XY follicle cells in the ovaries of XO/XY and XO/XY/XYY mosaic mice

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Summary

XO/XY and XO/XY/XYY mosaic hermaphrodites were generated from crosses involving BALB/cWt males. The distribution of Y-bearing cells in the gonads of these mice was studied by in situ hybridisation using the Y-specific probe pY353B. XY cells were found to contribute to all cell lineages of the ovary including follicle cells. The proportion of XY follicle cells was not significantly different from the XY contribution to other gonadal or non-gonadal cell lineages. However, this proportion was consistently low, all the hermaphrodites having a low XY contribution to the animal as a whole.

Because the XO- and Y-bearing cell lineages are developmentally balanced, the XY follicle cells cannot have formed as a result of a ‘mismatch’ in which the Y-directed testis determination process is pre-empted by an early acting programme of ovarian development. These results are discussed with respect to the hypothesis that Tdy acts in the supporting cell lineage, the lineage from which Sertoli cells and follicle cells are believed to be derived.

Key words: mosaic, hermaphrodite, XY follicle cells.

Introduction

Gonadal sex differentiation can first be recognised in mice at 12.5 days post coitum with the appearance of testicular cords in the gonads of XY fetuses. Female gonads show no gross morphological change from the indifferent state until 13.5 days post coitum when the germ cells first enter meiosis (McLaren, 1984). Studies on fetal rat testes by light and electron microscopy have revealed that the Sertoli cells can be recognised as a distinct cell type prior to their aggregation into cords (Magre and Jost, 1980). These cells are the first testicular cell type to appear. It is widely accepted that Sertoli cells differentiate from a pool of bipotential cells (the supporting cell lineage) which in females forms the follicle cells of the ovary. The derivation of Sertoli cells and follicle cells from a common lineage is supported in part by the fact that they share certain biochemical properties including production of the Mullerian inhibitor – AMH or MIS (Vigier et al. 1984; Donahoe et al. 1987). They also both share a lineage-specific cell surface antigen, as detected by cytotoxic T cell assay (Ciccarese and Ohno, 1978). Follicle cells have the ability to ‘transdifferentiate’ into Sertoli cells under special circumstances, as in the ovaries of ageing or hypophysectomised female rats (Crumeylerole-Arias et al. 1986) and in tissue derived from fetal ovaries grafted under the kidney capsule of adult mice (Takeoto-Hosotani et al. 1985; Takeoto-Hosotani and Sinclair-Thompson, 1987). Examination of oovestis sections from adult XO/XY mosaic hermaphrodites (Burgoynene and Palmer, unpublished data) and of T16/X5.xr hermaphrodites (Ward et al. 1988), has shown testis tubules which connect smoothly with follicles (which may contain oocytes) all bounded by a continuous basement membrane.

Burgoynene et al. (1988a) proposed that the primary function of the testis-determining gene (Tdy) is the cell-autonomous diversion of the supporting cell lineage to form Sertoli cells and that the remainder of testis development is directed by these Sertoli cells. In the absence of a Y (and thus Tdy), the supporting cell lineage differentiates into follicle cells. This ‘cell-autonomous action of Tdy’ model of sex determination, in its simplest form, predicts that follicle cells should be exclusively XX in XX<-»XY female chimaeras. This is because all supporting cells containing a Y chromosome are expected to be diverted into the Sertoli cell pathway. XY follicle cells have, however, been shown to exist in three XX<-»XY female chimaeras (Ford et al. 1974; Burgoynene et al. 1988b). The latter authors suggested that these XY follicle cells could be formed by a ‘timing mismatch’ mechanism in which Tdy was pre-empted by the ovarian determination process initiated by the XX component of the chimaera. In chimaeras, there is clearly the potential for a developmental mismatch between the two components and, in Ford et al.’s XX<-»XY females, the XY component was AKR, which other evidence suggests has a late-acting Y (Eicher and Washburn, 1986).

In this paper, we ask whether XY follicle cells can be formed in a situation where ‘mismatch’ can be ruled out: this is, in the ovaries of XO/XY and XO/XY/ XYY mosaic hermaphrodites that occur in crosses involving BALB/cWt males (Eicher et al. 1980). These mosaics arise through mitotic non-disjunction of the Y,
so there are no strain differences between the components. Furthermore, although XO fetuses with a paternal X are developmentally retarded (Burgoyne et al. 1983), XO fetuses with a maternal X develop as fast as their XY sibs (Thornhill and Burgoyne, unpublished data); so, on these additional grounds, we expect the XO and XY cell lines of these mosaics to be developmentally balanced.

