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Phil. Trans. R. Soc. Lond. B 1995 **350**, 229-233
doi: 10.1098/rstb.1995.0156

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Germ cells and germ cell sex

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SUMMARY

Whether germ cells succeed in making eggs or sperm depends both on their genetic constitution and on the tissue environment in which they develop. The decision as to whether it is oogenesis or spermatogenesis on which they initially embark depends only on their environment, however, and not at all on their own chromosomes. The foetal testis of the mouse produces an inhibitor of meiosis: germ cells that are exposed to it develop as prospermatogonia. Germ cells in the foetal ovary enter meiosis and develop as oocytes: this may represent the default pathway for germ cell sexual differentiation, or there may exist a meiosis-inducing substance. Experimental evidence suggests that any such substance must be present ubiquitously, not just in the ovary. The stage of foetal development at which meiosis is initiated may be programmed in the germ cell lineage.

1. INTRODUCTION

As the only route of biological transmission from one generation to the next, the germ cell lineage is of fundamental importance in studies of reproduction, development, genetics and evolution. From the time that a lineage-restricted pool of germ cells first emerges to the moment when fertilization is achieved and a new generation begins, the complex life-history of the germ cell encompasses migration, proliferation, and many successive stages of differentiation.

Most biologists who deal with germ cells see them only in their terminal stages, as gametes. In all multicellular organisms, female gametes (eggs) are notably different from male gametes (sperm), yet the early development of primordial germ cells is identical in male and female embryos. When does the difference in germ cell sex become apparent, and how is it brought about? These questions form the topic of this paper. For the mouse, the answer to the first question is known, and we are beginning to throw light on the second.

In the mouse, the primordial germ cells continue to proliferate for a few days after entering the genital ridges, the site of the future gonads. Up to this point, germ cells in male embryos are indistinguishable in appearance and behaviour from those in female embryos. But from about 13.5 days *post coitum* (DPC) onwards, the situation changes. In female embryos, the germ cells enter prophase of the first meiotic division, and pass through the stages of leptotene, zygotene and pachytene before arresting as diplotene oocytes embedded in primary follicles, at the time of birth. Germ cells in the genital ridges of male embryos also cease dividing at about 13.5 DPC, but instead of entering meiosis they remain arrested in the G1 stage of the cell cycle (McLaren 1984), as T-prospermatogonia, until shortly after birth. DNA replication then occurs and mitosis is resumed, with the first germ cells not entering meiosis until about a week later.

2. NATURE OR NURTURE?

Sry, the testis-determining gene on the mouse Y chromosome, is first expressed in the supporting cell lineage of the male genital ridge at about 10 DPC. By 12.5 DPC Sertoli cells have differentiated, testis cords have formed and the characteristic testicular pattern of vasculature has developed, so that male and female genital ridges can be readily distinguished. The germ cells in a normal male embryo are of course all XY in sex chromosome constitution, whereas those in a female embryo are all XX. So is it their own sex chromosome constitution that determines the phenotypic sex of germ cells, or is it their tissue environment?

This question could be approached by examining the fate of XX germ cells in a male genital ridge, or XY germ cells in a female ridge: situations that can be brought about in the mouse by appropriate genetic or embryological manipulation. For example, on average half of the chimeras made by aggregating together mouse embryos during the preimplantation stages of development will contain both XY and XX cells (McLaren 1978). Most of these will develop as males, but where the proportion of XY cells in the gonadal primordium is very low, the embryo will develop as a female. Female mice in which all the cells are XY in sex chromosome constitution may result if *Sry* is deleted (Gubbay et al. 1990) or is expressed too late (Eicher & Washburn 1986). XO embryos develop as females, but there are some XY male embryos in which, for genetic reasons, the Y chromosome is lost from a proportion of cells so that the testis contains XO as well as XY germ cells (Levy & Burgoyne 1986).

