Pre-Sertoli Specific Gene Expression Profiling Reveals Differential Expression of Ppt1 and Brd3 Genes Within the Mouse Genital Ridge at the Time of Sex Determination

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ABSTRACT

In mammals, testis determination is initiated when the SRY gene is expressed in pre-Sertoli cells of the undifferentiated genital ridge. SRY directs the differentiation of these cells into Sertoli cells and initiates the testis differentiation pathway via currently ill-defined mechanisms. Because Sertoli cells are the first somatic cells to differentiate within the developing testis, it is likely that the signals for orchestrating testis determination are expressed within pre-Sertoli cells. We have previously generated a transgenic mouse line that expresses green fluorescent protein under the control of the pig SRY promoter, thus marking pre-Sertoli cells via fluorescence. We have now used suppression-subtractive hybridization (SSH) to construct a normalized cDNA library derived from fluorescence-activated cell sorting (FACS) purified pre-Sertoli cells taken from 12.0 to 12.5 days postcoitum (dpc) fetal transgenic mouse testes. A total of 35 candidate cDNAs for known genes were identified. Detection of Sf1, a gene known for its role in sex determination as well as Vanin1, Vcp1, Sparc, and Aldh3a1, four genes previously identified in differential screens as gene overexpressed in developing testis compared with ovary, support the biological validity of our experimental model. Whole-mount in situ hybridization was performed on the 35 candidate genes for qualitative differential expression between male and female genital ridges; six were upregulated in the testis and one was upregulated in the ovary. The expression pattern of two genes, Ppt1 and Brd3, were examined in further detail. We conclude that combining transgenically marked fluorescent cell populations with differential expression screening is useful for cell expression profiling in developmental systems such as sex determination and differentiation.

INTRODUCTION

The SRY gene was identified more than 14 yr ago and is considered the dominant positive genetic factor responsible for initiating testis development in eutherian mammals [1–3]. It is generally accepted that Sry triggers the differentiation of Sertoli cells from a cell population that would otherwise become follicle cells. Surprisingly, the molecular mechanisms by which SRY accomplishes this remain enigmatic. In the mouse, Sry expression within the genital ridge is first detected at 10.5 days postcoitum (dpc); expression peaks at 11.5 dpc and ends at 12.5 dpc [4, 5]. Lineage tracing experiments demonstrate that Sry is expressed within pre-Sertoli cells [6, 7]. Pre-Sertoli cells induce cell proliferation, vascularization, and testis cord formation within the developing testis [8, 9] as well as the migration of myoid, endothelial, and interstitial precursor cells into the gonads from the underlying mesonephros [10, 11]. Sertoli cells provide morphogenetic signaling to other cell types, inducing steroidogenic precursor cells to differentiate into Leydig cells, causing germ cells to enter mitotic arrest, and resulting in the elimination of paramesonephric duct cells via apoptosis.

Most of the genes currently known to play a role in the process of sex determination and differentiation have been identified from human and mouse models. Studying human patients showing sex reversal or dysgenesis of the gonads has revealed roles for genes such as Sry itself, and Sox9, Dmrt1, Dax1, Wt1 [1, 12–15]. Phenotypic anomalies in sex determination observed in knockout mouse models have further implicated genes such as M33, Lhx9, and Sfi1 [16–18]. One gene, Dmrt1, was identified via a combination of homology with Drosophila and Caenorhabditis elegans genes [19] as well as human dysgenesis of the gonads [20]. More recently, to identify additional candidate genes as well as signaling pathways implicated in these processes, differential expression-based screening approaches were undertaken by several labs [21–25]. Although procedures varied somewhat, all of the screens reported to date have in common the tissue level comparison of developing testis with developing ovaries at a given developmental stage.

To characterize in more detail the expression profile of pre-Sertoli cells at the beginning of sex differentiation as well as to identify new candidate genes involved in sex determination and differentiation, we used a transgenic mouse model expressing green fluorescent protein (GFP) under the control of 4.5 (kilobases) kb of the pig SRY 5' flanking region (pSRYp-GFP) [7] to isolate and purify pre-Sertoli cells via fluorescence. RNA from 12.0 to 12.5 dpc pre-Sertoli cells was subtracted against nonfluorescent cells from the male genital ridge using suppression subtraction hybridization (SSH). Gene sequencing followed by whole-mount in situ hybridization (WISH) on selected clones al-
lowed us to identify new candidate genes involved in mammalian sex determination and differentiation and to confirm the utility of using transgenically labeled cell populations for SSH applications in development.

