Development 101 Supplement, 143–149 (1987)
Printed in Great Britain © The Company of Biologists Limited 1987



# The use of Y-chromosome-specific repeated DNA sequences in the analysis of testis development in an XX/XY mouse

LALJI SINGH, SHOICHI MATSUKUMA and K. W. JONES

*Department of Genetics, University of Edinburgh, King's Buildings, West Mains Rd, Edinburgh EH9 3JN, UK*

## Summary

**A study, by means of Y-chromosome-specific repeated DNA probes, of mouse (ST) with small testes is reviewed. Mouse ST was shown to be a somatic mosaic of 10 % XY and 90 % XX cells. The cellular composition of the azoospermic testis reflected the overall proportions of XX and XY cells but it was found that XY cells predominated in the Sertoli cells of the testis tubules. These findings have been interpreted to indicate a fundamental role for the Sertoli cell in inducing testis organization in the indifferent gonadal rudiment, involving the expression of the Y chromosome.**

Key words: Y-chromosome, mosaicism, mouse, testis development, repeated DNA sequences, DNA, small testes (ST).

## Introduction

The role of the Y chromosome in testis development remains to be clarified. However, we have obtained some evidence (Singh, Matsukuma & Jones, 1987) to suggest that the Y chromosome may be required in the lineage that differentiates into the Sertoli cells. Two DNA probes were used to detect the Y chromosome in the cells of an XX/XY mosaic mouse. One probe (2(8)) consists of repeats of the tetranucleotide GATA (Singh, Phillips & Jones, 1984), which has previously been used to detect the sex-determining pericentromeric region of the mouse Y chromosome (Jones & Singh, 1981*a*,*b*; Singh & Jones, 1982). However, since GATA sequences are also found elsewhere in the mouse genome, a second mouse Y-specific clone (M34), whose sequences are distributed only on the Y chromosome but outside the sex-determining region, was also used. The study using 2[8] and M34 analysed the histological structure of testis in a mosaic mouse (ST) containing only 10 % of XY and 90 % of XX cells.

## The origin of the small testis mouse (ST)

The parents of ST developed from zygotes microinjected with Bkm-associated male-specific mouse DNA in an independent study of experimental sex reversal. ST, one of the second litter (4 males, 3 females), was found to have pale yellow small testes (4×2 mm) on dissection at the age of 30 days. Two male litter mates dissected on the same occasion showed normal-sized testes (6·0×4·0 mm, 7·0×4·0 mm) and all other siblings (43 males, 52 females) were anatomically normal. Other anatomical features of ST were normal, including accessory sex organs and epididymis.

## DNA analysis of the small testis mouse (ST)

Southern blots of ST liver DNA probed with 2(8) showed hybridization in the high molecular weight region (>23 kb) similar to that of a normal male but very much reduced in intensity (Fig. 1). This reduction could either have been due to inheritance of integrated male-specific DNA from the microinjected father in the absence of a Y chromosome, or to mosaicism of XY and XX cells. To resolve these two possibilities, ST DNA was probed with Y-specific repeated sequence probe M34 (L. Singh & K. W. Jones, unpublished data) which is not represented in the sex-determining region of the mouse Y chromosome or in the male-specific DNA used for microinjection of the parents. The hybridization pattern of M34 proved to be the same as that of control male DNA, except that the intensity of the hybridization was much reduced (not shown). This confirmed the presence of a Y chromosome in the ST genome and

suggested mosaicism as the cause of reduced intensity of hybridization. The proportion of Y-bearing cells in ST was estimated at 10% by comparison on a Southern blot with DNA from normal female and male mice mixed in various proportions (Fig. 2).

### Chromosome analysis

To confirm the presence of a Y chromosome, bone marrow chromosome preparations were examined using *in situ* hybridization with $^{3}$H-labelled 2(8) probe (Fig. 3). 100% (47/47) of control normal male preparations showed the expected single concentrations of autoradiographic grains in the pericentric region of the Y chromosome, which was also visible in 83% of interphase nuclei but absent in control female preparations. In contrast, out of a total of 140 chromosome preparations examined from ST, only 9% of euploid metaphases were found with high-density grains in the pericentric region of the Y chromosome.

