vetro et al., 2011).

**FGF9**

Fibroblast growth factor 9, *FGF9*, is a peptide regulatory factor belonging to the family of fibroblast growth factors (FGF) involved in multiple biological processes during embryogenesis and adult life and is located on human chromosome 13q12.11. In 1993, the glia-activating factor (GAF) was purified from the culture supernatant of a human glioma cell line (Naruo et al., 1993). This same year, by using oligonucleotide probes, the cDNA of GAF was isolated, and sequence comparison analyses revealed that GAF was the ninth member of the FGF family, and therefore it was called FGF9 (Miyamoto et al., 1993). The human FGF9 cDNA encodes a polypeptide consisting of 208 amino acids. The sequence similarity between FGF9 and other members of the FGF family is around 30%. FGF9 mediates its biological response by acting as an extracellular protein which binds to and activates cell surface tyrosine kinase FGF receptors (Pellegrini et al., 2001).

Human mutations of FGF9 have been associated with skeletal diseases and cancer (Abdel-Rahman et al., 2008; Wu et al., 2009). During mouse embryogenesis, *Fgf9* expression is detected in several tissues, including intermediate mesoderm of late-stage gastrulation, ventricular myocardium, pleura, skeletal myoblasts in the early limb bud, spinal cord motor neurons, olfactory bulb, and gut luminal epithelium (Colvin et al., 1999). In addition, during gonadal development, *Fgf9* is expressed in the bipotential gonad of both sexes shortly before the sex determination stage. Later, it becomes male specific in the Sertoli cells and in the coelomic epithelium (Colvin et al., 2001; Kim et al., 2006). Analysis of *Fgf9*−/− mice revealed male-to-female sex reversal, indicating an important role for the gene in testis determination (Colvin et al., 2001). Subsequent studies in mice showed that FGF9 and WNT4 act as opposing signals during sex determination. In this context, SRY initiates a feed-forward loop between Sox9 and *Fgf9*, which up-regulates *Fgf9* expression and represses WNT4 signaling leading to testis differentiation (Kim et al., 2006). In addition, FGF9 is necessary in the fetal testis to prevent germ cells from entering meiosis and for germ cell survival (DiNapoli et al., 2006; Bowles et al., 2010).

**PTGDS**

The lipocalin-type prostaglandin D2 synthase (L-PGDS, PTGDS) is an enzyme that catalyzes the conversion of prostaglandin H2 (PGH2) to Prostaglandin D2 (PGD2). The latter is synthesized in many tissues, and acts as a signaling molecule involved in the regulation of a number of biological processes such as platelet aggregation, broncho-constriction and allergic diseases (Matsuoka et al., 2000; Breyer and Breyer, 2001). In humans and mice, abnormal function of PTGDS has been associated with glucose intolerance, nephropathy, atherosclerosis and Alzheimer disease (Ragolia et al., 2005; Kanekiyo et al., 2007).

PTGDS has a male specific pattern of expression and PGD2 can partially masculinize female embryonic gonads in culture (Adams and McLaren, 2002). PGD2 signaling up-regulates SOX9 in both an autocrine and paracrine manner (Wilhelm et al., 2005) and facilitates SOX9 translocation to the nucleus (Malki et al., 2005). Furthermore, SOX9 can activate PTGDS through the binding to a conserved element in its promoter (Wilhelm et al., 2007). Thus, SOX9 establishes a feed-forward loop with the PGD2 signaling to ensure male development (Wilhelm et al., 2007; Moniot et al., 2009). This loop acts independently to that formed between SOX9 and FGF9 (Moniot et al., 2009).

**SOX8**

SOX8 (SRY-related HMG box gene 8), together with *SOX9* and *SOX10*, belongs to the group E of SOX genes. The SOX8 protein has 47% amino acid sequence identity with SOX9, and within the HMG domain, these two transcription factors differ by just one amino acid. The SOX8 protein has a similar domain structure to that described for SOX9 (for a review see Barriónuevo and...
Scherer, 2010).