MATERIALS AND METHODS

Transgenic Mice, Cell Collection, and RNA Isolation

The cloning and characterization of 4.5 kb of pig SRY S' flanking sequences and the generation of pSRyp-GFP transgenic mice have been described previously [7, 26]. Mice were housed and handled according to guidelines established by the Guide for Care and Use of Laboratory Animals (copyright 1996, National Academy of Science) and according to the standards of the institution, and protocols were approved by the institutional ethics committee on use of animals in research. For staging of embryonic development, noon on the day of vaginal plug detection was designated as Day 0.5 postcoitum (0.5 dpc). To isolate male genital ridge cells from 12.0–12.5 dpc embryos, timed matings were performed. Genital ridges were dissected, male ridges were identified via fluorescence, and mesonephric removed. The male genital ridges were then pooled and digested for 30 min at 37°C in M2 media (Sigma, St. Louis, MO) supplemented with collagenase (50 U ml−1) (Gibco, Canadian Life Technologies, Burlington, ON, Canada) and dispase (2.4 U ml−1) (Gibco). Fluorescent cells were separated from male and nonfluorescent cells (remainder of genital ridge) were separated using fluorescent-activated cell sorting (FACS) isolation on a FACSTAR-Plus machine (Becton Dickinson, Mountain View, CA) equipped with an EGFP filter. In total 21 400 fluorescent cells and 22 000 nonfluorescent cells were recovered. Total RNA was extracted using RNeasy total RNA extraction kit (Qiagen, Misssissauga, ON, Canada). The concentration of total RNA was quantified by measurement of optical density at 260 nm and was deemed sufficient to continue with the procedure (data not shown).

Suppression Subtractive Hybridization (SSH)

The SSH procedure was performed essentially as previously described [27], with changes appropriate to the mouse model system as described herein. SSH was used to compare gene expression between fluorescent and nonfluorescent cells of 12.0–12.5 dpc pSRyp-GFP transgenic mice testis. To generate sufficient amounts of double-stranded cDNA for SSH, fluorescent and nonfluorescent cDNAs were amplified separately using the SMART polymerase chain reaction (PCR) cDNA synthesis kit according to the manufacturer’s instructions (BD Biosciences Clontech, Mississauga, ON, Canada). To generate the first strand cDNA, 0.3 μg of total RNA from fluorescent and nonfluorescent cells was reverse-transcribed with an oligo-dT30 primer (CDS: 5'-9

[57x171]struct the fluorescent minus nonfluorescent (F-NF) subtracted cDNA li-

[57x185]were cloned into the pT-Adv plasmid (BD Biosciences Clontech), to con-

[57x306]were further characterized by DNA sequencing and WISH.

DNA Sequencing and Sequence Analysis

The cDNA clones identified as differentially expressed by the (F-NF) cell cDNA probe were amplified by PCR for 15 cycles with the PCR-nested 1 and PCR-nested 2 oligos from the PCR product generated initially (QiAquick PCR purification kit; Qiagen) and verified by agarose gel analysis for the presence of a single band. The cDNA clones were then sequenced using the oligo PCR-Nested 1 and PCR-nested 2 oligos from the PCR product generated initially (Qiagen) and verified by agarose gel analysis for the presence of a single band. Sequencing reactions were performed on cDNA clones via the dyeoxy sequencing method (Big dye terminator 3.0; ABI prism; Applied Biosystems, PE, Branchburg, NJ) using the oligo PCR-Nested 1 (1 mM). Sequencing reactions were analyzed on ABI Prism 3100 sequencer (Applied Biosystems). Nucleic acid sequences were analyzed by BLAST (Basic Local Alignment Search Tool) against GenBank data banks (NR, EST, and mouse genome). A cDNA sequence was considered homologous to a GenBank sequence when at least 100 bp matched with an E probability value of less than e−10.

