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# Somatic and Germ-Cell Sex in Mammals [and Discussion]

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# Somatic and germ-cell sex in mammals

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The phenotypic sex of an individual mammal is determined by the sex of its gonads, i.e. testes or ovaries. This in turn is determined by the presence or absence of a small region of the Y chromosome, located near the X–Y pairing region in man and on the short arm of the Y chromosome in the mouse. The testis-determining region of the Y appears to exert its primary effect by directing the supporting-cell lineage of the gonad to differentiate as Sertoli cells, acting at least in part cell-autonomously.

The phenotypic sex of a germ cell, i.e. whether it undergoes spermatogenesis or oogenesis, is determined at least in the mouse by whether or not it enters meiotic prophase before birth. This depends not on its own sex chromosome constitution, but on its cellular environment. A germ cell in or near normal testis cords (made up mainly of Sertoli cells) is inhibited from entering meiosis until after birth; one that escapes this inhibition will develop into an oocyte even if it is in a male animal and is itself XY in chromosome constitution.

## The sex-determining switch

Most animals have two sexual forms (e.g. female and male, or male and hermaphrodite), with rather few intermediates. The developmental decision between the two forms depends on a switch, which may be environmental, chromosomal or genic. For those animals (*Caenorhabditis*, *Drosophila*) for which the genetic basis of sex determination has been most thoroughly worked out, the switch activates a succession of sex-determining genes. The final gene in the series then call into play the various genetic loci concerned with the differentiation of sexual organs and structures.

In this symposium, Judith Kimble gives a brief review of how this whole system works in *Caenorhabditis*, with a passing glance at *Drosophila*. For both these groups, the switch consists of the X:autosome ratio; i.e., for a diploid chromosome set, two X chromosomes rather than one. Some genetic evidence exists that the sex-determining switch in birds too may be based on the X:autosome ratio (Sittmann 1984).

Mark Ferguson describes an environmental sex-determining switch used by some reptiles, namely the temperature at which the fertilized eggs are reared.

In mammals, a Y-linked gene is used as a switch. The central role of the Y chromosome in mammalian sex determination was first established in 1959. In that year Jacobs & Strong described the first human sex chromosome abnormality, an XXY chromosome constitution in a male patient; a few months later Ford *et al.* (1959) reported an XO chromosome constitution in a female patient; and Welshons & Russell (1959) reported that XO mice, like XX, were fertile females. As Welshons & Russell wrote 'Since a fertile female can be of the X/X or X/O constitution, it follows that the Y-chromosome of the mouse is male-determining. This may apply to other, perhaps all, mammals, including man.' This result was unexpected because in *Drosophila* XO individuals were known to be male and XXY individuals female.

The mammalian sex-determining genes that are activated by the Y genic switch are likely to be located on autosomes or on the X chromosome rather than on the Y. Ulrich Wolf's paper discusses various models for the genetic control of sex determination; Karl Fredga gives an example of X chromosomal involvement, as well as discussing some unusual variations on the basic XX/XY pattern; Eva Eicher deals with autosomal genes concerned with sex determination in the mouse.

## Testis determination and sex hormones

One distinctive feature of mammals is that sexual differentiation is mostly controlled, not by direct gene action, but by hormones. In the male, these sex hormones are produced largely by the testis. In a classic paper published in 1947, Jost showed that male rabbit foetuses deprived of their testes before birth develop female secondary sexual characteristics. An analogous situation is seen in testicular feminization (*Tfm*), in which female characteristics develop because the male target organs are unable to respond to the male sex hormones produced by the testis (Lyon & Hawkes 1970). Thus for most, if not all, of our sexual phenotype, sex determination is equivalent to testis determination. An intriguing exception is presented in the paper by Marilyn Renfree and Roger Short. Some sexual characteristics in marsupials appear to be determined directly by the sex chromosomes, by-passing the gonads (O *et al.* 1988); whether this will also hold true for eutherian mammals remains to be seen.

