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26 January 1982; revised 10 May 1982

Mus poschiavinus Y Chromosome in the C57BL/6J Murine Genome Causes Sex Reversal

Abstract. *When the Y chromosome from* Mus poschiavinus *(Y^{POS}) is transferred onto the C57BL/6J genome, XY individuals develop as females with two ovaries, or as hermaphrodites. No XY individual develops normal testes. Although C57BL/6J-Y^{POS} XY females are rarely fertile, most hermaphrodites with normal male genitalia sire offspring. Thus, the* Mus poschiavinus *Y chromosome carries a form of the Y-linked testis-determining locus different from that present in the C57BL/6J inbred strain. This gene interacts abnormally with autosomal or X-linked testis-determining loci of the C57BL/6J genome to prevent normal testicular differentiation. Divergence of the Y-linked testis-determining gene may be involved in mammalian speciation.*

In mammals, the presence of a Y chromosome (XY, XYY, XXY) causes the undifferentiated gonad to develop as a testis. Individuals lacking a Y chromosome (XX, XO) develop ovaries. The simplest explanation for this is that one or more Y-linked genes are involved in testis determination, and in their functional absence, the uncommitted gonad develops as an ovary (*1*). Not surprisingly, exceptions to this hypothesis have been reported.

In wood lemmings, some XY individuals develop functional ovaries (*2*). This sex reversal is caused by an X-linked gene that overrides the Y-linked testis-determining gene (*3*). The gene "polled" in goats is an example of an autosomal mutation that, in the homozygous state, causes development of testicular tissue in the XX individual (*4*). What seems clear from these examples is that there are autosomal and X-linked genes involved in primary sex (gonad) determination. This conclusion does not conflict with the hypothesis that a Y-linked locus is responsible for testis induction if we envision that the Y-linked gene is the first gene (or one of the first) that functions in the series of genetic events necessary for testis development.

We report the discovery that the transfer of the Y chromosome from the mouse species *Mus poschiavinus* (*5*) to the genome of the C57BL/6J inbred strain causes disruption of the normal testis determination process and results in partial or complete sex reversal of XY individuals. We hypothesize that the Y-linked testis-determining locus carried by *M. poschiavinus* is significantly different from that carried on the Y chromosome of C57BL/6J and functions abnormally when present in the C57BL/6J genome.

While we were transferring onto the inbred mouse strain C57BL/6J a triethylenemelamine-induced α-thalassemia (*6*) that had occurred in a male from the inbred strain designated POS A [see (*7*) for origin of POS A], we noticed that two males sired an excess of female offspring. The N3 backcross generation male produced 69 females and 28 males; the N4 backcross generation male sired 19 females and 2 males. Their common N2 ancestor had sired 22 females and 11 males. Closer inspection of a number of adult offspring from the N3 and N4 generation males revealed that many sons were true hermaphrodites (both ovarian and testicular tissue were present) and that some daughters, although morphologically normal females, were chromosomally XY (*8, 9*). Because the N3 and N4 backcross generation males were related and produced XY hermaphrodites and XY females, we suspected both carried a mutation that interfered with primary sex determination.

Inheritance of the hypothesized mutation was determined as follows. The N3 backcross generation male, number 1643, was mated to several C57BL/6J females. Offspring were classified at weaning as females if they had normal-appearing external female genitalia, including mammae-associated pigment, or as males if they had normal external male genitalia. Offspring were classified as abnormal if they had ambiguous genitalia (for example, hypospadias, an underdeveloped scrotal sac, or an enlarged clitoris), with or without mammae-associated pigment, or if they had normal male genitalia and mammae-associated pigment. The chromosome constitution (*8, 9*) of a number of the females was ascertained. Some offspring were autopsied as adults, the type and condition of their internal sex organs were noted, and their gonads were histologically analyzed. Male 1643 sired 31 female, 17 male, and 11 abnormal offspring (Table 1). Of the 11 females karyotyped, 9 were XX and 2 were XY. These results rule out autosomal recessive or X-linked inheritance of the mutation because XY females and hermaphrodites were produced by male 1643 when he was mated to unrelated females.

To determine whether the mutation was inherited as an autosomal dominant or Y-linked locus, we mated 14 sons and 9 XX daughters of male 1643 and 4 XX daughters of a son of male 1643 known to carry the mutation (number 08 in Table 1) to mice of the C57BL/6J strain. None of the 13 XX females produced externally abnormal XY individuals, and the sex ratio of the progeny was 1:1 (77 females and 80 males), suggesting that they did not carry the mutation. Ten sons produced XY females or morphologically abnormal offspring, or both, indicating that all ten males carried the mutation (Table 1). Three other males were shown indirectly to carry the mutation: male 06 proved on autopsy to be a hermaphrodite, and sons from males 10 and 13 produced XY female offspring when mated to a C57BL/6J female. Another male, number 03, died after siring one litter of nine females, none of which was karyotyped. Because the probability of a normal male siring nine daughters and no sons is highly unlikely ($P = .002$), male 03 probably carried the mutation. In ad-

0036-8075/82/0806-0535$01.00/0

dition to the tabulated data presented in Table 1, 30 other male descendants of male 1643 have now been mated to C57BL/6J females. These males have either sired XY females and hermaphrodites or, if sterile, were found on autopsy to be true hermaphrodites. All of the above results are compatible with Y linkage, but not with autosomal dominant inheritance.