Whole-Mount In Situ Hybridizations

The cDNA clones of the known genes that were differentially expressed in the SSH experiment were further characterized via WISH. To
FIG. 1. Abundance of specific cDNA transcripts in subtracted versus nonsubtracted cDNA pools of fluorescent (pre-Sertoli) cell cDNAs as estimated by PCR analysis. Subtraction was performed using cDNA from nonfluorescent genital ridge cells. (A) Reduction in the abundance of Gapdh transcript in subtracted compared with nonsubtracted fluorescent cell cDNA pools (compare cycles 15); (B) enrichment of Sox9 transcript within the subtracted cDNA pool (compare cycles 21); (C) enrichment of GFP transcript within the subtracted cDNA pool (compare cycles 15). Mw, Molecular weight markers.

FIG. 2. Example of differential screening results by macro arrays of the fluorescent subtracted by nonfluorescent (F-NF) cell cDNA library using three different cDNA probes as follows: (A) fluorescent cell subtracted by nonfluorescent cell cDNA probe; (B) fluorescent cell (unsubtracted) cDNA probe; and (C) nonfluorescent cell (unsubtracted) cDNA probe. Controls include sequences for Gapdh (1) and for GFP (2). Arrows indicate the position of clone representing Sf1 sequences.

provide a standardized method for generating linear template DNA, clones were amplified by PCR using SP6-PCR-Nested1 (5′-TAATACGACTCACTATAGGGACGCTGCTCGGGCCGAGGT-3′) and T7-PCR-Nested2 (5′-ATTATAGTACACTATAGAATCGAGCGGCCGCCGAGGTA-3′). The PCR products were used as templates to generate digoxigenin-labeled antisense riboprobes for WISH as previously described [28]. The PCR products were transcribed using Sp6 or T7 RNA polymerase as appropriate. Expression was analyzed in 11.5, 12.5, 13.5, and 14.5 dpc gonads, using two pairs of embryos of each sex per gene.

Depletion of Germ Cells by Busulfan

Two cDNA sequences were selected for an expression analysis in embryos lacking germ cells. These embryos were generated by injecting pregnant females with 40 mg/kg busulfan at 9.5 dpc and then harvesting embryos at 12.5–13.5 dpc [29]. As a control for this experiment, expression of the Chk1 gene, known to be expressed in germ cells but not in pre-Sertoli cells, was verified in germ cell-depleted embryos.

RESULTS

Isolation of Pre-Sertoli Cells

The pSRYp-GFP-positive embryos from timed matings were dissected at 12.0–12.5 dpc and fluorescent (pre-Sertoli) cells and nonfluorescent cells from the genital ridge were efficiently separated via FACS purification, with an efficiency of 98%. Reverse transcription-polymerase chain reaction (RT-PCR) was performed on RNA from fluorescent cells to reveal the presence of endogenous Sry transcripts and the absence of Oct4 transcripts (data not shown), consistent with the fluorescent cells being of pre-Sertoli cell lineage and not of germ cell lineage as previously shown [7].

Production of Subtracted Libraries and Assessment of Normalization and Subtraction Efficiencies

To verify the efficiency of the SSH procedure, several control experiments were performed. To ensure that normalization of cDNAs had occurred, the quantity of the housekeeping gene Gapdh was assessed in subtracted (F-NF) and unsubtracted (F) cDNA pools. As expected, Gapdh abundance was decreased following subtraction (Fig. 1A). To assess the efficiency of enrichment of differentially expressed genes, the abundance of transcripts for GFP and Sox9 (pre-Sertoli cell expressed gene) was examined. Both GFP and Sox9 transcripts were enriched in the pre-Sertoli cell subtracted (F-NF) cDNA pools (Fig. 1, B and C). Interestingly, GFP but not Sox9 transcripts were still visible in PCR cycles taken from the unsubtracted cDNA pool. This can be explained by the PCR cycling conditions chosen and possibly also reflects relative differences in concentrations of message at the start of the procedure.