### Histology of small testis

The testes from ST and a normal male litter mate were fixed in formalin and processed for histological study. The seminiferous tubules in the small testes on both sides showed a single layer of cells within the limiting membrane (Fig. 4A,B). The cytoplasmic processes of these cells extended into the lumen in

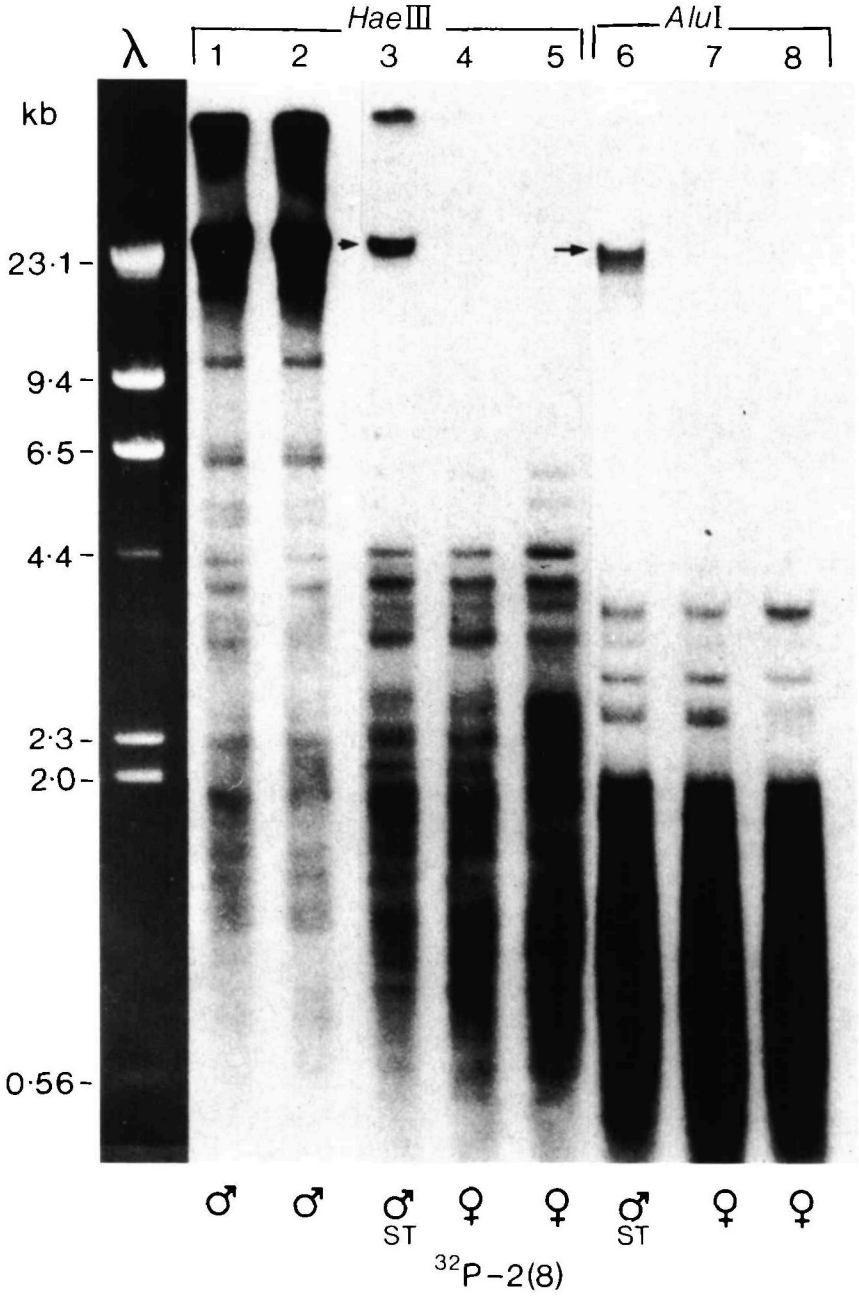


**Fig. 1.** Southern blot hybridization of *Alu*I and *Hae*III restriction digests of 10 μg aliquots of genomic DNA of ST mouse and several male and female litter mates with single-stranded $^{32}$P-2(8) probe. Note hybridization in the >23 kb region of male DNA (tracks 1 and 2) and its absence in female DNAs (tracks 4, 5, 7 and 8). ST DNA (tracks 3 and 6) shows conspicuously reduced >23 kb hybridization.