Because the POS A male ancestor of male 1643 had been treated with triethylenemelamine, the possibility existed that the inherited Y-linked sex reversal was induced by a mutagen. To test this, we mated a C57BL/6J female to an untreated POS A male and backcrossed two of the F_1 sons to C57BL/6J females. Both F_1 males sired XY females and hermaphrodites, indicating that the POS A Y chromosome carried the variant gene. We designate this Y-linked locus (or Y-linked loci) as Y-linked testis determining (symbol *Tdy*) and denote the variant *Tdy* locus and the Y chromosome carried by POS A as Tdy^{POS} and Y^{POS}, respectively, where POS stands for *M. poschiavinus*, the origin of the POS A Y chromosome (*7*).

A gross anatomical examination was conducted on 309 XY and 148 XX adult mice ranging in age from 1 to 27 months. These mice were from the N4 to N7 generations of backcrossing the Y^{POS} chromosome onto the C57BL/6J strain. On the basis of external morphology, as previously defined, all XX mice were normal females. Of the 309 XY mice, 118 were female, 112 were male, and 79 were externally abnormal. Examination of their gonads revealed that 117 of the XY females had two ovaries; the other female had one ovary and one ovotestis. The gonads of the 88 externally normal male mice appeared to be normal, except that often one or both testes were smaller than those of adult C57BL/6J males. The 79 externally abnormal and the remaining 24 male-appearing mice were overt hermaphrodites possessing bilateral ovotestes or an ovotestis accompanied by an ovary. Because of the fetal data presented below, we believe that the testes observed in the 88 normal-appearing males were ovotestes. A preliminary histological examination of these gonads supports this conclusion.

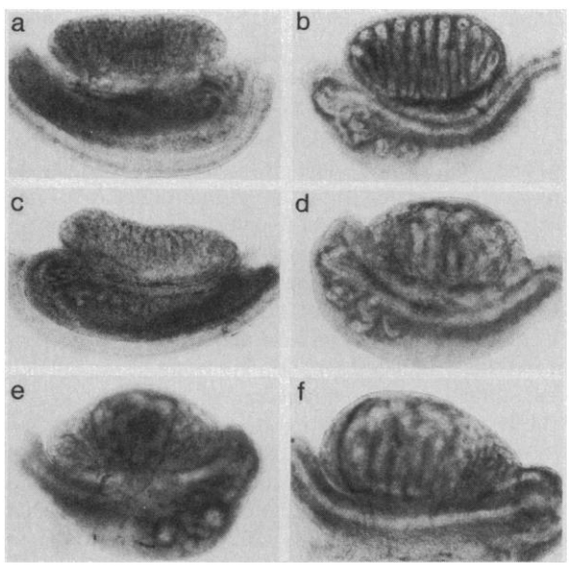

Fig. 1. Appearance of gonads from 14½- to 16-day-old C57BL/6J-Y^{POS} fetuses. Each freshly dissected gonad with attached mesonephros was photographed with a Zeiss inverted microscope. (a) Ovary from XX fetus. (b) Testis from normal C57BL/6J male fetus. (c) Ovary from XY female. (d to f) Ovotestes from XY hermaphrodites, each containing areas of ovarian and testicular tissue.

Table 1. Breeding record of male 1643 and of 14 of his sons. Offspring are characterized as male, female, or abnormal according to external genitalia. N.A., chromosomes not analyzed.

Generation		Female			Male	Abnormal
N3	N4	XX	XY	N.A.	N.A.	N.A.
1643		9	2	20	17	11
	03	0	0	9	0	0
	04	5	2	0	4	5
	05	21	6	0	6	10
	06* Sterile	–	–	–	–	–
	07	8	1	0	7	10
	08*	4	1	21	4	4
	10	3	0	0	1†	0
	11	17	9	1	4	7
	12	3	1	0	2	3
	13	0	0	26	2†	0
	17	0	0	2	0	1
	18*	0	0	16	1	1
	19*	0	0	7	3	1
	20*	17	4	0	5	6

*On autopsy found to be an overt hermaphrodite. †Produced XY females and hermaphrodites when mated to C57BL/6J females.

To better characterize primary sexual development in C57BL/6J-Y^{POS} mice (*10*), we analyzed gonads from fetuses 14½ to 16 days of age. A previous study has shown that at this stage of gonad development a small area of ovarian (or testicular) tissue in an ovotestis can be easily detected (*11*). A total of 80 fetuses were karyotyped (*12*) and phenotypically classified as female, male, or hermaphrodite on the basis of gonadal architecture (Fig. 1). Forty-two fetuses were chromosomally XX and possessed two normal-appearing ovaries. The other 38 fetuses were chromosomally XY; of these, 16 had two ovaries, 13 had two ovotestes, and nine had one ovary and a contralateral ovotestis. No individual had a normal testis. In addition, we noted no abnormalities of the Y chromosome (G-banded) nor did we find evidence of an XO cell population in any of the XY fetuses.