Once the subtraction efficiency was shown to be satis-
factory, a differential hybridization screening procedure was performed on 658 bacterial colonies randomly selected from the (F-NF) subtracted cDNA library. The (F-NF) subtracted cDNA pool as well as (F) and (NF) nonsubtracted cDNA pools were used to generate three different hybridization probes for the differential screening of the selected clones spotted on three identical sets of microarrays. Representative differential screening results are illustrated in Figure 2. The differential screening procedure identified 154 cDNA clones as true positives, i.e., showing enhanced expression via the (F-NF) probe compared with the (F) probe. One hundred ten of these clones were analyzed by sequencing and 90 generated adequate sequencing results. Comparison of the cDNA sequences obtained with GenBank sequence databases revealed that 38.8% (35/90) corresponded to 32 known genes (taking into account redundancy of clones), 16.6% (15/90) corresponded to GFP, and 44.4% (40/90) of the sequenced clones corresponded to novel genes (analysis to be presented elsewhere). Only six sequences were identified more than once, except for GFP.

| Function, gene symbol* | Gene name | GenBank | UniGene cluster (Mm) | Expression 12.5 dpc (testes, ovaries, both, n.d.)² |
|------------------------|-----------|---------|---------------------|--------------------------------------------------|
| Cellular cycle and growth | Cdc91L1 | Cdc91 cell division cycle 91-like 1 XM130696 | 5434 | n.d. |
| Mrgx | Mrgx pending NM019768 | 27 218 | Both |
| Sept2 | Septin 2 NM010891 | 242 324 | Both |
| Intracellular transport | Abcd3 | ATP binding cassette family member D3 NM008991 | 194 462 | n.d. |
| Dctn4 | Dynactin 4 NM026302 | 256 520 | Both |
| Myo10 | Myosin X NM019472 | 60 590 | Both |
| Actn4 | Actinin alpha 4 NM021895 | 276 042 | Both |
| Vcp | Valosin-containing protein NM009503 | 262 053 | Both |
| Pex14 | Peroxisomal biogenesis factor 14 NM019781 | 184 172 | Both |
| Mtap1A | Microtubule-associated protein 1 A XM194040 | 227 093 | Both |
| Transport | Cfr (n = 2) | ATP binding cassette subfamily C NM021050 | 15 621 | Both |
| Transcription | Sf1 | Stereodogenic factor-1 NM139051 | 31 387 | Testes (cords) |
| Brd3 | Bromodomain-containing 3 NM023336 | 28 721 | Overexpressed in testes (cords) |
| Enzymes | Ldh2 (n = 2) | Lactate dehydrogenase 2 NM008492 | 9745 | Both |
| Siat1 | Sialyltransferase 1 NM145933 | 213 222 | Both |
| Fdr | Ferredoxin reductase NM007997 | 4719 | Both |
| Ppt1 (n = 2) | Palmytoil-protein thioesterase NM008917 | 207 339 | Testes, Cords |
| Gatm | Glycine amidinotransferase NM025961 | 29 975 | Both |
| Tnx1 | Threonine-like NM016792 | 19 169 | Both |
| Vnn1 | Vanin 1 NM011704 | 27 154 | Overexpressed in testes (cords) |
| Kars | lysI-7Rn synthetase NM053092 | 196 544 | Both |
| Hmgcs2 | 3-hydroxy-3-methylglutaryl-Coenzyme A synthase 2 NM008256 | 289 131 | Both |
| Aldh3a1 | Aldehyde dehydrogenase family 3, subfamily A1 NM007436 | 4257 | Both |
| Ctsb | Cathepsin B NM007798 | 236 553 | Both |
| Gus | Beta-glucuronidase NM010368 | 3317 | Both |
| Sarl | IQ motif containing GTPase activating protein NM080476 | 207 619 | n.d. |
| Signal transduction | Rgs2 | Regulator of G-protein signaling 2 NM009061 | 28 262 | Ovaries |
| Pptn 1 | Tyrosine phosphatase non-receptor type 1 NM011201 | 277 916 | Both |
| Sel1L | Sel-1 suppressor of lin-12-like XM127076 | 250 605 | Testes (cords, interstitium) |
| Structure | Catna | Catenin alpha 1 NM009818 | 18 962 | Overexpressed in testes (cords) |
| Cgn | Cingulin NM131052 | 87 634 | Both |
| Sparc | Secreted acidic cysteine rich glycoprotein NM009242 | 291 442 | Overexpressed in testes (cords, interstitium) |

* Genes detected twice by SSH are represented by (n = 2).
² Genes detected by SSH but not by WISH are indicted by n.d.
which was identified 15 times. Among the known expressed genes identified was Sf1, a gene expressed in the genital ridge and implicated in sex determination/differentiation. In addition, Vanin-1, Vcp1, Sparc, and Aldh3a1 were identified, representing genes that were previously identified in screens of fetal testes versus ovaries [21, 22, 25].