The hormonal links between testis and male phenotype have been exhaustively studied. In contrast, little or nothing is known of the links between the Y chromosomes and the testis, either at the molecular level or in terms of developmental process. One hypothesis that has been widely quoted is the 'H-Y hypothesis' (Wachtel *et al.* 1975), which postulated that the male-specific H-Y antigen, known to be controlled by the Y chromosome, acted as a diffusible inducer to switch cells of the indifferent gonad into the testicular pathway. Although initially very attractive (no other function for H-Y antigen was known, and no other inducer of testis determination had been proposed), this hypothesis is no longer tenable. Ellen Goldberg's paper reviews the history and present status of male-specific antigens in relation to testis determination.

## Sex-reversed mice

In the mouse, much of what we know about testis determination stems from the discovery by Cattanach *et al.* (1971) of a mutation generating XX males. This sex-reversed (Sxr) condition turned out to be due to a rearranged Y chromosome (Singh & Jones, 1982), in which the region containing the testis-determining gene (*Tdy*) appears to have been duplicated and transposed to the distal end of the long arm, beyond the region where the X and Y chromosomes pair. Because in every male meiosis a cross-over occurs between an X and a Y chromatid (Burgoyne 1982), one copy of the transposed region will be transferred to one of the X chromatids. When a sperm carrying the X with the transposed region attached fertilizes an egg, the resulting XX embryo develops as a male; hence the transposed region is now referred to as the Sxr region. However, the masculinizing effect of *Tdy* may be in part or whole negated by the preferentially expressed X-autosome translocation T16H. T16H/XSxr embryos may develop as fertile females in spite of the presence of Sxr (McLaren & Monk 1982). T16H/XSxr females and XX Sxr males express H-Y antigen (Simpson *et al.* 1984), so not only *Tdy* but also

*Hya*, the gene responsible for H-Y expression, must be located within the Sxr region. A variant form of the Sxr region, termed Sxr′, has been reported (McLaren *et al.* 1984), which retains *Tdy* but appears to have lost *Hya*. XXSxr′ mice are male, but do not express H-Y antigen. Because testis determination occurs in the absence of H-Y antigen, the H-Y hypothesis cannot be sustained.

But if H-Y antigen is not involved, what is the mechanism of testis determination? Alfred Jost's paper outlines what the development of the testis actually involves, at the cellular level; and Paul Burgoyne presents what we know of male-specific gene expression during early testis development.

Colin Bishop's paper describes molecular studies on the Sxr region, and establishes that the transition from Sxr to Sxr′ involved a small deletion. He and his colleagues have shown by *in situ* hybridization that the homologous region to Sxr on the normal Y chromosome is located on the very minute short arm (Roberts *et al.* 1988), a conclusion in full agreement with our own genetic findings (McLaren *et al.* 1988).

## The human Y chromosome

In man, the location of the testis-determining region on the short arm of the Y chromosome was first established by Jacobs & Ross (1966). The gene responsible for expression of H-Y antigen is now known to be on the long arm of the Y (Simpson *et al.* 1987), so in man as in the mouse, H-Y antigen cannot be the testis inducer. Immediately distal to the testis-determining region lies the region of X–Y pairing, where the single obligatory cross-over occurs during male meiosis. Genes located in or beyond this region are not completely sex-linked, but show recombination with sex ranging up to 50% near the telomere. They are therefore termed pseudoautosomal because in the extreme case they cannot be distinguished by linkage testing from autosomal genes.

Jean Weissenbach discusses how these different regions of the human Y chromosome have been mapped, with particular reference to the pseudoautosomal region; Malcolm Ferguson-Smith's paper illustrates the hazards of having the testis-determining region directly adjacent to the region of X–Y recombination; Peter Goodfellow describes in more detail the boundary region, comprising the proximal part of the pseudoautosomal and the distal part of the testis-determining region; and David Page tells how he and his colleagues have cloned a gene that seems likely to be *TDF*, the human testis-determining gene.