No human syndrome has been associated with loss of SOX8 function, although the gene was shown to be deleted in one patient with alpha-thalassemia/mental retardation (ATR-16) syndrome who carried a deletion of approximately 2 Mb on distal 16p (Pfeifer et al., 2000). In mice, Sox8 starts to be expressed in Sertoli cells shortly after the sex determination stage and shortly after Sox9, and this expression is maintained in Sertoli cells throughout embryonic and postnatal testis development (Schepers et al., 2003). Despite this, Sox8 seems to be dispensable for embryonic testis development, as Sox8-/- mice are viable and initially fertile (Sock et al., 2001), but they become sterile at around 5 months because of impaired Sertoli cell function (O’Bryan et al., 2008). Ablation of Sox9 in embryonic Sertoli cells at 14.0 dpc, shortly after the sex determination stage, does not perturb normal testis function at embryonic and postnatal stages, but late sterility occurs at about 5 months of age (Barriouneau et al., 2009). Thus, Sox8 appears to compensate for Sox9 function in the embryonic and postnatal testis. Inactivation of both genes in Sertoli cells after the sex determination stage led to perinatal cessation of testis cord differentiation and thus to primary infertility (Barriouneau et al., 2009), confirming that the combined function of both transcription factors is necessary for the maintenance of testicular function. Due to their similar expression profile and protein structure, SOX8 and SOX9 can be expected to activate similar target genes during testogenesis. This has been shown to be the case for Amh, which is activated by SOX8 in vitro, as well as by Sox9, although to a lesser extent (Schepers et al., 2003). In vivo, in the absence of either Sox8 or Sox9, sufficient levels of Amh transcripts are still produced to induce Müllerian duct regression, whereas the lack of both genes causes such low levels of Amh transcripts that a rudimentary uterus is occasionally visible (Barriouneau et al., 2009).

**SOX10**

The SRY-related HMG-box gene 10, SOX10, the third member of the group E of SOX genes, is located on human chromosome 22q13.1. It was isolated in 1998 from both human and mouse cDNA and genomic libraries (Pusch et al., 1998; Kuhlbrodt et al., 1998). The SOX10 protein has a 54% amino acid sequence identity with SOX9, and within the HMG domain the two proteins differ by only four residues. The domain structure of SOX10 is similar to that described for SOX9 and SOX8 (Barriouneau and Scherer, 2010). In humans, mutations in SOX10 have been associated with Waardenburg (deafness with pigmentary abnormalities) and Hirschsprung (aganglionic megacolon) disease (Pingault et al., 1998). In mice, Sox10 is expressed in the bipotential gonads of both sexes, becoming male-specific after the sex determination stage (Polanco et al., 2010). Mutations leading to SOX10 loss of function have not been associated with abnormalities of sexual development either in humans or in mice. A possible explanation for this is that SOX9 and/or SOX8 can compensate for the absence of SOX10. However, cases of masculinized or incompletely feminized XX patients with duplication of a chromosomal region containing the SOX10 gene have been reported (Aleck et al., 1999; Seeherunvong et al., 2004), indicating that overexpression or misexpression of the gene may induce XX sex reversal. Indeed, ectopic expression of Sox10 causes XX sex reversal in mice (Polanco et al., 2010), and therefore Sox10 can substitute for Sry or Sox9 in testis determination. In conclusion, it is difficult to know the precise role (if any) of SOX10 during testis determination and differentiation, as it seems to be redundant to that of the other SOX E genes.

**SOX3**

The SRY-related HMG-box gene 3, SOX3, is another gene belonging to the SOX family of transcription factors which is located in human chromosome Xq27.1. SOX3 is an intronless gene, highly conserved amongst mammalian species (Stevanovic et al., 1993). Because of its location on the X chromosome, it has been proposed that SRY evolved from SOX3 (Foster and Graves, 1994). Mutations in the human SOX3 gene have been associated with X-linked mental retardation with isolated growth hormone deficiency (Laumonnier et al., 2002) and with X-linked panhypopituitarism (Solomon et al., 2004). Both XY patients with a SOX3 mutation and Sox3 null XY mice are males, indicating that SOX3 is dispensable for testis determination (Laumonnier et al., 2002; Weiss et al., 2003; Solomon et al., 2004). However, SOX3 is necessary for later testis differentiation, as Sox3 null mutant mice show reduced testis weight, Sertoli cell vacuolization, loss of germ cells, reduced sperm counts, and disruption of the seminiferous tubules (Weiss et al., 2003). On the other hand, ectopic expression of Sox3 in the murine bipotential gonad causes complete XX male sex reversal, and three human XX males have been described with rearrangements within the SOX3 regulatory region. Furthermore, SOX3 is able to up-regulate Sox9 expression by binding to a conserved element in its promoter in a fashion similar to that of SRY (Sutton et al., 2011). Taken together, these data are consistent with the notion that SRY evolved from SOX3 through a regulatory mutation that led to SRY expression in the bipotential XY gonad.