In all these examples, the answer is clear-cut. The embryonic germ cells differentiate according to the sex of the gonad in which they reside, not according to their own sex chromosome constitution. XY germ cells in XX/XY female chimeras or in XY females (Eicher & Washburn 1986) enter meiosis at 13.5 DPC and develop as oocytes; XX or XO germ cells in XX/XY

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male chimeras (Palmer & Burgoyne 1991) or XO/XY male mosaics (Levy & Burgoyne 1986) enter mitotic arrest at 13.5 DPC and develop as prospermatogonia.

In contrast, the fate of germ cells after birth and their ability to form gametes depends crucially on their own sex chromosome constitution. Many XY oocytes in an ovary die shortly after birth but some survive, so that XY female mice may produce one or more litters. XX prospermatogonia in a testis die a few days after birth. XO germ cells in a testis last a little longer, but most die as differentiating type B spermatogonia. The role of the Y chromosome in spermatogenesis has been investigated by Burgoyne (1992).

The decision of the germ cell to embark on oogenesis or spermatogenesis appears to be independent not only of the germ cell's own sex chromosome constitution but also, in XX embryos, of its X-chromosome inactivation status. In a normal XX embryo, by the time germ cells start their migration towards the genital ridges they will have undergone random X-inactivation, along with the rest of the epiblast-derived cells. The silent X chromosome becomes reactivated shortly after the germ cell has entered the genital ridge i.e. before or at the time of the onset of meiosis (Monk & McLaren 1981). It has been suggested that X-chromosome reactivation and entry into meiosis might be in some way related; however it has recently been shown that female germ cells remaining outside the genital ridge, although they are known to enter meiosis at the normal time, fail to undergo reactivation of the silent X (Tam et al. 1994). Conversely, XX germ cells in the testis of sex-reversed male embryos have been reported to undergo X-chromosome reactivation even though they are developing as prospermatogonia (McLaren & Monk 1981). Thus two active X chromosomes are neither necessary nor sufficient for entry into meiosis before birth.

3. INDUCER OR INHIBITOR?

If the initial difference in development of germ cells as between male and female embryos depends upon the tissue environment in which they are located rather than upon their own genotype, is it the female genital ridge that is inducing germ cells within it to enter meiosis at 13.5 DPC, or the male genital ridge that is inhibiting them from so doing? Or both?

Fortunately, an experiment of Nature gives us part of the answer to this question. In the mouse embryo, the urogenital ridge gives rise not only to the genital ridge that develops into the gonad, but also to the mesonephros (the mesonephric kidney is never functional in the mouse), the definitive metanephric kidney and the adrenal primordium. At the time when the primordial germ cells start colonizing the urogenital ridge, the metanephric kidney primordium has already separated off from the ridge; but the adrenal, the mesonephros and the genital ridge are still all connected by mesenchyme. Most of the germ cells enter the genital ridge, but some remain in the adjacent mesonephric region, and some enter the adrenal primordium. In female embryos, all the germ cells outside the genital ridge enter meiosis at about the same time as do those inside the ridge, namely at or shortly after 13.5 DPC. In male embryos, the same is true of the germ cells that have entered the adrenal primordium, even though they are all of course XY in chromosome constitution, while in the mesonephric region some enter meiosis and some undergo mitotic arrest, developing as prospermatogonia (McLaren 1984; Francavilla & Zamboni 1985).

The XY meiotic germ cells in the adrenal primordium lack the so-called 'sex vesicle' characteristic of meiotic germ cells (spermatocytes) in the postnatal testis (Hogg & McLaren 1985). This observation suggests that the sex vesicle is a feature of germ cells undergoing spermatogenesis rather than a feature of meiotic germ cells as such. The meiotic germ cells in the adrenal, whether in a female or a male embryo, undergo oogenesis, differentiating after birth into growing oocytes, complete with zona pellucida (Zamboni & Upadhyay 1983).