the manner of Sertoli cells and, although one or two degenerating cells which may have been spermatocytes were seen (arrow, Fig. 4B), sperm were absent. In contrast, the normal control testes showed well-developed seminiferous tubules filled with numerous spermatogenic cells and contained some sperm (Fig. 4C,D). The histiotypic composition of the interstitial cell population appeared to be normal, suggesting that Leydig cell differentiation was unaffected. However, the interstitial cell population was found to be considerably reduced compared with the normal sib testis.

### The XY cell content of the small testis

To assess the contribution of XY cells to the testis of ST, histological sections were studied by *in situ* hybridization with clone M34. Hybridization due to the Y chromosome was visible in about 70 % of cells distributed at random in the normal control testis sections (Fig. 5). This reflected the fact that 50 % of postmeiotic germ cells lack a Y chromosome and that the condensation of the Y chromosome may vary in different stages in chromosomal activity. In the ST testis sections, a Y chromosome could be detected by

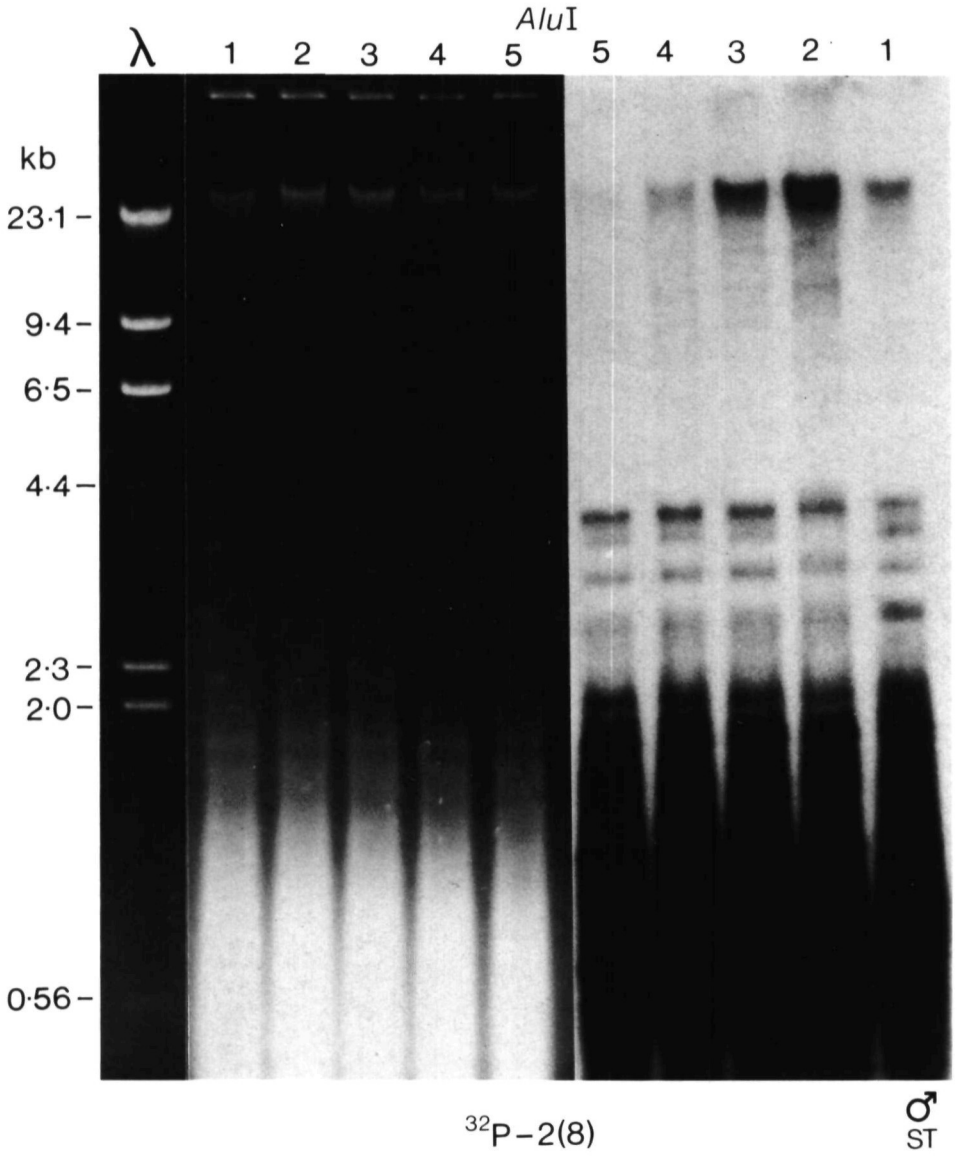