**DAX1**

Human XY individuals carrying a duplication of a 160 Kb region on the short arm of the X chromosome exhibit gonadal dysgenesis and either female or ambiguous external genitalia, a situation known as dosage-sensitive sex reversal (DSS; Bardoni et al., 1994). The gene DAX1 (DSS-AHC critical region on the X chromosome), also known as NR0B1 (nuclear receptor
subfamily type 0, group B, gene 1), was identified in this chromosomal region at Xp21.2 (Muscatelli et al., 1994; Zanaria et al., 1994). Mutations in DAX1 result in an X-linked syndrome known as adrenal hypoplasia congenita (AHC), which causes adrenal insufficiency and hypogonadotropic hypogonadism in XY individuals (Zanaria et al., 1994). Like Sf1, this gene is expressed in several cell types throughout the hypothalamic-pituitary-gonadal axis (Ikeda et al., 1996). In the mouse gonad, Dax1 expression appears in the gonadal primordia of both sexes shortly after Sf1 expression, increases in Sertoli cells of the XY gonads at 12.5 dpc but decreases rapidly, to increase again in the interstitial cells between 13.5 and 14.5 dpc, when it decreases again (Ikeda et al., 2001). In vitro studies have shown that Dax1 transcription is activated by β-catenin, the effector protein of the WNT4 pathway in the XX gonad (see below), acting in synergy with SF1 (Mizusaki et al., 2003). Although several findings suggested that DAX1 would antagonize SF1 function in both steroidogenesis and gonadogenesis (Nachtigal et al., 1998; Tremblay and Viger, 2001), other studies have shown a cooperation between these two proteins to activate testis-promoting genes (Park et al., 2005).

The function of DAX1 has been highly controversial. Data from both DSS patients (Bardoni et al., 1994) and transgenic mice over-expressing DAX1 (Swain et al., 1998) suggested an anti-testis role for this gene (Jiménez et al., 1996; Jiménez and Burgos, 1998). In contrast, other studies suggested that this gene is necessary for testis development, as XY mice hemizygous for a Dax1 null allele had abnormal testes, whereas Dax1 homozygous null XX females were normal and fertile (Yu et al., 1998). The fact that both high and low levels of Dax1 expression have similar effects on testis development indicate that this gene must be tightly regulated during gonad development as its protein levels must be maintained between two precise thresholds (Ludbrook and Harley, 2004). No significant advances have been made in recent years in our knowledge on the involvement of DAX1 in gonad development, so its precise role in this process remains elusive.

**PDGFRα**

Platelet derived growth factors (PDGFS) were first identified as serum factors that stimulate the proliferation of myoid cells of arteries (Ross et al., 1978), but they also have an essential role in other biological processes, such as cell proliferation, cell survival, cell migration and extracellular matrix deposition. This family consists of four ligands, PDGFA, PDGFB, PDGFC and PDGFD, which can form different combinations of homo- and heterodimers that bind to one of the PDGF receptors: PDGFRα or PDGFRβ. Generally, PDGF ligands are synthesized by epithelial or endothelial cells, while the receptors are usually found in mesenchymal cells. The proliferation and migration of mesenchymal cells in response to these factors contributes to the morphogenesis and integrity of various organs (Hoch and Soriano, 2003).

**PDGFRα** encodes a receptor that can bind to PDGFA, PDGFB and PDGFC homodimers, and to PDGFA-PDGFB heterodimers. This receptor is essential for the proliferation of mesenchymal cells during the early development of the intestine, skin, lungs, kidneys and gonads (Karlsson et al., 1999, 2000; Sun et al., 2000; Li and Hoyle, 2001; Brennan et al., 2003). In all these cases, PDGFRα is expressed in the mesenchyme and its function is activated by local paracrine signals (Hoch and Soriano, 2003).

