Meiosis Onset Is Postponed to Postnatal Stages during Ovotestis Development in Female Moles

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Abstract

In mammals, germ cells are important both during development and for the function of female gonads, whereas male gonads may develop in the absence of germ cells. The gonads of female moles (genus \textit{Talpa}) develop according to a testis-like pattern which results in the formation of ovotestes. In this paper, we studied the expression pattern of several pre-meiotic and meiotic germ cell markers, in order to establish the precise time of meiosis onset in the mole species \textit{T. occidentalis}, and to investigate the location and possible role of germ cells in ovotestis organogenesis. Our results evidenced that: (1) the asymmetrical distribution of primordial germ cells, which concentrate in the cortex of the XX gonad, is brought about by germ cell depletion from the medulla between the s5a and s5b stages, (2) XX germ cells enter meiosis postnatally, which is quite exceptional among eutherian mammals, and (3) XX but not XY germ cells of moles express \textit{DMRT1} during premeiotic stages of development, an expression pattern not described previously in vertebrates.

In mammals, the genetic sex is established at conception and depends on whether the male gamete transmits either an X or a Y chromosome. In XX embryos, a Y-linked gene, \textit{SRY}, induces testis differentiation and the subsequent production of male hormones is responsible for the somatic masculinization of the body. When \textit{SRY} is absent (in XX embryos, normally) ovaries rather than testes are formed and the individual will then develop as a female (for a review see Viger et al., 2005). Phenotypic sex of mammals is thus determined by gonadal sex, which in turn is determined by genetic sex.

The gonad is a bipotential organ, as it can follow two different developmental pathways: testis or ovary. Gonad differentiation involves multiple complex biological aspects, including gene interactions, cell proliferation and...
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migration, and paracrine signaling (reviewed by Ross and Capel, 2005). Also, several cell types differentiate in both the testes and ovaries. Germ cells are one of the most important cell types in the developing gonad, as they are responsible for transmitting the genetic information to the next generation. These cells appear in the epiblast and migrate to the growing gonads through the gut mesentery, where they proliferate.

In XX gonads, germ cells initiate meiosis, which is followed by the rest of the events of ovarian differentiation. Contrarily, in XY gonads the SRY-expressing pre-Sertoli cells initiate testicular differentiation before germ cells enter meiosis and, once in a testicular environment, germ cells differentiate as spermatogonia. Thus, testicular but not ovarian differentiation can occur in the absence of germ cells, which are also essential for maintaining the ovarian structure (Merchant, 1975; McLaren and Southee, 1997). Also, meiotic XX cells antagonize testicular development by interfering with testis cord differentiation and mesonephric cell migration into the gonad. Hence, once germ cells initiate meiosis, testicular differentiation may no longer take place (Yao et al., 2003).

The timing of meiosis initiation is quite different in males and females (see Koubova et al., 2006). In females of most mammals, meiosis begins prenatally, coinciding with the onset of ovarian development, whereas in males the germ cells are sequestered in the testis cords formed by Sertoli cells, where they undergo mitotic arrest until after birth. Meiosis begins to take place at puberty, although a portion of the spermatogonia continue dividing by mitosis thus maintaining a pool of renewed germinal cells.

Female moles of the genus Talpa are exceptional among mammals because they develop ovotestes instead of normal ovaries and, nevertheless, they are fertile (Jiménez et al., 1993; Sánchez et al., 1996). These ovotestes are composed of a small portion of normal ovarian tissue and a generally large portion of disgenic testicular tissue. In the species T. occidentalis the medullar region of the XX gonads develops according to a testis-like pattern which includes cord formation, mesonephric cell migration, profuse vascularization and Leydig and peritubular-myoid cell differentiation (Barrionuevo et al., 2004a). Although these features define most of the main events of testis organogenesis (Brennan and Capel, 2004), ovotestes of female moles lack fully differentiated Sertoli cells, as they do not express typical Sertolian markers such as AMH (anti-Mullerian hormone; Zurita et al., 2003) or SOX9 (our unpublished data). However, Leydig cells in the ovotestes of non-breeding females produce abundant testosterone in postnatal stages of development and during the non-breeding season.