**Fig. 2.** Experiment to determine the proportion of Y-bearing cells in the ST mouse by mixing various proportions of male and female DNA for comparison with ST DNA after *Alu*I digestion and 2(8) hybridization. Left panel shows the ethidium-bromide-stained gel and *Hin*dIII size marker. Left panel: track 1, 10 μg ST DNA; track 2, 8 μg female + 2 μg male DNA; track 3, 9 μg female + 1 μg male DNA; track 4, 9·5 μg female + 0·5 μg male DNA; track 5, 9·75 μg female + 0·25 μg male DNA. Note the comparable intensity of >23 kb hybridization in tracks 1 and 3, indicating that ST contains approximately 10 % of Y-bearing cells.

*in situ* hybridization in cells that were dispersed amongst a majority population of Y-negative cells (Fig. 6), consistent with the somatic proportion of 10% XY cells in the liver. However, the distribution of cells in which the Y chromosome hybridized unequivocally was found not to be random but to predominate in the testis tubules in what appeared to be Sertoli cells. Since in the normal sib no such histological difference was observed in the distribution of cells in which a Y chromosome could definitively be detected, it was concluded that selection had occurred in favour of XY cells within tubules of ST. It was impossible to determine whether this represented preferential recruitment or survival of XY cells. The proportions of XY cells in different tubules appeared to be highly variable.

## Discussion

Previous work has shown clearly that XX/XY chimaeras develop as males (McLaren, 1972). However, much remains to be done in order to understand fully