It has long been known that both PDGFRs and PDGFs are involved in testicular development (Gnassi et al., 1995). Mutant mice lacking Pdgfa show very small testes with few adult Leydig cells and spermatogenic arrest one month after birth (Gnassi et al., 2000). It has also been shown that PDGFA and PDGFB homodimers and heterodimers are involved in mesonephric cell migration into the XY gonad (Brennan et al., 2003; Ricci et al., 2004). During mouse gonadogenesis, PDGFRα is expressed in the gonadal primordia of both sexes at 11.5 dpc, and its expression increases rapidly in the interstitial cells of XY gonads and decreases in XX gonads at 12.5dpc (Brennan et al., 2003; Ricci et al., 2004). Mice homozygous for a null allele of Pdgfra die after the sex determination stage, making it possible to analyze the effect of this gene on early gonadal development. XY mutant embryos show a high disorganization of the gonadal vasculature (Brennan et al., 2003). Coelomic blood vessels are irregular and have few branches. In addition, testicular development is delayed and there is an abnormal development of testicular cords and interstitial cells, including Leydig and peritubular myoid cells. These observations indicate that PDGFRα is necessary for cell migration from the mesonephros and thus for the development of the vasculature and the interstitial compartment of the testis. Nevertheless, during migration, Pdgfra expression is only detected in the cells of the gonad but not in mesonephric cells. This suggests that PDGF signals do not directly induce cell migration, although they are necessary as second messengers (Brennan et al., 2003). These authors also suggested that PDGFRα may act in parallel with DHH (desert hedgehog) in the induction of Leydig cell differentiation.

**DMRT1**

The DMRT1 gene (DSX and MAB3 Related Transcription Factor 1) is involved in sex determination and gonadal development of several metazoan phyla. This was the first case described of a sex regulatory gene conserved in both vertebrates and invertebrates (Vollh et al., 2003; Zarkower, 2001). DMRT proteins are transcription factors sharing a DNA binding domain, similar to a zinc finger, called the DM domain, which was first identified in the proteins MAB3 of *C. elegans*
and doublesex (DSX) of *D. melanogaster* (Raymond et al., 1998). Several subtelomeric deletions in the p arm of the human chromosome 9 have been associated with sex reversal and gonadal dysgenesis in XY individuals. The smallest chromosomal region capable of causing sex reversal in this chromosome was referred to as TD9 (Hoo et al., 1989). *DMRT1* was identified in this region (Raymond et al., 1998, 1999b) and, despite the fact that no mutations in this gene were described, it was suggested to have a role in sex determination only because it was shared by all of these deletions (Ottolenghi and McElreavey, 2000; Raymond et al., 1999b).

The human *DMRT1* is located in band 9p24.3 (Raymond et al., 1998, 1999b) and it is part of a group of *DMRT* genes (*DMRT1, DMRT2, DMRT3*) conserved in mouse, fish and birds (Brunner et al., 2001; Kim et al., 2003; Nanda et al., 1999, 2000; Ottolenghi et al., 2002; Smith et al., 2002).

Expression of *DMRT1* has been detected in the human undifferentiated XY gonadal primordium at the sixth week of gestation. At the seventh week, when testicular differentiation has begun, its expression is located in the testicular cords, probably in the pre-Sertoli cells. No expression of *DMRT1* has been found at any stage of female embryonic gonad development (Moniot et al., 2000). However, in mice *Dmrt1* is expressed in the gonadal primordia of both sexes at 10.5 dpc, and at 12.5 dpc its expression decreases until it disappears in the ovary and increases in the testis, maintaining high levels of expression until adulthood within Sertoli cells and premeiotic germ cells (Raymond et al., 1999a, 2000; De Grandi et al., 2000). Unexpectedly, *Dmrt1-/-* mutant mice do not show abnormalities in sex determination and gonadal development in the embryo, suggesting that this gene has no relevant role in the early stages of gonadogenesis. Nevertheless, major defects were observed in testicular differentiation after birth. Sertoli cells failed to complete their differentiation and proliferated more than usual, filling the testicular cords. These immature Sertoli cells die, causing a high disorganization of the testis with few seminiferous tubules in the adult. Germ cells, in turn, did not migrate to the periphery of the seminiferous tubules and died shortly after 7dpp. These data indicate a critical role of DMRT1 in post-natal testicular development (Raymond et al., 2000). Accordingly, studies carried out in the mole species *Talpa occidentalis* suggest that intratesticular levels of testosterone could regulate circannual spermatogenic variations of seasonal breeders by modulating the expression of *DMRT1* to control spermatogonial proliferation (Dadhich et al., 2010).