Primordial germ cells (PGC) show an asymmetrical distribution in mole ovotestes. They concentrate in the cortex of the developing XX gonad, so that the medulla soon becomes sterile (Barrionuevo et al., 2004a). In this study, based mainly on morphological analyses of tissue sections, we established that ovarian development in these gonads is considerably delayed if compared with the rest of mammals, and that meiosis onset in female moles occurs postnatally, which would be another uncommon feature in the reproductive biology of these singular mammals.

In the present paper, we investigate the expression patterns of several gene products, including pre-meiotic and meiotic germ cell markers, in order to investigate the localization and possible role of germ cells in gonadal development in the mole, and to establish the precise time of meiosis onset in this species.

Materials and Methods

A total of 42 embryos, fetuses, infants, juveniles and adults of the insectivorous mole species Talpa occidentalis were collected in poplar groves around Santa Fe (Granada province, southern Spain) over several annual breeding seasons. The animals were captured under annual permission granted by the Andalusian Environmental Council. Handling followed the guidelines and approval of the ‘Ethical Committee for Animal Experimentation’ of the University of Granada. Developmental stages considered in this study were those described by Barrionuevo et al. (2004a, b) for this species. Table 1 summarizes the main developmental features of each stage. These data are necessary to understand the significance of the results of the present study. Procedures concerning sample collection, age determination, paraffin- and resin-embedding of tissue for histological preparations, embryo sexing, and immunostaining were performed as described previously (Barrionuevo et al., 2004a). Gene-expression analyses were performed by immunofluorescence, using the primary antibodies summarized in table 2. FITC- (green) and Cy3- (red) conjugated anti-rabbit and anti-goat secondary antibodies were obtained from Sigma. Double immunostaining was performed in some instances by applying the immunofluorescence technique sequentially with two different primary antibodies on the same histological preparations.

Results

PGC Location during Early Gonad Development

To study the distribution of primordial germ cells in the mole gonads during early development, we used semithin gonadal sections, which provided clear light-micro-
Table 1. Chronology and key events of gonadal development in *Talpa occidentalis*

<table>
<thead>
<tr>
<th>Stage</th>
<th>Age $^a$</th>
<th>Key features related to sex development $^b$</th>
</tr>
</thead>
<tbody>
<tr>
<td>s4</td>
<td>14–16 dpc</td>
<td>Formation of the indifferent gonad in both XY and XX embryos</td>
</tr>
<tr>
<td>s5a</td>
<td>17 dpc</td>
<td>Regionalization of the XX gonad thus differentiating medulla and cortex</td>
</tr>
<tr>
<td>s5b</td>
<td>18 dpc</td>
<td>Cord formation in the gonadal medulla of both XY and XX embryos</td>
</tr>
<tr>
<td>s5c</td>
<td>19 dpc</td>
<td>Vascularization in the gonadal medulla of both XY and XX embryos</td>
</tr>
<tr>
<td>s6</td>
<td>19–21 dpc</td>
<td>Leydig cells appear in XY gonads; pronounced regionalization of XX gonads</td>
</tr>
<tr>
<td>s7</td>
<td>21–23 dpc</td>
<td>Tunica albuginea appears in XY gonads; two cortical layers present in XX gonads</td>
</tr>
<tr>
<td>s8</td>
<td>24–28 dpc</td>
<td>Rete testis formation in XY gonads; PGCs proliferate in the cortex of XX gonads</td>
</tr>
<tr>
<td>s9 (birth)</td>
<td>0–1 dpp</td>
<td>Testicular spherules appear in the fast-growing medulla of XX gonads</td>
</tr>
<tr>
<td>s10</td>
<td>5 dpp</td>
<td>Leydig cell differentiation in the medulla and meiosis onset in the cortex</td>
</tr>
<tr>
<td>s11</td>
<td>10 dpp</td>
<td>Peritubular myoid cells appear in the medulla of XX gonads</td>
</tr>
<tr>
<td>s12</td>
<td>15 dpp</td>
<td>Folliculogenesis initiation in the cortex of XX gonads</td>
</tr>
<tr>
<td>s13</td>
<td>20 dpp</td>
<td>Massive growing of the medulla and Leydig cell hyperplasia (XX)</td>
</tr>
<tr>
<td>s14</td>
<td>25 dpp</td>
<td>Massive growing of the medulla (XX testicular tissue)</td>
</tr>
<tr>
<td>s15a</td>
<td>30 dpp</td>
<td>Rete testis formation in the testicular portion of the XX ovotestis</td>
</tr>
<tr>
<td>s15b</td>
<td>30 dpp</td>
<td>Meiosis initiation in pubertal males; proestrus in pubertal females</td>
</tr>
<tr>
<td>adult</td>
<td>1 year</td>
<td>Seasonal breeding</td>
</tr>
</tbody>
</table>