Materials and methods

CXBH/By females were mated to BALB/cWt males to produce XO/XY and XO/XY/XYY mosaics. The mice were killed 8–11 days after birth and examined internally for signs of hermaphroditism. A sample of bone marrow was removed from the hermaphrodites and air-dried metaphase spreads were prepared by standard methods. The gonads were fixed in 3:1 ethanol/glacial acetic acid at 4°C overnight, washed in two changes of absolute ethanol and cleared in three changes of diaminobenzidine. Positive hybridisation, estimates of relative cell numbers are subject to bias due to the amount of false negative cells that occur in tissue sections. These ovaries were from a 2-week-old XXY female mouse carrying the mutant 129Y of Lovell-Badge and Robertson (1990), which has lost Tdy (Gubbay et al. 1990).

Results

Table 1. The proportion of Y-bearing cells in bone marrow and gonadal cell lineages of XO/XY and XO/XY/XYY mosaics

<table>
<thead>
<tr>
<th>Mosaic</th>
<th>Age (dpp)</th>
<th>Gonads*</th>
<th>Bone marrow</th>
<th>Ovary</th>
<th>Testis or ovotestis</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1</td>
<td>OT O</td>
<td>20 (30)</td>
<td>17.2±1.0</td>
<td>19.6±1.78</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>O OT</td>
<td>20 (30)</td>
<td>15.5±1.53</td>
<td>20.0±1.53</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>O T</td>
<td>15 (28)</td>
<td>15 (594)</td>
<td>15 (594)</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>O OT</td>
<td>22 (510)</td>
<td>25 (735)</td>
<td>20 (502)</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>T O</td>
<td>21 (560)</td>
<td>25 (654)</td>
<td>84 (735)</td>
</tr>
<tr>
<td>Mean±s.e.</td>
<td></td>
<td></td>
<td>20.0±2.9</td>
<td>17.2±1.0</td>
<td>19.6±1.78</td>
</tr>
</tbody>
</table>

* O, ovary; T, testis; OT, ovotestis.
† Not recorded due to technical failure.
‡ 11% of the Y-bearing cells were XYY.
Fig. 1. (A) Section of ovary from a 2-week-old XXY female. Counts of labelled and unlabelled cells from these sections produced the correction factors for the mosaic ovaries. (B) Section of ovary from mosaic 5 showing three single-layered follicles, which include labelled XY follicle cells. (C) Multilayered follicle of a 3-week-old BALB/cWt mosaic ovary showing a patch of labelled XY follicle cells. (D) Testis section from mosaic 5, the contralateral gonad to the ovary shown in B. This testis had virtually no germ cells due to the high XO contribution but the Sertoli cells are nearly all XY. The patch of labelled cells that appear to be outside a tubule (bottom left) are Sertoli cells from a tangentially cut tubule. Bar, 20 μm.
How do we account for the formation of XY follicle cells in these mosaics? In an accompanying paper, we have shown that the ‘cell-autonomous action of Tdy’ model is incorrect: XX Sertoli cells do occur in fetal, prepuberal and adult XX→XY testes, albeit at a low frequency (Palmer and Burgoyne, 1991). The strong bias in favour of XY Sertoli cells, even in fetal XX→XY testes (and also in the ovotestes and testes analysed here) confirms the original conclusion of Burgoyne et al. (1988a) that Tdy acts in the lineage that forms Sertoli cells, but the fact that some XX Sertoli cells are formed means that, at some point between Tdy expression and the formation of fetal Sertoli cell cords, there is a step that is able to recruit a few XX cells. If some XX cells can be recruited by XY cells to form Sertoli cells, could some XY cells fail to be recruited when their numbers fall below a certain threshold? If the source of the ‘recruiting factor’ is the XY supporting cells, it seems implausible that they fail to recruit themselves. An alternative possibility is that the XY supporting cells are triggered to form fetal Sertoli cells by the action of Tdy but subsequently ‘transdifferentiate’ into follicle cells under the influence of ovarian factors. It may be a characteristic of Sertoli cells and follicle cells that they retain the ability to transdifferentiate into the reciprocal cell type. Burgoyne (1988, 1991) has argued that the XX Sertoli cell cords that form in ovarian tissue in various situations (in all cases preceded by oocyte loss) are the result of transdifferentiation of follicle cells. Transdifferentiation of Sertoli cells to follicle cells through contact with oocytes has recently been suggested as an explanation for the occurrence of oocytes surrounded by ‘granulosa-like’ cells in some T16/XSxr testes (McLaren, 1991). An in situ Y-probe analysis of fetal XO/XY gonads might help to resolve this question.

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References


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