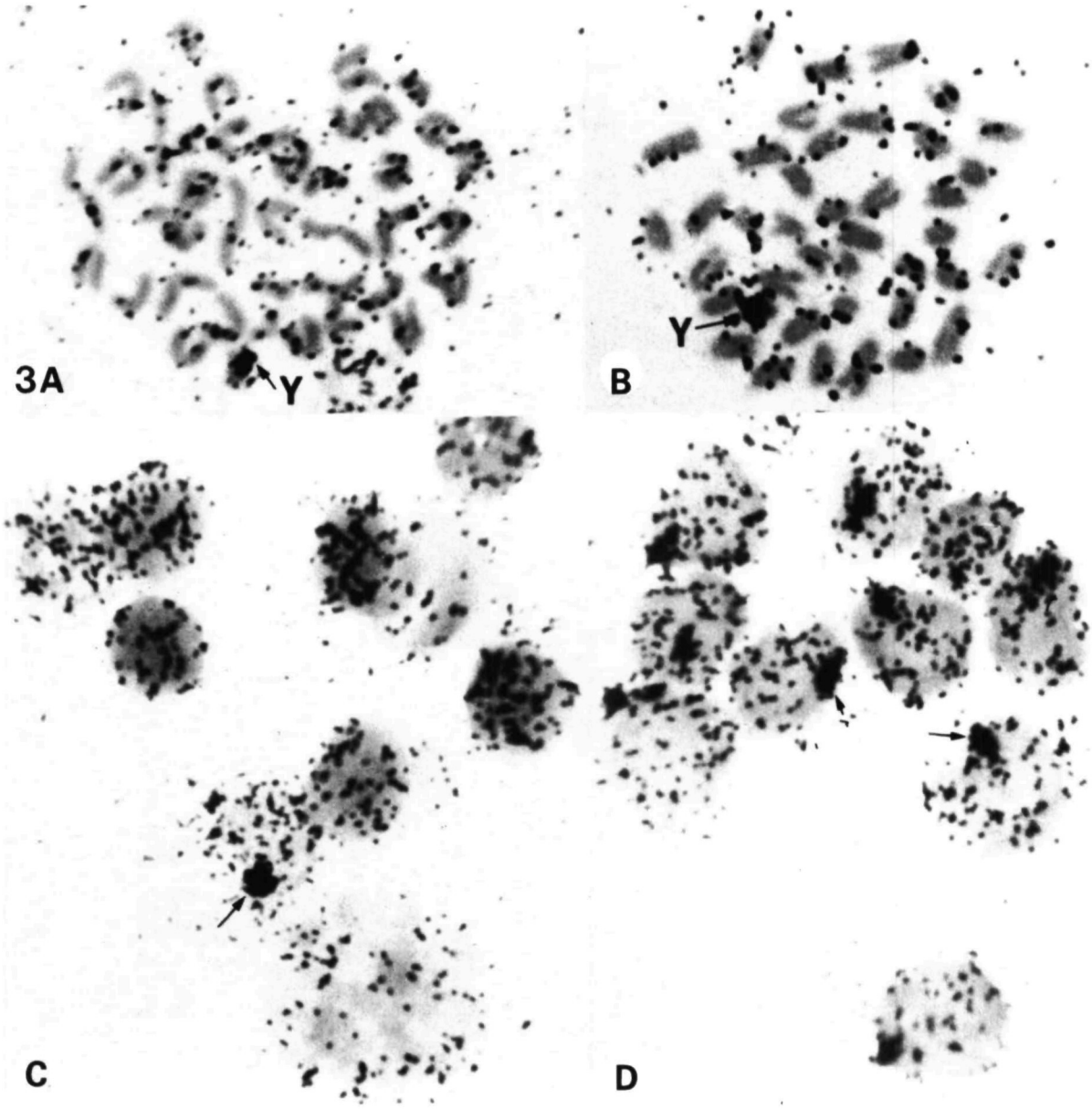


**Fig. 3.** Chromosomes and interphase nuclei of control normal male and ST male mouse hybridized *in situ* with $^{3}$H-labelled 2(8) probe. (A) A Y-bearing metaphase spread of ST mouse, note hybridization concentrated on the Y chromosome. (B) Metaphase spread of normal male mouse, arrow points to Y chromosome. (C) Interphase nuclei of ST mouse, note only one out of eight shows a cluster of grains compatible with the presence of the Y chromosome. (D) Interphase nuclei of normal male mouse, note most nuclei show a cluster of grains indicating the Y chromosome.

the effects of XX/XY mosaicism on testicular development. As shown here, Y-chromosome-specific probes can be useful in this type of study. The results obtained with the ST mouse show that the somatic proportion of XY cells, determined from the liver, was reflected in the proportion of XY cells making up the testis soma. This suggests that the uncommitted embryo cell mass consisted of a random mixture of XX and XY cells and that 10% of male cells is sufficient to trigger testis development and to induce basic testis architecture. This conclusion is consistent with the rarity of hermaphrodite and female phenotypes in experimentally produced XY–XX chimaeric mice and supports a previous conclusion that most, but not all, sex-chromosome chimaeras develop as males (McLaren, 1972). Whether primordial germ cells that migrate into the genital ridges at the time of gonadal differentiation operate as the primary inductor has not yet been settled in mammals. The histological structure of the testis tubules in the ST mouse, however, suggested that the cellular content was mainly of Sertoli cells together with a few poorly defined germ cells in which it was not possible to be certain of the genotype. There may have been interference with spermatogenesis by the environment established by the predominating XX testis somatic cells, consistent with previous findings of premature meiosis and degeneration of XY germ cells in sex-chromosome chimaeras (McLaren, 1972). Alternatively, there may have been a scarcity of XY germ cell precursors. XX cells are unable to undergo spermatogenesis but oocytes have been reported in the testes of sex-reversed $XX_{Sxr}$ mice (McLaren, 1980). However, these do not persist and, since none were