$^a$ dpc: Days post coitum; dpp: days post partum (data from Barrionuevo et al., 2004a).

$^b$ Developmental features previously described by Barrionuevo et al. (2004b).

Table 2. Primary antibodies used in this study

<table>
<thead>
<tr>
<th>Gene product $^a$</th>
<th>Description/utility</th>
<th>Antibody source</th>
<th>References $^b$</th>
</tr>
</thead>
<tbody>
<tr>
<td>MVH</td>
<td>ATP-dependent RNA helicase. Generic germ cell marker.</td>
<td>rabbit polyclonal, raised against mouse protein</td>
<td>Dr. Toshiaki Noce</td>
</tr>
<tr>
<td>STAG3</td>
<td>Meiosis-specific cohesin. Meiotic germ cell marker.</td>
<td>rabbit polyclonal, raised against human protein</td>
<td>Dr. José Luis Barbero</td>
</tr>
<tr>
<td>SYCP3</td>
<td>Synaptonemal complex protein. Meiotic germ cell marker.</td>
<td>rabbit polyclonal, raised against human protein</td>
<td>Dr. José Luis Barbero</td>
</tr>
<tr>
<td>γH2AX</td>
<td>Recombination-related histone. Meiotic germ cell marker.</td>
<td>mouse polyclonal, raised against human protein</td>
<td>Dr. Paul Burgoyne</td>
</tr>
<tr>
<td>DMRT1</td>
<td>Zinc-finger-like DNA-binding motif protein. Sertoli and germ cell marker.</td>
<td>rabbit polyclonal, raised against mouse protein</td>
<td>Dr. Silvana Guioli</td>
</tr>
<tr>
<td>OCT4</td>
<td>POU family homeodomain protein. Pre-meiotic germ cell marker.</td>
<td>goat polyclonal, raised against human protein</td>
<td>Santa Cruz Biotechnology Ref. sc-8628</td>
</tr>
<tr>
<td>αSMA</td>
<td>Smooth muscle protein. Myoid cell marker.</td>
<td>mouse polyclonal, raised against rabbit protein</td>
<td>Sigma Ref. A2547</td>
</tr>
</tbody>
</table>

$^a$ Gene product names: MVH = mouse VASA homolog; STAG3 = stromal antigen 3; SYCP3 = synaptonemal complex protein 3; γH2AX = phosphorylated histone H2AX; DMRT1 = dsx- and mab3-related transcription factor 1; OCT4 = octamer-binding transcription factor 4; αSMA = smooth muscle α-actin.

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scope images of germ cells at these stages, identifiable by their large clear cytoplasm and round nucleus (fig. 1). These observations demonstrated that PGCs are scarce in s4 embryos. No more than 1 or 2 PGCs were normally observed per transversal section of the gonads, frequently located near the coelomic epithelium. In s5a females, PGCs were more abundant and located in both cortex and medulla. Similarly, PGCs may be observed in deep and peripheral locations of the gonads from s5a males, but two gonadal regions were not distinguished in this case. In s5b stage the distribution of germ cells differed sharply in males and females. In the latter, PGCs were located exclusively in the cortex and none was observed either inside or outside the already-formed medullar cords. On the other hand, s5b male gonads contained all PGCs inside the just differentiated testicular cords and none of them remained in the peripheral region.