**AMH**

The observation that Müllerian ducts regressed in XY fetuses led Jost (1953) to propose the existence of a second fetal hormone different from testosterone, which was called AMH (Anti-Müllerian hormone) or MIS (Müllerian-inhibiting Substance). Furthermore, freemartinism suggested that this hormone should also have a role in sexual differentiation. Freemartins are masculinized XX cattle that have developed together with XY twins. It was suggested that this phenotype could be due to the transfer of AMH from the XY fetus to the female mate through placental anastomosis. Freemartin animals lacked Müllerian ducts and showed masculinized ovaries, which contained structures resembling testicular cords and a very small number of oocytes (Jost et al., 1975). Later, once Cate et al. (1986) had cloned and sequenced the bovine and human AMH gene, Jost’s suggestion was confirmed by Vigier et al. (1987) by culturing fetal rat ovaries exposed to bovine AMH, and by Behringer et al. (1990) using transgenic mice with AMH over-expression.

The human *AMH* gene has five exons (Cate et al., 1986) and is located at the 19p13.3 band (Cohen-Haguenauer et al., 1987). Human patients with missense mutations in the *AMH* gene showed persistent Müllerian duct syndrome (Knebelmann et al., 1991). Human *AMH* is expressed in the early gonad of both sexes at the time of sex determination, but shows a male-specific expression at the seventh week of gestation, when Sertoli cells differentiate and testicular cords are formed. During this period, AMH protein is located in the cytoplasm of Sertoli cells, being one of the first detected Sertolian markers. AMH secretion causes the irreversible regression of the Müllerian ducts, which is completed by the ninth week of human gestation. High levels of AMH are maintained until puberty, when they decrease (De Santa Barbara et al., 2000; Taguchi et al., 1984).

In the mouse, expression of *Amh* has not been observed in the undifferentiated gonadal primordium. In the male, expression starts at 12.5 dpc in Sertoli cells, when testicular differentiation has begun. As in humans, this expression continues throughout testicular development until puberty, coinciding with the first round of spermatogonia entering meiosis. In female mice, *Amh* expression was not found during ovarian development, but after 6 dpp there were low transcript levels in follicle cells throughout the entire fertile period, although the meaning of this expression is still unknown (Münsterberg and Lovell-Badge, 1991). Since high levels of AMH in the female gonad are coincident with a decrease in the number of oocytes and these levels decrease in the testis when spermatogonia enter meiosis, McLaren (1990) proposed that AMH could be cytotoxic for meiotic germ cells. In addition, it has been proposed that androgens may contribute to decrease AMH expression at puberty (Al-Attar et al., 1997).

Many studies have focused on the regulation of *AMH* expression. SF1 is known to be directly involved in the activation of the promoter of this gene (Giuli et al., 1997; Shen et al., 1994), in cooperation with other factors such as WT1, SOX9, GATA4 and SOX8 (De
Santa Barbara et al., 1998; Nachtigal et al., 1998; Arango et al., 1999; Tremblay and Viger, 1999; Watanabe et al., 2000; Schepers et al., 2003; Chaboissier et al., 2004). In vitro experiments suggested that DAX1 could repress these interactions (Nachtigal et al., 1998; Tremblay and Viger, 2001), although further studies have questioned this hypothesis, suggesting that DAX1 might even be necessary for normal AMH expression (Park et al., 2005). FOG2 is responsible for the inhibition of AMH transcription during ovarian development in females and at puberty in males (Tremblay et al., 2001; Anttonen et al., 2003).

Neurotropins

Neurotropins, such as neurotropin-3, 4 and 5 (NT3, NT4, NT5), brain-derived growth factor (BDGF) and nerve growth factor (NGF) and their receptors, either the p75 low affinity neurotrophin receptor (p75NTR) or the cell membrane tyrosine kinase receptors (trk A, B and C receptors) not only promote neuronal survival and differentiation, but also target non-neuronal cells and are involved in mesenchymal-epithelial cell interactions. The human NGF gene maps to the 1p13 band (Middleton-Price et al., 1987; Garson et al., 1987). NGF, together with NT3, alters the expression of locally produced growth factors such as TGFA and TGF8 (transforming growth factors A and B) by stimulating postnatal testicular growth. NT3, NGF and their receptors are expressed in cells essential for seminiferous cord formation and are important regulators of testis morphogenesis (Cupp et al., 2000) and spermatogenesis (Parvinen et al., 1992).