**Analysis of mRNA Expression**

To verify that genes identified by SSH are differentially expressed between pre-Sertoli cells and the Leydig/endothelial and germ cell compartments and to test whether these genes are also differentially expressed between male and female embryos, WISH was performed on male and female embryos. Initial screening was carried out using 12.5-dpc embryos of both sexes for the 32 known genes. Most of these genes (23) were expressed in both sexes; 5 genes were overexpressed in testis; 1 gene was overexpressed in ovary; and no expression was detected for 3 genes (Table 1). Representative examples of WISH results are shown in Figure 3 for the genes Catna, Sparc, Fdxr, Hmgcs2, and Rgs2. For the clones showing sex-specific or overexpression patterns in gonads of one sex, additional in situ experiments were performed at 11.5, 12.5, 13.5, and 14.5 dpc. We present here the expression patterns for Bromodomain-containing 3 (Brd3) (Fig. 4, A–D) and Palmitoyl-protein thioesterase 1 (Ppt1) (Fig. 4, E–H). At the detection level of the WISH procedure, Brd3 is ubiquitously expressed but is more strongly expressed in the testis compared with the ovary (see Fig. 4B) and to other tissues (data not shown). In contrast, Ppt1 was only detected within the developing testis. To further confirm that these genes are expressed in the pre-Sertoli cell population and not within germ cells, WISH was performed on embryos lacking germ cells after treating pregnant females.
with busulfan (Fig. 5). Expression of Brd3 and Ppt1 was not diminished within busulfan-treated compared with nontreated testes.

DISCUSSION

In mammals, Sertoli cells are thought to be the first cell lines of the genital ridge to commit to the male differentiation pathway and additionally to recruit other cell types to the male pathway via paracrine and contact signaling. Characterization of the specific subset of genes differentially expressed in pre-Sertoli cells compared with the other cell types present within the genital ridge at the moment of sex determination should provide further understanding of the molecular processes involved in sex determination and sex differentiation. To further study these processes, a mouse transgenic line expressing GFP under the control of 4.5 kb of pig Sry promoter [7] was used to isolate pre-Sertoli cells at 12.0–12.5 dpc. These cells were purified by FACS, and a SSH screen was performed to identify genes that were differentially expressed in fluorescent cells (F) compared with nonfluorescent cells (NF).

Screening the (F-NF) subtracted cDNA library by membrane hybridization allowed the identification of 32 known gene candidates, the analysis of which is presented herein. These genes included Sry, known for its role in sex determination/differentiation, as well as Vanin-1, Sparc, Vcp1, and Aldh3a1, four genes previously identified in published screens for genes overexpressed in developing testis compared with ovary [21, 22, 25]. Furthermore, we confirmed by RT-PCR that Sry is expressed in fluorescent cells and that Sox9 is overexpressed within the (F-NF) subtracted cDNA pool compared with the (F) nonsubtracted cDNA pool. The identification of these genes provides an important confirmation of the biological validity of our current model. Other genes known to be implicated in sex determination, such as Wt1, Dax1, Lhx9, or Gata4 were not identified in our current hybridization screen, and although Sry and Sox9 were subsequently identified by RT-PCR, they were not identified within the hybridization screen. This is possibly due to the relatively small sample size of clones screened as well as the potentially large complexity and low redundancy of the cDNA population of the pre-Sertoli cell. These observations are consistent with the findings of other reported genital ridge differential screens [21, 22, 25] but are in contrast with expression profiling experiments reported for other tissues such as granulosa cells of the developing bovine follicle [27].

The known genes identified by SSH were further characterized via WISH to determine differential expression patterns within the developing testis and also between developing testis and ovary. A high proportion of the genes tested by WISH (21/32) were expressed in both developing testis and ovary. This is not surprising due to the common origin of Sertoli and granulosa cells [6] and the fact that our subtraction was not between testicular and ovarian origin of Sertoli and granulosa cells [6] and the fact that no WISH signal detected could be explained by the moderate sensitivity of the WISH method or could simply be due to technical failure.