**PGCs in Males**

In s5c testes PGCs are still scarce (fig. 2a). PGCs proliferate afterwards, so that they may be seen inside the testis cords in newborn (s9) and s10 males (fig. 2b, c). Here, they can occupy any location, including peripheral and deep places. However, by stage s11, germ cells move to the periphery of the testis cords and are no longer seen in deep locations. This situation is maintained from this stage on, as shown in s12 (fig. 2d) and s13 (fig. 2e). In this plate, sequential immunostaining with the PGC marker MVH and the peritubular myoid marker αSMA showed that PGCs are close to myoid cells and thus in the outermost periphery of the cords. In adult males, the condition of germ cells depends on the timing of the seasonal breeding cycle. During the non-breeding season (summer), germ cells again occupy deep locations in the seminiferous tubules. In a non-breeding male ini-
tiating testicular reactivation, only a few germ cells, marked by red cytoplasmic MVH immunostaining (fig. 2f), would have re-initiated meiosis, as evidenced by the presence of green foci of γH2AX immunostaining (arrow), whereas others have not (arrowhead). Contrari-

ously, most germ cells in adult breeding males (winter season) are meiotic spermatocytes occupying peripheral locations in the now wider seminiferous tubules, as shown by immunostaining with the meiotic markers SYCP3 (fig. 2g) and γH2AX (fig. 2h). In the latter case, two different staining patterns may be observed in spermatocytes. In some cases a single, strong focus of γH2AX fluorescence is evident (arrow), whereas in others a more homogeneous staining may be observed (arrowhead), corresponding to different stages of the first meiotic prophase.

XX Germ Cells Located in the Cortex of Female Gonads

Figure 3 shows the distribution of germ cells in the gonads of 4 representative developmental stages of female moles, including the last prenatal (s8; fig. 3a) and 3 postnatal stages (s10, s12 and s15a; fig. 3b, c and d, respectively). Immunostaining using an anti-MVH antibody shows in all cases that germ cells locate at the cortical region of the gonad, which in s12 and s15a young moles (fig. 3c, d) may be called the ovarian portion of the ovotestis. Contrarily, no germ cell is present in the medullary region of the gonad (the testicular portion of the ovotestis; fig. 3c, d).
**XX Germ Cells Enter Meiosis Postnatally between s10 and s11 Stages**

To establish the precise timing of the meiosis onset in female moles, a combination of pre-meiotic (OCT4 and MVH) and meiotic prophase (γH2AX, SYCP3 and STAG3) germ-cell markers were used in immunofluorescence analyses performed throughout female gonad development. Figure 4a–l shows just the results found with OCT4, γH2AX and SYCP3 in the first 4 stages of postnatal development (s9–s12), whereas figure 4m graphically summarizes the results of all stages with all markers (including DMRT1, which is also expressed by XX germ cells of moles; see below). In newborn females (fig. 4a–c; s9 stage) germ cells express OCT4 but not γH2AX or SYCP3, thus showing that none of them had initiated meiosis. In XX gonads at s10 stage, some germ cells still express OCT4 (arrow; fig. 4d) but others already show a weak expression or no expression of this gene (arrowhead). Similarly, some germ cells located in the deepest layer of the gonadal cortex begin to express γH2AX and SYCP3 (arrows; fig. 4e, f), whereas those located in the outermost layer of the cortex still remain non-immunoreactive for these meiotic markers (arrowheads). This is particularly clear in the case of γH2AX immunostaining. At s11 stage, no OCT4 expression was detected (fig. 4g) and all germ cells appeared to be immunoreactive for the two meiotic markers (arrows; fig. 4h, i), showing that all of them are in meiosis by this time. The same situation was detected in female gonads at s12 stage (fig. 4j–l). Germ cells were absent from the medullary region of the XX gonad in all stages. These results clearly show that meiosis starts in the germ cells of the deep cortex of s10 embryos, and that a wave of meiosis onset moves from there towards the external cortex and finishes at s11 stage.