The fact that it is only in the male genital ridge and to some degree in the closely adjacent mesonephric region that the germ cells fail to enter meiosis, suggests the presence of an inhibitor, produced within the ridge but capable of diffusing a short distance outside it (McLaren 1991). At 13.5 DPC the male genital ridge already contains testis cords, formed by a peripheral layer of Sertoli cells surrounded by peritubular myoid cells. The great majority of germ cells in the testis are contained within the cords: some remain outside, all normally enter mitotic arrest. The earliest known gene product of Sertoli cells is anti-Mullerian hormone (AMH, also known as MIS, Mullerian inhibiting substance); AMH mRNA can already be identified at 12.5 DPC (Munsterberg & Lovell-Badge 1991). The interstitial region between the cords consists only of mesenchyme and vascular tissue; no Leydig cells have yet begun to differentiate.

The identity of the postulated meiosis inhibiting factor is not known. Testosterone does not inhibit mouse germ cells from entering meiosis *in vitro* (A. McLaren & D. Southee, unpublished observations), and in any case it seems unlikely that testosterone, a product of Leydig cells, could be synthesized in the male genital ridge as early as 13.5 DPC. AMH is perhaps a more likely candidate, though the protein has not been shown to be present earlier than 14.5 DPC. When the *Amh* gene is knocked out, germ cells in the testis still undergo spermatogenesis normally (Behringer *et al.* 1994), but possibly some other member of the TGFβ family is substituting for AMH. However, further evidence against AMH being an inhibitor of meiosis comes from the experiments of Vigier et al (1987), in which a high concentration of purified AMH failed to inhibit female germ cells in the embryonic rat ovary from entering meiosis *in vitro*, although it did cause substantial germ cell degeneration.

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4. A MEIOSIS-INDUCER ALSO?

The demonstration of a meiosis-inhibiting factor specific to the testis does not of course rule out the presence of a meiosis-inducing substance in the embryonic ovary, as postulated by Byskov & Saxen (1976). Such a substance could not be specific to the ovary, as we have seen that germ cells also enter meiosis before birth in the adrenal and in the mesonephric region, in male embryos as well as in female. But gonad, adrenal and mesonephros all have a closely similar embryological origin, being derived from the urogenital ridge which is itself derived from intermediate mesoderm. Perhaps the whole of the urogenital ridge produces a meiosis-inducing substance, and it is thus only germ cells exposed to the testis-specific meiosis-inhibiting factor that fail to enter meiosis before birth, and hence fail to develop as oocytes.

We therefore decided to examine the fate of mouse germ cells exposed to a tissue environment of very different embryological origin (A. McLaren & D. Southee, unpublished observations). The endodermally derived embryonic lung proved to be a suitable tissue. Germ cells can be isolated from the genital ridge with no more than 10–20% somatic cell contamination, mainly erythrocytes (De Felici & McLaren 1982). These were mixed with a large excess of lung tissue, reduced by trypsinization to a single-cell suspension. The mixture was centrifuged to form a pellet, which was then cultured in a standard organ-culture system (McLaren & Buehr 1990). Lung from embryos 13.5 DPC was used throughout: younger did not aggregate well, and older was more resistant to trypsinization. The sex of the embryo from which the lung was derived proved to be irrelevant. After a few hours in culture the pellet rounded up to form an aggregate; at the end of the culture period the aggregate was fixed and sectioned. 'Donor' germ cells were identified either by staining for alkaline phosphatase activity, or more often just by their appearance after staining with haematoxylin and eosin: control lung aggregates contained no cells positive for alkaline phosphatase activity, nor any cells that resembled either prospermatogonia or meiotic germ cells. Somatic cells introduced with the germ cells are unlikely to have affected the results as they were few in number, not associated with the germ cells, and enormously diluted by the mass of lung tissue.

When the donor germ cells were taken from female genital ridges 11.5–13.5 DPC and cultured for 4–5 days, all germ cells identified in the aggregates were in meiosis. Meiotic progression through leptotene and zygotene, and into pachytene, appeared to be retarded by up to 24 h relative to germ cells of the same chronological age *in vivo*. When the culture period was extended to 2 weeks, growing oocytes were found. After 3 weeks, the oocytes were larger and material resembling zona pellucida was seen.