## Determination of germ-cell sex

The emphasis of the present meeting is on sex determination of the somatic component of the gonad. We often take for granted that male gonads contain male germ cells and female gonads contain female germ cells; it is therefore worth considering briefly how germ-cell sex is determined.

By germ-cell sex, I mean whether a primordial germ cell embarks on spermatogenesis, as in a normal testis, or oogenesis as in an ovary. The decision for most mammals is taken well before birth. In the mouse, all the germ cells in the ovary enter meiotic prophase up to a week before birth: there is thus no stem-cell population in the female, and no more germ cells are ever made. The germ cells in the testis enter mitotic arrest at the same stage of gestation as the


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ovarian germ cells enter meiosis. The male germ cells start dividing again immediately after birth and the first wave to enter meiotic prophase do so about a week later.

Whether a mouse germ cell embarks on oogenesis or spermatogenesis does not in any way depend on its own chromosome constitution. Its chromosomes may determine how well it does, how far it gets, but they do not determine in which direction it goes.

In a foetal testis, both XX and XO germ cells go into mitotic arrest rather than entering meiosis; XX die shortly after birth because of the presence of the second X chromosome, XO do better but few survive into the adult because they lack a Y chromosome gene required for normal spermatogenesis (Levy & Burgoyne 1986). Like *Hya*, this gene has been lost in the transition from Sxr to Sxr′ (Burgoyne *et al.* 1986), raising the possibility that H-Y antigen may itself play a role in spermatogenesis. In an XXSxr foetal testis, a few germ cells enter meiosis and develop as oocytes (McLaren 1980), probably because the testis is not entirely normal in its somatic development. They do rather better than the majority that take the male pathway, but they do not survive into the adult.

In the ovary of an XY female mouse, the XY germ cells may form oocytes that can be fertilized and give rise to normal progeny (Robin Lovell-Badge, personal communication). Even when the Y chromosome is entirely normal, as in a female XX↔XY chimera, the XY germ cells can enter meiosis and give rise to oocytes. T16H/XSxr females are fully fertile (McLaren & Monk 1982): both X chromosomes are known to be expressed during oogenesis, so evidently the presence of the testis-determining gene attached to an active X is fully compatible with normal oogenesis. In *Drosophila* too, sex-determining genes appear to control sex in somatic tissues only, and do not affect germ cells (Baker & Belote 1983); but in *Caenorhabditis*, as Judith Kimble makes clear in her paper, most sex-determining genes affect both somatic and germ-line tissues.

If it is not the chromosomes of the germ cells that determine their sex, it must be their environment, i.e. the environment provided by the gonad or its precursor, the genital ridge, in which they develop. What happens if we disturb that environment, or put them into a different environment? Disturbing the ovarian environment has little effect; if the germ cells survive, they enter meiosis on schedule and develop as oocytes. But if the genital ridge of a male mouse embryo is transplanted to the kidney or cultured *in vitro* from a sufficiently early age ($10\frac{1}{2}$ days *post coitum*, before Sertoli cell differentiation), some of the germ cells become diverted into the female pathway and undergo oogenesis (McLaren 1985). We can also examine germ cells outside the gonad, because during the course of their migratory phase, some germ cells fail to enter the gonads and end up either in the directly adjacent mesonephric region, or in the nearby adrenal primordium. In the adrenal, all the germ cells enter meiosis before birth and develop as oocytes even if the embryo is male; in the mesonephric region of a male embryo, some germ cells enter meiosis before birth but others enter mitotic arrest as they would in the testis (Zamboni & Upadhyay 1983; A. McLaren, unpublished observations). The XY germ cells in the male adrenal not only enter meiosis before birth, but having done so they follow the female pathway and develop into large growing oocytes surrounded by zona pellucidas. So for germ-cell sex, it seems that germ cells are female (that is they follow the female pathway of development) because they have entered meiosis before birth, rather than entering meiosis before birth because they are female.