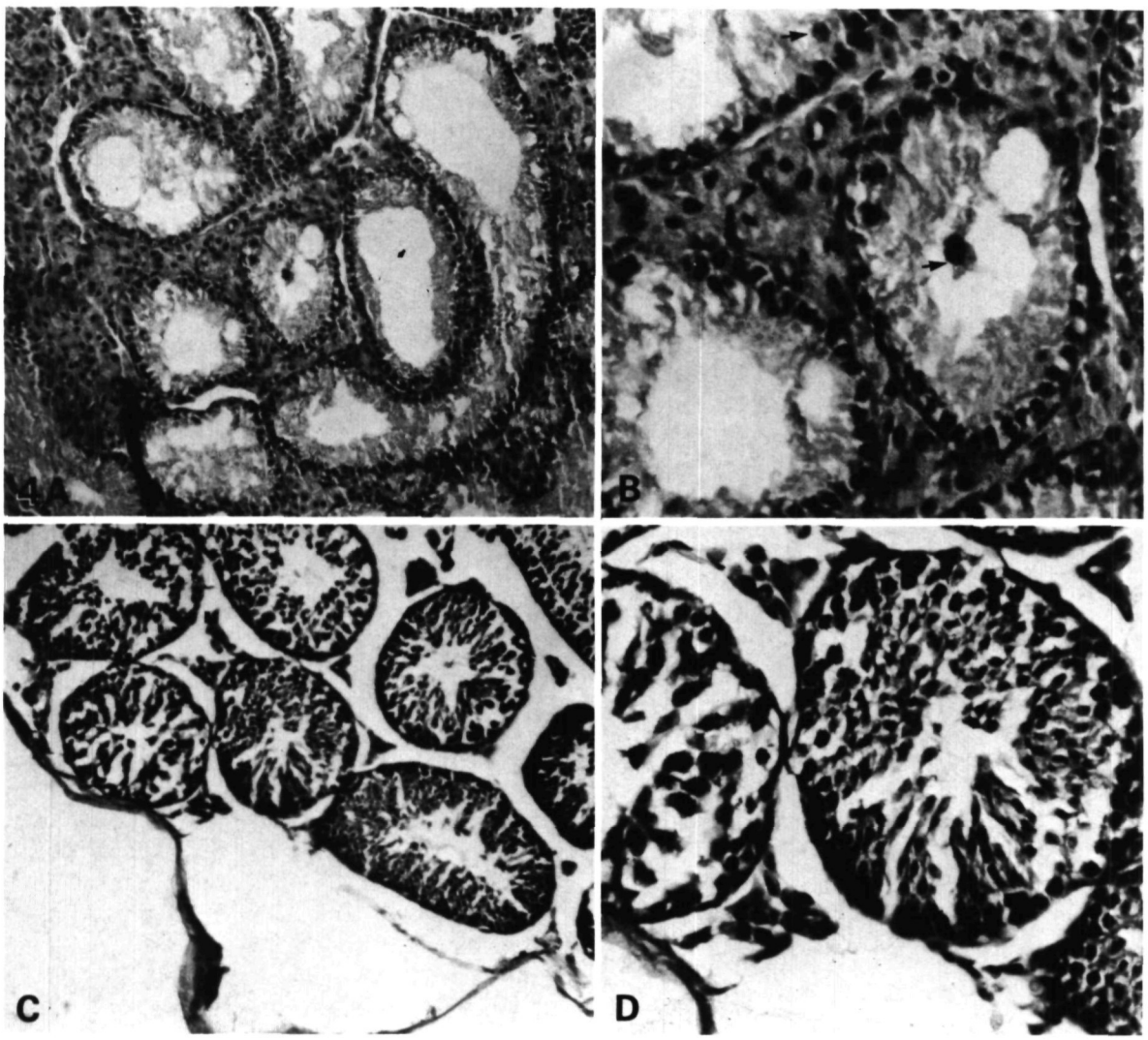


**Fig. 4.** Histological sections of testes. (A) ST mouse showing single-layered seminiferous tubules. Note paucity of germ cells and absence of spermatids and spermatozoa. (B) Higher magnification of A; arrows indicate degenerating cells which may have been spermatocytes. (C) Normal litter mate of ST showing well-developed seminiferous tubules with numerous spermatogonia. (D) Higher magnification of C.