For the six genes showing a differential expression between testes and ovaries, Sry, Vanin-1, and Ppt1 were only expressed in pre-Sertoli cells, Brd3 and SelLL were more strongly expressed in pre-Sertoli cells compared with in-
terstitial cells, while Catna1 and Sparc were expressed equally in pre-Sertoli cells and interstitial cells. Two genes, Fdxr and Hmgcs2, were expressed in both testicular and ovarian genital ridges. One gene, Rgs2, was found to be overexpressed in ovaries compared with testes at 12.5 dpc but on closer examination was observed to be downregulated in ovaries at 13.5 dpc, underscoring the fact that sex differentiation occurs later in ovaries than in testis.

Ppt1 and Brd3 were selected for more detailed characterization of genital ridge expression. Ppt1 encodes a soluble lysosomal enzyme that hydrolyzes the thioester bond that attaches long-chain fatty acids, mostly palmitate, to the cysteine residue of S-acylated proteins [32, 33]. Palmitoylation has been shown to be important in different cellular processes, including neural cell differentiation (review: [34]) and possibly in insulin-regulated cellular functions [35]. Recently, nonlysosomal roles for Ppt1 were described in synaptosomes [36] and during apoptosis [37]. It was previously reported that Ppt1 is ubiquitously expressed in adult tissue, with increased expression within the testis [32, 38], while in Drosophila, Ppt1 shows testicular expression levels five time that of other tissues [39]. Here, we report that, within the mouse genital ridge, Ppt1 was only expressed in pre-Sertoli cells of the developing testis via SSH and WISH but was not seen in developing ovary via WISH. These observations suggest that Ppt1 may be involved in mammalian sex determination and differentiation.

Brd3 is a potential transcription factor containing two bromodomains. Members of this family include Brd2, a gene that is strongly expressed in ovary and testis (both Sertoli and germ cells) [40] and BrdT [41], a gene only expressed in the germ cells of the testis. Significantly, this family of genes is related to the female sterile homeotic protein gene in Drosophila, a gene required maternally for proper expression of other homeotic genes, such as Ubx, which is involved in pattern formation. Ubiquitous expression of UBX protein within the mesoderm in Drosophila results in the formation of ectopic gonad tissue anterior to the normal location of the gonads [42]. We now provide evidence that Brd3 may play a role in testis formation in mammals. Identification of Brd3 expression within the male genital ridge, with increased expression within pre-Sertoli cells compared with other cell types, provides evidence that Brd3 may play a role in testis formation in mammals and may help to define the developmental tissue code required for testis formation. Further studies are now required to either confirm or refute these ideas.

In summary, we have combined fluorescent marking of cell populations in transgenic models with differential expression screening to study gene expression in pre-Sertoli cells of the mammalian genital ridge. Using these methods, we have identified new candidate genes, including Ppt1 and Brd3, the genital ridge expression patterns of which suggest they may be involved in the processes of sex determination and differentiation.

REFERENCES

1. Sinclair AH, Berta P, Palmer MS, Hawkins JR, Griffiths BL, Smith MJ, Foster JW, Frischhauf AM, Lovell-Badge R, Goodfellow PN. A gene from the human sex-determining region encodes a protein with homology to a conserved DNA-binding motif. Nature 1990; 346:240–244.

2. Gubbay J, Collignon J, Koopman P, Capel B, Economou A, Munsterberg A, Vivian N, Goodfellow P, Lovell-Badge R. A gene mapping to the sex-determining region of the mouse Y chromosome is a member of a novel family of embryonically expressed genes. Nature 1990; 346:245–250.
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3. Koopman P, Gubbay J, Vivian N, Goodfellow P, Lovell-Badge R. Male development of chromosomally female mice transgenic for Sry. Nature 1990; 351:117–121.

4. Hacker A, Capel B, Goodfellow P, Lovell-Badge R. Expression of Sry, the mouse sex determining gene. Development 1995; 121:1603–1614.

5. Bullejos M, Koopman P. Spatially dynamic expression of Sry in mouse genital ridges. Dev Dyn 2001; 221:201–205.

6. Albrecht KH, Eicher EM. Evidence that Sry is expressed in pre-Sertoli cells and Sertoli and granulosa cells have a common progenitor. Dev Biol 2001; 240:92–107.

7. Daneau I, Pilon N, Boyer A, Behdani R, Overbeek PA, Viger R, Lussier J, Silversides DW. The porcine SRY promoter is transactivated within a male genital ridge environment. Genesis 2002; 33:170–180.

8. Tilmann C, Capel B. Mesonephric cell migration induces testis cord formation and Sertoli cell differentiation in the mammalian gonad. Development 1999; 126:2883–2890.