**DMRT1 Expression in Pre-Meiotic XX Germ Cells and XY Sertoli Cells of Moles**

In female moles, DMRT1 shows a non-conventional expression pattern. Several layers of gonadal cells adjacent to the coelomic epithelium are immunoreactive against a DMRT1 antibody at the s4 stage (fig. 5a). However, only germ cells express this gene from s5b stage on (fig. 5b). Double, sequential immunostaining with DMRT1 and OCT4 antibodies, as shown in s8 (fig. 5c) and s9 (fig. 5d) gonads, demonstrated that cells expressing DMRT1 are in fact germ cells, as they also express OCT4. At the s10 stage, it is evident that germ cells that had already initiated meiosis (immunoreactive for γH2AX, fig. 5e) did not express DMRT1. In s12 gonads, neither OCT4 nor DMRT1 expression was detected (fig. 5f). These data reflect that DMRT1 expression in XX germ cells of moles is down-regulated when meiosis begins between the s10 and s11 stages.

In males, on the other hand, our results are consistent with expression of DMRT1 by Sertoli cells. Many cells of the undifferentiated s4 gonad show DMRT1 immunoreactivity in the medullary region (fig. 5g), according to a pattern which is almost exactly the opposite of that observed in the gonads of s4 females (fig. 5a). DMRT1 expression is restricted to the cells inside the cords in the differentiated testes of s5c males (fig. 5h). Double immunostaining with DMRT1 and OCT4 shows that the DMRT1-positive cells in the testis cords are Sertoli cells.
(red) rather than germ cells (green), as shown in the testes of an s10 male (fig. 5i). However, the observation of some yellow nuclei or red cytoplasm in particular germ cells in these preparations does not permit to exclude the possibility that some XY germ cells also express DMRT1.

**Discussion**

In most mammals, the first sign of gonadal differentiation appears in the male, as testis differentiation in XY embryos precedes ovarian differentiation in XX littermates (Merchant, 1975; Wartenberg, 1978, 1982; Mer-
Fig. 5. *DMRT1* expression is detected in pre-meiotic XX but not XY germ cells of moles and in XX and XY somatic cells. In females (a–f), the DMRT1-immunostaining pattern (red) is consistent with expression by germ cells, as suggested by both the location of the positive cells in the gonad cortex (shown in stages s4 (a) and s5b (b)), or the colocalization with the germ-cell marker OCT4 (green) (shown in stages s8 (c) and s9 (d)). Note that most of the DMRT1-positive cells observed in s4 (a) and s5b (b), are probably somatic cells as the number of germ cells is low in these stages. In s10 females, only non-meiotic germ cells still express *DMRT1* (red cells), whereas meiotic cells expressing γH2AX (green cells) show no red fluorescence (e). In the s12 stage, all germ cells have initiated meiosis (as evidenced by the lack of OCT4 expression; green) and no *DMRT1* expression remains (red) (f). In males (g–i) *DMRT1* is expressed by cells of the medulla in the undifferentiated gonad (s4 stage, g), by most cells in the cords of the just differentiated testes (s5c stage, h), and by Sertoli cells in postnatal testes (red immunostaining; shown in an s10 testis); green cells in this picture are germ cells expressing OCT4 (i). Transversal sections are shown in all figures. MN, mesonephros; G, gonad. Scale bar = 100 μm in b, f, g and h, 80 μm in a, 50 μm in c, 40 μm in e and i, 30 μm in d.