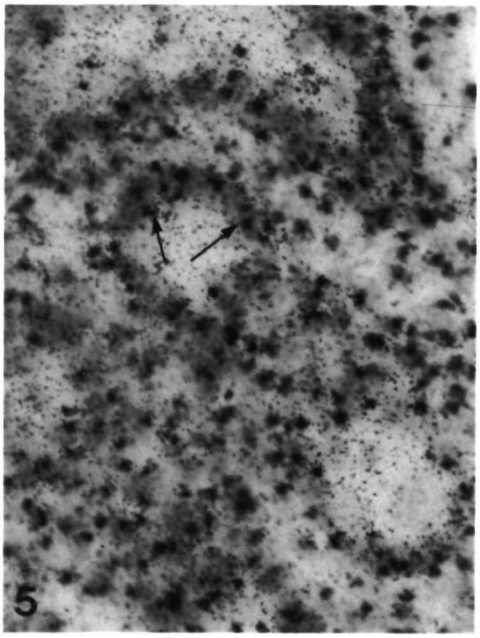

**Fig. 5.** Frozen section of testis of a normal male mouse hybridized *in situ* with $^{3}$H-labelled probe M34. Arrows indicate clustering of grains in the nuclei of cells containing a Y chromosome.

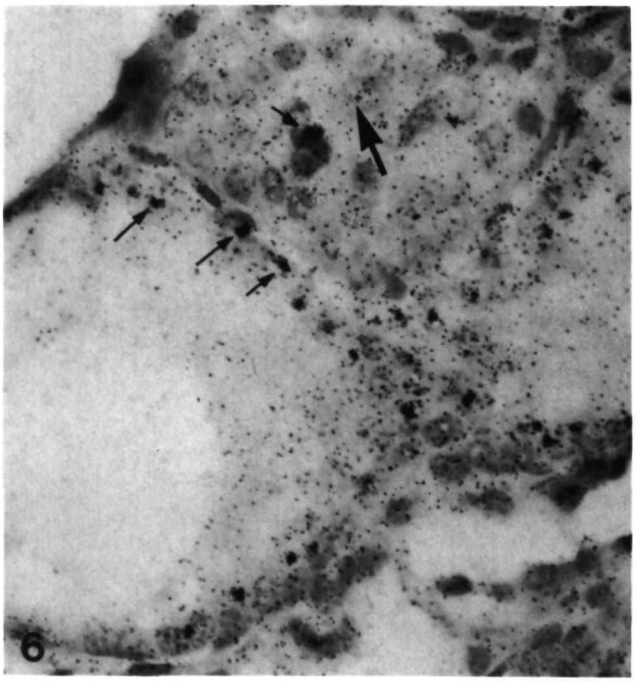

**Fig. 6.** Frozen testis section of ST mouse hybridized as in Fig. 5 showing a minor population of XY (hybridization to Y chromosome indicated by small arrows) cells amongst XX cells. There is a predominant location of XY cells within the testis tubules. The large arrow indicates an area of interstitial cells lacking a Y chromosome.

positively identified in ST, it was suggested that they may have already degenerated by the time this mouse was examined.

Whilst there was no evidence of overall recruitment of XY cells to form the testis itself, the proportion of definitive XY cells was distinctly higher in testis tubules when compared with the surrounding testis interstitial cell population. This suggested either preferential recruitment of Y-bearing cells during tubule formation or their differential survival in the tubule environment. This observation accords with the fact that the sexual genotype of the cell is functionally important for normal germline differentiation, although it is not clear in which cells, or even which tissue, the decisive influence of the Y chromosome is exercised. However, since the XY cells in the ST testis sections were mainly Sertoli cells in the testis tubules, these cells must be considered as the probable locations in which the essential expression of Y-chromosome primary sex genes occurs. This would explain the longstanding observation that the formation of Sertoli cell cords appears to be the basic event in testicular differentiation. Thus, whilst these observations on ST confirm that the Leydig cell can differentiate in the absence of a Y chromosome, they suggest that the presence of a Y chromosome may be critically important for the differentiation and function of the Sertoli cell. The primary function of the Y chromosome in directing the indifferent gonadal primordium into the testis pathway may therefore be expressed through the Sertoli cell precursors whose differentiation may depend upon the presence of a competent Y chromosome.