9. Schmahl J, Eicher EM, Washburn LL, Capel B. Sry induces cell proliferation in the gonad. Development 2000; 127:65–73.

10. Martineau J, Nordqvist K, Tilmann C, Lovell-Badge R, Capel B. Male-specific cell migration into the developing gonad. Curr Biol 1997; 7:958–968.

11. Capel B, Albrecht KH, Washburn LL, Eicher EM. Migration of mesonephric cells into the mammalian gonad depends on Sry. Mech Dev 1999; 84:127–131.

12. Andersson M, Page DC, de la Chapelle A. Chromosome Y-specific DNA is transferred to the short arm of X chromosome in human XX males. Science 1986; 233:786–788.

13. Foster JW, Dominguez-Segrich MA, Guioli S, Kowk G, Weller PA, Stevanovic M, Weissenbach J, Mansour S, Young ID, Goodfellow PN, Brook JD, Schafer AJ. Campomelic dysplasia and autosomal sex reversal caused by mutations in an SRY-related gene. Nature 1994; 372:525–530.

14. Guo W, Mason JS, Stone CG Jr, Morgan SA, Madu SI, Baldini A, Lindsay EA, Biessel HG, Copeland NC, Horiick MNB, Pettigrew AL, Zanaria E, McCabe ERB. Diagnosis of X-linked adrenal hypoplasia congenita by mutation analysis of the DAX1 gene. JAMA 1995; 274:324–330.

15. Pelletier J, Bruening W, Li FP, Haber DA, Glaser RL, Tilmann C, Housman DE. WT1 mutations contribute to abnormal genital system development and hereditary Wilms’ tumour. Nature 1991; 353:431–434.

16. Katoh-Fukui Y, Tsuchiya R, Shiroishi T, Nakahara Y, Hashimoto N, Pelletier J, Tilmann C, Housman DE, Haber DA, Glaser RL, Viger R, Lussier J, Silversides DW, Pearson S, Young ID, Goodfellow PN, Brook JD, Schafer AJ. Campomelic dysplasia and autosomal sex reversal caused by mutations in an SRY-related gene. Nature 1994; 372:525–530.

17. Birk OS, Casiano DE, de la Chapelle A. Chromosome Y-specific DNA is transferred to the short arm of X chromosome in human XX males. Science 1986; 233:786–788.

18. Foster JW, Dominguez-Segrich MA, Guioli S, Kowk G, Weller PA, Stevanovic M, Weissenbach J, Mansour S, Young ID, Goodfellow PN, Brook JD, Schafer AJ. Campomelic dysplasia and autosomal sex reversal caused by mutations in an SRY-related gene. Nature 1994; 372:525–530.

19. Liu X, Eldey A, Germain K, Hovland DE, Tilmann C, Housman DE. WT1 mutations contribute to abnormal genital system development and hereditary Wilms’ tumour. Nature 1991; 353:431–434.

20. Katoh-Fukui Y, Tsuchiya R, Shiroishi T, Nakahara Y, Hashimoto N, Pelletier J, Tilmann C, Housman DE, Haber DA, Glaser RL, Viger R, Lussier J, Silversides DW, Pearson S, Young ID, Goodfellow PN, Brook JD, Schafer AJ. Campomelic dysplasia and autosomal sex reversal caused by mutations in an SRY-related gene. Nature 1994; 372:525–530.

21. Guo W, Mason JS, Stone CG Jr, Morgan SA, Madu SI, Baldini A, Lindsay EA, Biessel HG, Copeland NC, Horiick MNB, Pettigrew AL, Zanaria E, McCabe ERB. Diagnosis of X-linked adrenal hypoplasia congenita by mutation analysis of the DAX1 gene. JAMA 1995; 274:324–330.

22. Wertz K, Herrmann BG. Large-scale screen for genes involved in gonad development. Mech Dev 2000; 98:51–70.

23. Menke DB, Page DC. Sexually dimorphic gene expression in the developing mouse gonad. Gene Expression Patterns 2002; 2:359–367.

24. Smith L, Van Hateren N, Willan J, Romero R, Blanco G, Siggers P, Boockfor FR, Morris RA, DeSimone DC, Hunt DM, Walsh KB. Sertoli cell differentiation in the mammalian gonad. Development 1999; 126:2883–2890.