When germ cells were taken from male genital ridges 13.5 or 12.5 DPC, they developed as prospermatogonia. When they were taken at 11.5 DPC, however, they entered meiosis and progressed through the stages of meiotic prophase in a similar manner to those taken from female embryos. Prospermatogonia and meiotic oocytes were never seen in the same aggregate.

In a subsequent experiment, genital ridges from male embryos 11.5–13.5 DPC were disaggregated by trypsinization, then reaggregated and cultured for 4–5 days as for the lung aggregates. Again, germ cells in the 12.5 and 13.5 DPC reaggregates developed as prospermatogonia, but in the 11.5 DPC reaggregates all the germ cells entered meiosis and developed as oocytes.

We conclude from these experiments: (i) that if entry into meiosis before birth is dependent on exposure to a meiosis-inducing substance then this substance must be distributed very widely in both female and male embryos, not only in the genital ridges and adrenals, but also in the lungs, perhaps systemically; (ii) that inhibition of meiosis and commitment to a spermatogenic pathway occurs in the male genital ridge between 11.5 and 12.5 DPC and rapidly becomes irreversible; and (iii) that production of the meiosis-inhibiting factor in the male genital ridge is prevented by trypsinization and reaggregation at 11.5 DPC. It has been reported previously that partial disruption of testicular structure by culture or transplantation of early male genital ridges is associated with localized entry of germ cells into meiosis (Ozdzenski 1972; McLaren & Buehr 1990).

5. OR PROGRAMMED IN THE GERMLINE?

The possibility remains that entry into meiosis before birth is not induced, but represents the 'default' pathway which is programmed into the germ-cell lineage i.e. the pathway that all germ cells follow unless inhibited from so doing.

A further question would then arise. Is the timing of entry into meiosis (at 13.5 DPC or shortly thereafter) programmed, or is it a response to some environmental change, perhaps the lifting of some inhibitory influence exerted on the germ cells prior to 13.5 DPC?

As an approach to this question, germ cells from female embryos 13.5 DPC were cultured in lung reaggregates for 3 days, and compared with germ cells from female embryos 10.5 DPC cultured in lung aggregates for either 3 days or 6 days. The prediction was that if timing is programmed, the 10.5 DPC germ cells would hardly have entered meiosis after 3 days (given the 24 h delay associated with culture). However, if meiosis was initiated as soon as the germ cells are removed from the embryo, 10.5 DPC germ cells would reach much the same stage of meiotic prophase as 13.5 DPC germ cells cultured for the same length of time.

The first prediction turned out to be essentially fulfilled. After 3 days of culture, the 13.5 DPC germ cells were already in the zygotene or even early pachytene stage of meiotic prophase, corresponding to about 15.5 DPC *in vivo*; whereas the 10.5 DPC germ cells had not yet entered meiotic prophase but were mostly in the so-called preleptotene stage, which is the last post-mitotic stage before entry into meiotic prophase or mitotic arrest, characteristic of germ cells in both female and male *in vivo* genital ridges 12.5 DPC. That

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they were not just blocked in preleptotene was shown by the 10.5 DPC germ cells cultured for 6 days, which reached a similar stage to the 13.5 DPC germ cells cultured for 3 days.

At 10.5 DPC, the germ cells have only just started to enter the genital ridge and are mostly still in the mesentery. No attempt was made to isolate the germ cells: the whole mesentery was trypsinized together with the lung tissue. The 'donor' somatic cell relative to germ cell contribution was therefore much higher than in the case of germ cells isolated from the genital ridge, but would still have been exceedingly small relative to the amount of lung tissue. Unless it was affected by the presence of this small admixture of cells from the mesentery, the timing of germ-cell entry into meiosis must have been cell-autonomous, regulated perhaps by some counting mechanism such as the number of mitotic cycles since germline lineage restriction (7–8 cycles).