In the developing rat testis, the low-affinity receptor for neurotropins, p75NTR, is expressed at the mesonephros prior to seminiferous cord formation and later in the peritubular myoid cells until birth (Levine et al., 2000), whereas NGF and NT3 were mainly expressed in Sertoli and interstitial cells (Robinson et al., 2003). Treating human embryonic testis organ cultures with the Trk-specific kinase inhibitor K252a, these authors showed that neurotropins are involved in the progression of male sex differentiation and are critical for the induction of embryonic testis cord formation, having roles in the regulation of proliferation and survival of germ and peritubular cells. Consistently, Cupp et al. (2002) showed that both trkA and trkC neurotropin receptors influence germ cell population size and seminiferous cord formation during testis development. NT3 also promotes mesonephric cell migration into the gonad, suggesting that the developing Sertoli cells express NT3 as a chemoattractant for mesonephric cells, which express the high-affinity trkC receptor (Cupp et al., 2003). It can be concluded that neurotropins and their receptors are regulators of paracrine cell-cell interactions necessary for testis organogenesis and perinatal testis growth.

Ovary-promoting genes and aberrant testis differentiation

RSPO1

R-spondins constitute a small family of secreted proteins (RSPO1-4), which are ligands for canonical WNT signaling (Kamata et al., 2004; Nam et al., 2006). Mutations of the human RSPO1 gene, which is located on 1p34.3, have been associated with palmoplantar hyperkeratosis, squamous skin cell carcinoma and female-to-male sex reversal (Parma et al., 2006). This was the first gene known to cause this particular type of sexual disorder in the absence of SRY and, contrary to the classical view of the ovary as the default pathway in gonad differentiation, evidenced the existence of ovary-promoting genes which, as in the case of the testis, are responsible for the activation of the ovarian differentiation pathway (Capel, 2006; Wilhelm et al., 2007; Chassot et al., 2008a; DiNapoli and Capel, 2008).

Whereas Rspo1 over-expression in XY mice appears not to disturb testis development (Buscara et al., 2009), its absence in null mutant XX mice partially reproduces the human XX sex reversal described by Camerino and colleagues (Parma et al., 2006). XX Rspo1-/- mice have masculinized external genitalia, testosterone-producing ovaries which contain a coelomic vessel and persisting Wolffian ducts, but do not exhibit complete sex reversal (Chassot et al., 2008a; Tomizuka et al., 2008). Interestingly, the phenotype of these Rspo1-/- XX mutant mice recapitulated that previously described for Wnt4 -/- XX mice (see below), suggesting that the function of Wnt4 and other genes under its control was impaired in Rspo1-/- XX gonads, and thus that RSPO1 could suppress the male pathway in the absence of Sry by activating WNT4 signaling. Although the relationship between RSPO1 and WNT4 proteins in the activation of the ovarian pathway is not completely understood, it is currently assumed that they both cooperate to regulate the intracellular status of β-catenin (see below). Overall, available data suggest that RSPO1 is in fact the factor that tips the balance towards the female pathway during sex differentiation in XX mammals (Capel, 2006).

WNT4

The wingless-related MMTV integration site (WNT) proteins constitute a family of signaling molecules involved in multiple functions during development (see Huang and He, 2008, for a review). Duplications of distal 1p chromosomal region, where WNT4 is located (1p36.12), were found in patients who exhibited diverse sexual anomalies, from cryptorchidism to male-to-female sex reversal (Elejalde et al., 1984; Jordan et al., 2001), although those duplications do not always include the WNT4 locus (Wieacker and Volleth, 2007). The opposite phenotype, i.e. female-to-male sex reversal, resulted from homozygosity for a WNT4 loss-of-function missense mutation in 46,XX patients with SERKAL
syndrome, which consists of multiple anomalies, including ambiguous genitalia and gonads presenting as ovotestes or ovaries (Mandel et al., 2008). An intermediate defect of sexual development with absence of Müllerian structures and androgen excess was observed in women with heterozygous WNT4 mutations (Biasson-Lauber et al., 2004). The correlation of WNT4 activity levels with sexual phenotype is a prominent example of the general view that sex differentiation is a matter of dosage (for review, see Biasson-Lauber and Konrad, 2008).