25. McClure PJ, Hurley TM, Sarrat MA, van den Bergen JA, Sinclair AH. Subtractive hybridisation screen identifies sexually dimorphic gene expression in the embryonic mouse gonad. Genesis 2003; 37:84–90.

26. Pilon N, Daneau I, Paradis V, Hamel F, Lussier JG, Viger R, Silversides DW, Porcine SRY promoter is a target for steroidogenic factor 1. Biol Reprod 2003; 68:1098–1106.

27. Guardiola M, Koopman P. Spatially dynamic expression of Sry in mouse genital ridges. Dev Dyn 2001; 221:201–205.

28. Albrecht KH, Eicher EM. Evidence that Sry is expressed in pre-Sertoli cells and Sertoli and granulosa cells have a common progenitor. Dev Biol 2001; 240:92–107.

29. Daneau I, Pilon N, Boyer A, Behdani R, Overbeek PA, Viger R, Lussier J, Silversides DW. The porcine SRY promoter is transactivated within a male genital ridge environment. Genesis 2002; 33:170–180.

30. Tilmann C, Capel B. Mesonephric cell migration induces testis cord formation and Sertoli cell differentiation in the mammalian gonad. Development 1999; 126:2883–2890.

31. Schmahl J, Eicher EM, Washburn LL, Capel B. Sry induces cell proliferation in the gonad. Development 2000; 127:65–73.

32. Martineau J, Nordqvist K, Tilmann C, Lovell-Badge R, Capel B. Male-specific cell migration into the developing gonad. Curr Biol 1997; 7:958–968.

33. Capel B, Albrecht KH, Washburn LL, Eicher EM. Migration of mesonephric cells into the mammalian gonad depends on Sry. Mech Dev 1999; 84:127–131.

34. Andersson M, Page DC, de la Chapelle A. Chromosome Y-specific DNA is transferred to the short arm of X chromosome in human XX males. Science 1986; 233:786–788.

35. Foster JW, Dominguez-Segrich MA, Guioli S, Kowk G, Weller PA, Stevanovic M, Weissenbach J, Mansour S, Young ID, Goodfellow PN, Brook JD, Schafer AJ. Campomelic dysplasia and autosomal sex reversal caused by mutations in an SRY-related gene. Nature 1994; 372:525–530.

36. Guo W, Mason JS, Stone CG Jr, Morgan SA, Madu SI, Baldini A, Lindsay EA, Biessel HG, Copeland NC, Horiick MNB, Pettigrew AL, Zanaria E, McCabe ERB. Diagnosis of X-linked adrenal hypoplasia congenita by mutation analysis of the DAX1 gene. JAMA 1995; 274:324–330.

37. Pelletier J, Bruening W, Li FP, Haber DA, Glaser RL, Tilmann C, Housman DE. WT1 mutations contribute to abnormal genital system development and hereditary Wilms’ tumour. Nature 1991; 353:431–434.

38. Katoh-Fukui Y, Tsuchiya R, Shiroishi T, Nakahara Y, Hashimoto N, Pelletier J, Tilmann C, Housman DE, Haber DA, Glaser RL, Viger R, Lussier J, Silversides DW, Pearson S, Young ID, Goodfellow PN, Brook JD, Schafer AJ. Campomelic dysplasia and autosomal sex reversal caused by mutations in an SRY-related gene. Nature 1994; 372:525–530.

39. Liu X, Ikeda Y, Parker KL. A cell-specific nuclear receptor is essential for adrenal and gonadal development and sexual differentiation. Cell 1994; 77:481–490.

40. Raymond CS, Kettlewell JR, Hirschl B, Bardwell VJ, Zarkower D. Expression of Dmrt1 in the genital ridge of mouse and chicken embryos suggests a role in vertebrate sex development. Dev Biol 1999; 215:208–220.

41. Raymond CS, Parker ED, Kettlewell JR, Brown LG, Page DC, Kusz K, Jareulska J, Reinger Y, Flejter WL, Bardwell VJ, Hirschl B, Zarkower D. A region of human chromosome 9p required for testes development contains two genes related to known sexual regulators. Hum Mol Genet 1999; 8:989–996.

42. Bowles J, Bullejos M, Koopman P. A subtractive gene expression screen suggests a role for vanin-1 in testis development in mice. Genesis 2000; 27:124–135.