6. MEIOSIS AND GERMLINE SEX DETERMINATION

Other papers in this symposium may suggest that somatic sex determination is rather flexible and not very strongly conserved in evolution. Germ-cell sex determination, on the other hand, is very closely linked in mammals with the initiation of meiosis, and the control of entry into meiosis one would expect to be highly conserved. However, when one looks at other species the situation is not clear. In *Caenorhabditis elegans* too, germ-cell sex determination is linked with the initiation of meiosis, or at least with the cessation of mitotic proliferation (Barton & Kimble 1990). There is a region of germ-cell proliferation in the vicinity of the distal tip cell that is common to both males and hermaphrodites. In males, germ cells beyond this region undergo spermatogenesis. In hermaphrodites, the first postmitotic germ cells undergo spermatogenesis although the germ cells that later leave the proliferative zone enter meiosis as oocytes. The control of mitotic proliferation and hence of entry into meiosis is exerted by two molecules: the signalling factor *lag*-2 (see Clifford *et al.* 1994) and its germ-cell receptor *glp*-1 (Austin & Kimble 1987, 1989). Both these genes have homologues in *Drosophila*, *delta* and *notch* respectively, but the expression of *delta* and *notch* is not confined to the germline (Artavanis-Tsakonos & Simpson 1991). I know of no mammalian homologues. In yeast, a variety of cyclin-dependent kinases and other molecules have been implicated in the initiation of meiosis. Mammalian homologues are being energetically pursued but to date the study of germ-cell sex determination in mammals still remains surprisingly untouched by molecular biology.

REFERENCES

Artavanis-Tsakonas, S. & Simpson, P. 1991 Choosing a cell fate: a view from the *Notch* locus. *Trends Genet.* **7**, 403–408.

Austin, J. & Kimble J. 1987 *glp-1* is required in the germ line for regulation of the decision between mitosis and meiosis in *C. elegans*. *Cell* **51**, 589–599.

Austin, J. & Kimble J. 1989 Transcript analysis of *glp-1* and *lin-12* homologous genes required for cell interactions during development of *C. elegans*. *Cell* **58**, 565–571.

Barton, M. K. & Kimble, J. 1990 *fog-1,* a regulatory gene required for specification of spermatogenesis in the germ line of *Caenorhabditis elegans*. *Genetics* **125**, 29–39.

Behringer, R. R., Finegold, M. J. & Cate, R. L. 1994 Mullerian-inhibiting substance function during mammalian sexual development. *Cell* **79**, 415–425.

Burgoyne, P. S. 1992 Y chromosome function in mammalian development. *Adv. dev. Biol.* **1**, 1–29.

Byskov, A. G. & Saxen, L. 1976 Induction of meiosis in foetal mouse testis *in vitro*. *Devl Biol.* **52**, 193–200.

Clifford, R., Francis, R. & Schedl, T. 1994 Somatic control of germ cell development in *Caenorhabditis elegans*. In *Soma-germline interactions* (ed. A. McLaren). *Semin. devl Biol.* **5**, 21–30.

De Felici, M. & McLaren, A. 1982 Isolation of mouse primordial germ cells. *Expl Cell. Res.* **142**, 477–482.

Eicher, E. M. & Washburn, L. L. 1986 Genetic control of primary sex determination in mice. *A. Rev. Genet.* **20**, 327–360.

Francavilla, S. & Zamboni, L. 1985 Differentiation of mouse ectopic germinal cells in extra- and peri-gonadal locations. *J. exp. Zool.* **233**, 101–109.

Gubbay, J., Collignon, J., Koopman, P. *et al.* 1990 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, Lond.* **346**, 245–250.

Hogg, H. & McLaren, A. 1985 Absence of a sex vesicle in meiotic foetal germ cells is consistent with an XY chromosome constitution. *J. Embryol. exp. Morph.* **88**, 327–332.

Levy, E. R. & Burgoyne, P. S. 1986 The fate of XO germ cells in the testes of XO/XY and XO/XY/XYY mouse mosaics: evidence for a spermatogenesis gene on the mouse Y chromosome. *Cytogenet. Cell Genet.* **42**, 208–213.