During mouse gonadogenesis, Wnt4 is expressed in mesonephric mesenchymal cells before the formation of the genital ridge. Then, expression also appears in the somatic cells of the gonadal primordia of both sexes until 11.5 dpc. At later stages, Wnt4 expression increases in the XX and decreases in the XY gonad and is also observed in the Müllerian ducts (Vainio et al., 1999). Gonadal expression of Wnt4 could be regulated by the KTS isoforms of WT1 (Sim et al., 2002) and some members of the p53 tumor suppressor gene family (Osada et al., 2006). Also, Wnt4 could be negatively regulated by SOX9 as the gonads of XY mice where Sox9 was conditionally ablated showed Wnt4 expression (Barrionuevo et al., 2006), and the ectopic expression of Sox9 in XX gonads induces Wnt4 down-regulation (Qin and Bishop, 2005).

To investigate the function of Wnt4 in ovarian development, homozygous null mutants (Vainio et al., 1999) and XY transgenic mice over-expressing this gene (Jeays-Ward et al., 2003; Jordan et al., 2003) were studied. XX, Wnt4 -/- mouse die shortly after birth due to renal insufficiency and show several defects in ovarian development. Their sex ducts appeared clearly masculinized with complete regression of the Müllerian ducts and continued development of the Wolffian ducts. In the gonads of these animals most oocytes degenerate (Vainio et al., 1999) and expression of typical testicular markers like Sox9 and Fgf9 was detected (Kim et al., 2006). Since Wnt4 expression is up-regulated in the gonads of mice homozygous for null mutations of either Sox9 (Barrionuevo et al., 2006) or Fgf9 (Kim et al., 2006), it was hypothesized that both WNT4 and FGF9 act as antagonistic signals during the process of sex determination (Kim et al., 2006; Kim and Capel, 2006). Moreover, mice over-expressing Wnt4 showed low levels of serum testosterone and abnormal development of the testicular vasculature, but did not undergo complete sex-reversal. According to these data, the role of WNT4 in the XX gonad could be to inhibit important testis-specific processes, including migration of endothelial cells from the mesonephros (Jeays-Ward et al., 2003) and steroidogenesis, either by repressing Sfl (Jordan et al., 2003) or by precluding the recruitment of steroidogenic cell precursors (Jeays-Ward et al., 2003). Furthermore, premature down-regulation of WNT4 in the gonads of female moles of the species Talpa occidentalis has been associated with ovotestis formation in these animals (Carmona et al., 2009a,b). Whereas these results make clear that WNT4 is a pro-ovary (or an anti-testis) gene, there are however additional data suggesting that it could also have a role in early steps of testis development (Jeays-Ward et al., 2004).

**CTNNB1**

The cadherin-associated protein beta 1 gene, CTNNB1, which encodes the β-catenin protein, is located on 3p21.1. Both in vitro (Binnerts et al., 2007; Wei et al., 2007; Kim et al., 2008) and in vivo gain- and loss-of-function experiments (Chang et al., 2008; Chassot et al., 2008b; Manuylov et al., 2008; Liu et al., 2009) have shown that β-catenin is the common effector of both RSPO1 and WNT4 signaling. Conclusive evidence that the canonical WNT signaling pathway is involved, which implies the stabilization of the intracellular regulator β-catenin, came from experiments with transgenic mice carrying a stable, undegradable form of β-catenin (Maatouk et al., 2008). These authors showed that constitutive stabilization of β-catenin in the somatic cells of the gonads of XY mice is sufficient to induce male-to-female sex reversal, as indicated by down-regulation of testis-specific gene markers such as Sox9 and Amh, and activation of ovary-specific markers such as Foxl2, Bmp2, Wnt4 and Fst, as well as the lack of testicular cords. Furthermore, selective mutation of the β-catenin gene in somatic cells of the gonads in XX mice led to the down-regulation of these ovary-promoting genes, but did not result in the activation of testis-specific ones, such as Sox9, Amh and Dhh, and hence, no gonadal sex reversal was produced (Manuylov et al., 2008).