chant-Larios and Taketo, 1991; Burgoyne and Palmer, 1993, among others). Contrarily, morphological studies of mole gonad organogenesis (Barrionuevo et al., 2004a) revealed that the formation of two gonadal regions, cortex and medulla, in XX embryos precedes testis differentiation in males. This event takes place at the s5a stage, one day before overt testis cord formation occurs in males. Regionalization of the indifferent gonad of female moles may probably be associated with the asymmetrical distribution of the primordial germ cells. We previously showed that germ cells concentrate in the cortex of the XX gonad of moles (Barrionuevo et al., 2004a), but these observations did not indicate whether this is the cause or a consequence of gonadal regionalization. Our present results clearly confirm the asymmetrical distribution of the germ cells and evidence that none of them is present in the medullary region in stages as early as s5b. In sheep, however, about 10% of germ cells still remain in the medullary region by the time of folliculogenesis, which takes place several days after the beginning of meiosis (Sawyer et al., 2002). In the mole, primordial germ cells were detected in both cortical and medullary regions of the XX gonad at s5a stage, but they were depleted from the medulla in just 1 day (s5b stage). We could not investigate the mechanism by which germ cells are eliminated from the medulla as mole embryos of these stages are scarce.
and very difficult to collect, but a plausible explanation is apoptosis, as this is a common cause of substantial germ cell loss in the mammalian ovary (Reynaud and Driancourt, 2000). Nevertheless, these data rule out the hypothesis that germ cells are asymmetrically distributed in XX mole gonads due to selective colonization of the gonadal primordium.

Germ cells of male moles behave very similarly to those of other mammals during gonadal development (for comparison with the mouse, see Brennan and Capel, 2004). They are rapidly enclosed into the testis cords at s5b stage, proliferate very actively around birth and migrate to the periphery of the testis cords during the first postnatal stages (s9 and s10). However, moles are seasonally breeders (Mathews, 1935; Jiménez et al., 1990), so that their gonads show some peculiarities in adult males. Roughly, moles are sexually active during the winter and inactive during the summer in the southern Iberian Peninsula. Moreover, they inactivate and activate their gonads during the intermediate seasons, spring and autumn, respectively. These processes of cyclic, seasonal gonad activation and inactivation involve profound changes in many parameters of the reproductive tract of both males and females that cause them to become fertile in the winter and sterile in the summer (Jiménez et al., 1996). Testes from winter (fertile) males have thick seminiferous tubules with spermatocytes in peripheral locations, whereas those showing single, interstitial and B spermatogonia or pre-leptotene to zygotene spermatocytes, whereas those showing single, bright γH2AX foci are in pachytene stage, where only the sex vesicle (the condensed XY bivalent) appears immunoreactive (Hamer et al., 2003; see fig. 2h).

One of the most striking features of the gonad development in female moles is that germ cells enter meiosis postnatally (Barrionuevo et al., 2004a). Here, we present evidence that the first cells to enter meiosis are those located near the cortex-medulla boundary of s10 mole infants, which are about five days old. This is very uncommon among eutherian mammals, as only the rabbit has been described up to now in which the gonads of newborn females still contain non-meiotic germ cells (Peters et al., 1965). In eutherian mammals, meiosis usually starts in prenatal stages of development (for an extensive review, see Mossman and Duke, 1973). However, postnatal meiosis onset is the norm in XX marsupials, as birth occurs in very early developmental stages in these mammals (see Renfree and Shaw, 2001). The mechanism controlling the differences between males and females concerning the timing of the onset of meiosis has been the subject of extensive research. Several experiments have suggested that XX germ cells initiate meiosis autonomously, according to an internal biological clock (Zamboni and Upadhyay, 1983; McLaren, 1984; McLaren and Southey, 1997). However, recent discoveries strongly support the Byskov and Saxen hypothesis (1976) that a ‘meiosis inducing substance’ present in the ovary environment is responsible for meiosis initiation. Recent studies indicate that retinoic acid (RA) produced by the mesonephros and many other embryonic tissues, activates the STRA8 gene (stimulated by retinoic acid gene 8) in germ cells, an event that seems to induce them to enter meiosis. In embryonic males, however, meiosis is retarded by the action of the RA-degrading enzyme CYP26B1 (the cytochrome P450, P450RAI-2) which is absent in fetal ovaries (Bowles et al., 2006; Koubova et al., 2006). Thus, assuming that this is also the genetic system controlling meiosis initiation in moles, delayed meiosis onset in female moles may be due to either (1) a delay in the arrival of RA to the XX gonad, or (2) ectopic expression of the CYP26B1 gene in the ovary that temporally mimics the situation in male gonads. The latter hypothesis is consistent with the fact that the medulla of XX mole embryonic gonads develops according to a testis-like pattern (see above). It is possible that somatic cells of the medullary region produce CYP26B1, as is assumed to occur in male testes. These possibilities are currently being investigated.