McLaren, A. 1978 Sexual differentiation in mammalian chimaeras and mosaics. In *Results and problems in cell differentiation. Genetic mosaics and cell differentiation* (ed. W. J. Gehring), pp 243–258. Berlin: Springer.

McLaren, A. 1984 Meiosis and differentiation of mouse germ cells. In *Controlling events in meiosis*. (ed. C. W. Evans & H. G. Dickinson), 38th Symposium of the Society of Experimental Biologists, pp. 7–23. Cambridge: Company of Biologists.

McLaren, A. 1991 Sex determination in mammals. *Oxf. Rev. reprod. Biol.* **13.** 1–33.

McLaren, A. 1992 Development of primordial germ cells in the mouse. *Andrologia* **24**, 243–247.

McLaren, A. & Monk, M. 1981 X chromosome activity in the germ cells of Sex-reversed mouse embryos. *J. Reprod. Fert.* **63**, 533–537.

McLaren, A. & Buehr, M. 1990 Development of mouse germ cells in cultures of foetal gonads. *Cell. Diff. Dev.* **31**, 185–195.

Monk, M. & McLaren, A. 1981 X-chromosome activity in foetal germ cells of the mouse. *J. Embryol. exp. Morph.* **63**, 75–84.

Munsterberg, A. & Lovell-Badge, R. 1991 Expression of the mouse anti-Mullerian hormone gene suggests a role in both male and female differentiation. *Development* **113**, 613–624.

Palmer, S. J. & Burgoyne, P. S. 1991 *In situ* analysis of foetal prepuberal and adult XX/XY chimaeric mouse testes: Sertoli cells are predominantly but not exclusively XY. *Development* **112**, 265–268.

Ozdzenski, W. 1972 Differentiation of the genital ridges of

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mouse embryos in the kidneys of adult mice. *Archiv. Anat. microscop. Morph. exp.* **61**, 267–278.

Tam, P. P. L., Zhou, S. & Tan, S.-S. 1994 X-chromosome activity of the mouse primordial germ cells revealed by the expression of an X-linked *lacZ* transgene. *Development* **120**, 2925–2932.

Vigier, B., Watrin, F., Magre, S. *et al.* 1987 Purified AMH induces a characteristic freemartin effect in foetal rat prospective ovaries exposed to it *in vitro*. *Development* **100**, 43–55.

Zamboni, L. & Upadhyay, S. 1983 Germ cell differentiation in mouse adrenal glands. *J. exp. Zool.* **228,** 178–193

Discussion

U. Mittwoch (*Department of Anatomy, Queen Mary & Westfield College, Mile End Road, London E1 4NS, U.K.*). After the germ cells have entered the genital ridges is their rate of proliferation the same regardless of whether they find themselves in a differentiating testis or ovary? The rate of cell proliferation could influence whether or not a germ cell enters meiosis because meiosis needs to be preceded by a long S-phase.

A. McLaren. On entering the genital ridges, germ cells in both female and male mouse embryos continue to proliferate at the same rate as during the previous migratory period (see P. Tam & M. H. L. Snow, 1981 *J. Embryol. exp. Morph.*). However, no information is available about the length of the cell cycle immediately preceding entry of female germ cells into meiosis, which is when a long S phase would be expected.

N. Josso (*Unité de Recherches sur l'Endocrinologie du Développement, Ecole Normale Supérieure, Département de Biologie, 1 rue Maurice Arnoux – 92120 Montrouge, France*). In relation to the role of AMH in meiosis retardation, the *in vitro* results of Vigier *et al.* 1987 (see *Development* **100**, 43) did not show a significant effect, but the germ cells were not happy in culture and many did not even reach the prophase of meiosis. More recent studies in transgenic mice have indicated that AMH does retard meiosis in foetal ovaries (see Lyet *et al.* 1995 *Biol. Reprod.* **52**, 444).

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