**FST and BMP2**

These genes encode follistatin and the bone morphogenetic protein 2, respectively. FST maps to 5q11.2 and BMP2 is located on 20p12.3. They are both signaling molecules secreted into the gonadal interstitium and are expressed throughout ovarian development in the mouse (Yao et al., 2004). The study of Bmp2 function has been difficult, as mice homozygous for null mutations of this gene die prematurely during embryonic development. However, recent studies have shown that the BMP2 protein acts cooperatively with FOXL2 to ensure correct expression of Fst in the developing ovary (Kashimada et al., 2011). Whereas mice homozygous for a null allele of the Wnt4 gene show no Fst or Bmp2 expression, those with two mutant copies of Fst retain Wnt4 expression, although they exhibit a sexual phenotype very similar to that of Wnt4 mutants. These results indicate that both Fst and Bmp2 act downstream of Wnt4 in the ovarian development pathway (Menke and Page, 2002; Yao et al., 2004). Moreover, the gonads of XX mice lacking Fst function also lack Leydig cells, suggesting that in addition to activating Fst, Wnt4 could also inhibit Leydig cell differentiation. Hence, available data...
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indicate that Wnt4 is responsible for the initiation but not for the maintenance of Fst expression. Since the loss of either Wnt4 or Fst results in ectopic formation of a coelomic vessel in the fetal ovary (Vainio et al., 1999; Yao et al., 2004), it was thought that the main FST function is to inhibit mesonephric cell migration into the gonad, precluding in this way the formation of a testis-specific vasculature. This view is supported by the finding that FST inhibits the formation of a testis-like vasculature by antagonizing activin B, a member of the transforming growth factor beta (TGFβ) superfamily, which probably induces mesonephric cell migration in the testis (Yao et al., 2006).

**FOXL2**

The forkhead box gene L2, FOXL2, belongs to the forkhead box family of transcription factors (Fkh), which includes 44 members sharing a common DNA binding domain of up to 110 amino acids (Lehmann et al., 2003). Mutations in the FOXL2 gene, which is located on 3q22.3, are associated with two human syndromes: Blepharophimosis Potosis Epicanthus Inversus Syndrome (BPES; Crisponi et al., 2001) and Premature Ovarian Failure Syndrome (POF; De Baere et al., 2001). In the goat, disrupted expression of this gene has been detected in both granulosa and theca cells (Schmidt et al., 2001). In the mouse gonad, Foxl2 expression is limited to the ovary, where it begins at 12.5 dpc in somatic cells and continues during further stages, including the adult female gonad. Here, expression is detected in both granulosa and theca cells (Schmidt et al., 2004). XX Foxl2-/- mutant mice are infertile females (Schmidt et al., 2004; Uda et al., 2004) with defective ovaries in which primordial follicles contain granulosa cells unable to undergo the squamous-to-cuboidal transition, and thus cannot initiate proper follicle maturation. These follicles undergo subsequent atresia, leading to infertility due to progressive follicular depletion (Schmidt et al., 2004; Uda et al., 2004; Uhlenhaut and Treier, 2006). Hence, Foxl2 is necessary for correct follicle development and female fertility.

More recent studies in the mouse have shown that sustained Foxl2 expression is necessary for repressing genetic reprogramming of the postnatal ovarian somatic cells to testicular cell types, and thus for maintenance of the adult female phenotype (Uhlenhaut et al., 2009). Using the tamoxifen-inducible Cre/loxp system, these authors produced an ovarian-specific mutation of the Foxl2 gene, which resulted in the transdifferentiation of ovarian granulosa and theca cells into testicular Sertoli and Leydig cells, respectively. Accordingly, these gonads produce high levels of testosterone. This transdifferentiation process is accompanied by the activation of most testis-promoting genes, including Sox9, and the silencing of ovary-promoting genes like Rspo1 and Wnt4. The authors also showed that FOXL2 directly represses the testis-specific enhancer of Sox9, core sequence (TESCO; see Sekido and Lovell-Badge, 2008) through a synergistic interaction with estrogen receptors α and β (Uhlenhaut et al., 2009). Hence, currently available data suggest that Sox9 expression must be continuously repressed in XX mice and that this repression is exerted first by the Wnt signaling pathway during the embryonic stage and then by Foxl2/ERα-β throughout adulthood (Sinclair and Smith, 2009).

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