The findings on germ cells in the mesonephric region and adrenal suggest that some diffusible signal coming from the testis is retaining the germ cells in the mitotic cycle, and


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inhibiting their entry into meiosis before birth. In the absence of the signal all germ cells, whether XX or XY, enter meiosis before birth and undergo oogenesis. Because the only differentiated cells in the testis at the critical stage of development are the Sertoli cells, the signal may be presumed to emanate from the Sertoli-cell population. The situation is formally similar to that described by Judith Kimble for *Caenorhabditis*, where the distal tip cell produces a signal that allows all germ cells within a certain range to continue in the mitotic cycle, but those out of range of the signal all enter meiosis.

Such analogies should not be pushed too far, yet surely the recent discovery of the germ-line proliferation gene (*glp*−1) in *Caenorhabditis* (Austin & Kimble 1987) must reinforce our view that students of sex determination in mouse and man have much to learn from the extensive and elegant analyses that have proved possible in the nematode.

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*Discussion*

M. A. Ferguson-Smith, F.R.S. (*Department of Pathology, University of Cambridge, U.K.*). Does Dr McLaren not agree that the recent observations by Page *et al.* (1987) that there is likely to be a functional homologue of *TDF* on the X chromosome in man, strongly suggest that sex is primarily determined by the number of active *TDF* loci rather than by a dominant Y-linked factor? In this scheme, X-inactivation results in one dose of TDF in females and two in males. This brings man back into line with *Drosophila* and *Caenorhabditis*.

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Anne McLaren. Although I can appreciate the attraction of bringing man 'back into line with *Drosophila* and *Caenorhabditis*', there seems little scientific justification at present for preferring a dosage rather than a dominant-male hypothesis for sex determination in mammals. I see no reason to expect the 'switch' mechanism to be evolutionarily stable: Hodgkin (1987) has pointed out that, even within *Caenorhabditis*, it only needs a mutation or two to shift the switch from X:autosome ratio to dominant male to dominant female to temperature dependence. Until further evidence is forthcoming (as it surely soon will be), I prefer to keep an open mind with regard to the four possible roles that Page *et al.* (1987) postulate for the putative *TDF* homologue that they have detected on the mammalian X chromosome.

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M. Adinolfi (*Paediatric Research Unit, Guy's Hospital, London, U.K.*). Dr McLaren has mentioned that Sertoli cells produce a factor that induces mitotic arrest. I wonder if Dr McLaren can tell us more about the physicochemical properties and characteristics of this factor? As she knows, it has been suggested that Sertoli cells release H-Y antigen capable of triggering the differentiation of the primordial gonads into testes. Paul Polani, Jo Zenthon and I have tried to repeat these experiments with little success. We have incubated mouse primordial gonads in serum-free media from mouse Sertoli cells cultured *in vitro*. The gonads were then transplanted under the kidney capsule of adult mice. When analysed histologically, we were able to observe a sort of arrest of the differentiation of the genotypically XX primordial gonads, but no evidence of testicular structures. I wonder if this effect may be attributed to the mitotic arresting factor Dr McLaren has mentioned?


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Anne McLaren. Because of the evidence that Burgoyne presents in this meeting, it is not surprising that Professor Adinolfi and his colleagues were unable to induce testis-cord formation in embryonic XX gonads by incubating them with Sertoli-cell-conditioned medium.

The arrest of differentiation of the XX gonads could have been due to the effect of anti-Müllerian hormone (AMH) known to be produced by Sertoli cells. Vigier *et al.* (1987), have recently shown that exposure to purified AMH *in vitro* causes depletion of germ cells in embryonic rat ovaries, and it is well known that follicle cells do not survive in the absence of oocytes. The gene for AMH has recently been cloned (Cate *et al.* 1986; Picard *et al.* 1986).

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