According to Eicher, Washburn, Whitney & Morrow (1982), the Y chromosomal gene *Tdy* suppresses the pathway to ovarian differentiation and at the same time activates testicular differentiation under the control of autosomal sex genes. Since this regulatory influence can extend to XX cells and be trans-specific (Muller, Singh, Grund & Jones, 1982), the Y-chromosomal regulatory gene must indirectly influence XX cells in the tissues of sex-chromosome chimaeras and mosaics by an evolutionarily conserved pathway. Whether HY antigen is the paracrine factor in testicular differentiation (Wachtel, Ohno, Koo & Boyse, 1975) has been questioned by the finding, in a variant of the sex-reverse mutation *Sxr* (*Sxr'*), that HY antigen deficiency presents no barrier to male development (McLaren *et al.* 1984). It is therefore presently uncertain what induces XX cells to participate in testis development.

The origin of the ST phenotype is unknown. Data from liver DNA and *in situ* hybridization to testis sections using the Y-specific probe M34 suggested that all tissues may have consisted of the same proportion of 90 % XX and 10 % XY cells. This may

have come about either chimaerically by the fusion of XX and XY embryos or by mosaicism. The former explanation could invoke the fact that one or other embryo cell population may be selectively discouraged (Tarkowski, 1961, 1963). The latter explanation would require two events such as the founder cell of the XX clone doubling its X chromosome while simultaneously losing its Y chromosome.

The work reviewed here was carried out with the financial assistance of MRC Grant No. G8306230CB to K.W.J.

## References

EICHER, E. M., WASHBURN, L. L., WHITNEY, J. B. & MORROW, K. E. (1982). *Mus poshiavinus* Y chromosome in the C57BL/6J murine genome causes sex reversal. *Science* **217**, 535–537.

JONES, K. W. & SINGH, L. (1981*a*). Conserved sex associated repeated DNA in vertebrates. In *Genome Evolution*, vol. 20 (ed. G. Dover & R. Flavell), pp. 135–154. London: Academic Press.

JONES, K. W. & SINGH, L. (1981*b*). Conserved repeated sequences in vertebrate sex chromosomes. *Hum. Genet.* **58**, 46–53.

MCLAREN, A. (1972). Germ cell differentiation in artificial chimeras of mice. In *Proc. Int. Symp. on The Genetics of the Spermatozoon* (ed. Cr. A. Beaty & S. Gluecksohn-Waelsch). Edinburgh.

MCLAREN, A. (1980). Oocytes in the testis. *Nature, Lond.* **283**, 688–689.

MCLAREN, A., SIMPSON, E., TOMONARI, K., CHANDLER, P. & HOGG, H. (1984). Male sexual differentiation in mice lacking H-Y antigen. *Nature, Lond.* **312**, 552–555.

MULLER, U., SINGH, L., GRUND, S. & JONES, K. W. (1982). Ovarian cells participate in the formation of tubular structures in Mouse/Rat heterosexual gonadal cocultures: A direct demonstration by *in situ* hybridization. *Differentiation* **22**, 136–138.

SINGH, L. & JONES, K. W. (1982). Sex reversal in the mouse (*Mus musculus*) is caused by a recurrent nonreciprocal crossover involving the X and an aberrant Y chromosome. *Cell* **28**, 205–216.

SINGH, L., MATSUKUMA, S. & JONES, K. (1987). Testis development in a predominantly XX mosaic mouse. *Devl Biol.* **122**, 287–290.

SINGH, L., PHILLIPS, C. & JONES, K. W. (1984). The conserved nucleotide sequences of Bkm which define Sxr in the mouse are transcribed. *Cell* **36**, 111–120.

TARKOWSKI, A. K. (1961). Mouse chimaeras developed from fused eggs. *Nature, Lond.* **190**, 857–860.

TARKOWSKI, A. K. (1963). Studies on mouse chimaeras developed from eggs fused in vitro. *Natn. Cancer Inst. Monogr.* **11**, 51–71.

WACHTEL, S. S., OHNO, S., KOO, G. C. & BOYSE, E. A. (1975). Possible role for HY antigen in the primary determination of sex. *Nature, Lond.* **257**, 235–236.