DMRT1 expression in mole gonads is somewhat peculiar. This gene has been shown to be expressed in somatic and germ cells of most vertebrates at different stages of gonadal development (Raymond et al., 1999, 2000; De Grandi et al., 2000; Moniot et al., 2000; Nanda et al., 2000, among others). Our present results clearly show that XX germ cells of moles express DMRT1 through premeiotic stages and that downregulation occurs just before the onset of meiosis. Current knowledge concerning the function of this gene for the moment does not reveal the significance of this expression pattern. Very recent studies on the human testis have evidenced that three different transcripts may be generated by alternative splicing for this gene, and that they show different expression levels in the testis (Cheng et al., 2006). These data probably reflect that regulation of DMRT1 expression is controlled by a complex mechanism and that different transcript isoforms may play different roles in gonadal development and function. The precise timing of DMRT1 downregulation in XX germ cells of moles, coinciding
with meiosis initiation, suggests that a product of this gene could play a role in premeiotic XX germ cells.

The most outstanding feature of gonads in female moles is the presence of a big portion of disgenic testicular tissue adjacent to the functional ovarian tissue (Jiménez et al., 1988, 1993, 1996; Sánchez et al., 1996; Zurita et al., 2003; Barrionuevo et al., 2004a). The demonstration that meiotic germ cells antagonize testis organogenesis during early gonadal development in the mouse (Yao et al., 2003), raises the possibility of a relationship between these two singular features of mole gonads. By culturing aggregates of XY somatic cells with XX germ cells from mouse gonads in different developmental stages, Yao et al. (2003) established that meiotic, but not pre-meiotic germ cells are able to disturb meiotic cell migration and cord formation in the mouse gonad at the time of testis differentiation. This implies that XX gonads are able to follow the male pathway of development during a temporal window that terminates when XX germ cells enter meiosis. These and other authors (Burgoyne and Palmer, 1993) have suggested that meiotic oocytes produce signals that antagonize the first steps of testis organogenesis, so that once meiosis is initiated the ovarian pathway is strongly canalized. Accordingly, the considerable delay of mole XX germ cells to enter meiosis could permit that a testis-like development, involving mesonephric cell migration and medullary cord formation (Barrionuevo et al., 2004a), can begin in the female gonad during such a long pre-meiotic period, in the absence of such oocyte-derived inhibitory factors. However, a premise for this hypothesis is that these events of early testis development should be able to be induced in XX gonads, in the absence of SRY. Yao et al. (2004) have evidenced that endothelial cell migration and the subsequent formation of a testis-type vascular system is specifically inhibited by Wnt4 (wingless-related MMTV integration site gene 4) and Fst (Follistatin) signaling in the mouse ovary, and that these typically testicular events are induced in the gonads of XX (Sry-negative) mice deficient for either of these two genes. According to new data (Kim et al., 2006), the participation of the Sex9 gene in mesonephric migration cannot be ruled out in XY gonads, but the testicular events observed in the gonads of XX mice deficient for Wnt4 and Fst appear in the absence of this pro-testis gene (Yao et al., 2004). Furthermore, evidence has been provided that meiotic cell migration induces cord formation in XX gonads co-cultured, as a sandwich, between XY gonads and either XY or XX mesonephroi (Tilman and Capel, 1999). Wnt4 and Fst signalling are not needed to initiate meiosis but are essential for survival of meiotic cortical germ cells at pachytene and diplonete stages of the mouse (Yao et al., 2004).

Hence, if WNT4 and/or FST signalling were absent during the long pre-meiotic phase of gonad development in XX moles, then testicular features could appear during this period in the medulla of these gonads. The expression patterns of WNT4 and FST in moles are currently being investigated and will probably help to elucidate the singular organogenesis of mole female gonads.

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References