It was of interest to determine if XY^{POS} females are fertile. Eight XY^{POS} females were placed with normal C57BL/6J males. All eight mated, as evidenced by the presence of mating plugs; however, only one female produced a litter—two males. Unfortunately, the origin of the Y chromosome (Y^{POS} or Y^{B6}) in these males could not be determined. To date, no further offspring have been observed from XY^{POS} females, suggesting that, with rare exceptions, these females are sterile. A preliminary histological study shows that sterility is caused by a rapid depletion of germ cells; ovaries from 4-week-old XY females appear normal, but by 8 weeks of age few, if any, germ cells remain.

We conclude that abnormal testis development occurs in XY mice whose autosomes and X chromosome are derived from the C57BL/6J inbred strain and whose Y chromosome is derived from *M. poschiavinus*. We hypothesize that *M. poschiavinus* carries a variant allele (compared to C57BL/6J) of the Y-linked testis-determining locus. Implied in this hypothesis is the presence of an autosomal or X-linked testis-determining locus (or loci) that interacts with the Y-linked locus (or loci) for normal testis determination and differentiation to pro-

ceed. We envision that variant alleles for the autosomal or X-linked locus (or loci) also exist between *M. poschiavinus* and C57BL/6J, and it is the mismatching of these genes with the Y-linked locus that causes the disruption of the initial testis determination steps.

Further investigation is under way to determine whether the *M. poschiavinus* Y-linked testis-determining gene functions abnormally when transferred to other inbred strain backgrounds (for example, AKR/J, DBA/2J, and BALB/cBy), because the possibility exists that only C57BL/6J carries X-linked or autosomal genes that interact abnormally with the Tdy^{POS} gene. In addition, experiments are in progress to determine whether the *M. poschiavinus* Tdy^{POS} gene functions normally when transferred to other *M. domesticus*, as well as *M. musculus*, genomes. These results will be of special interest because mutations that disrupt the testis determination pathway could be an effective mechanism for initiating speciation.

Mice from the C57BL/6J-Y^{POS} strain will be extremely useful for testing hypotheses concerning sex determination. For example, Wachtel *et al.* (*13*) suggested that the transplantation antigen molecule called H-Y initiates testis development in the uncommitted gonad. In conflict with this hypothesis is our finding that C57BL/6J-XY^{POS} individuals with two ovaries are positive for H-Y as determined by skin grafting, cell-mediated lympholysis, and the popliteal lymph node assays (*14*). Finally, the availability on a common genetic background (C57BL/6J) of two Y chromosomes that differ at their testis-determining locus will be instrumental in identifying Y-linked DNA sequences involved in primary sex determination.

Note added in proof: We have found that three Y chromosomes derived from *M. domesticus* cause sex reversal after transfer to the C57BL/6J genome. The three Y chromosomes were from mice trapped in northern Italy (Alpi Orobie, near Bergamo), central Italy (Apennine, Molise), and southern Italy (Lipari, Isole Eolie). In all cases, XY hermaphrodites were recovered in the first backcross generation. Thus, the Y-linked sex reversal phenomenon observed with the Y^{POS} chromosome is the result of transferring the *M. domesticus* Y chromosome to the C57BL/6J genome.

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27 November 1981; revised 20 April 1982

Intracellular Oxidation-Reduction State Measured in situ by a Multichannel Fiber-Optic Surface Fluorometer

Abstract. *The principles of the measurement in vivo of the oxidation-reduction state of intramitochrondrial pyridine nucleotides were used in establishing a multichannel fluorometer-reflectometer. This approach made possible the study of changes of mitochrondrial redox states in four different organs (brain, liver, kidney, and testis) of the same animal, as well as the monitoring of four different cortical areas of the same brain hemisphere. In the measurement of reduced nicotinamide adenine dinucleotide fluorescence, oximetric and movement artifacts are negligible, but blood volume changes and tissue absorption properties are a source of error. The corrected fluorescence is obtained by subtracting the reflectance from the fluorescence signal in 1:1 ratio. During graded hypoxia, the corrected fluorescence showed a gradual increase and was maximal during anoxia in all four organs tested.*

The first detailed study on surface microfluorometry of organs in situ was reported in 1962 by Chance *et al.* (*1*). Since then, the same basic approach has been used to study the oxidation-reduction states of various tissues in various animal models, including the human brain [for review, see (*2*)]. Because there have been discrepancies in results, as well as in their interpretation (*3*, *4*), we discuss in detail results obtained with fiber-optic fluorometry-reflectometry during the last 9 years.

Since the first light-guide fluorometer was built at the end of 1972, fiber optics have been used in various types of fluorometers (*5*, *6*). The direct-current (d-c) fluorometer-reflectometer containing a Y-shaped light guide has been of value in most of the studies in which reduced nicotinamide adenine dinucleotide (NADH) fluorescence is measured. Mayevsky and Bar-Sagie (*7*) described the use of the two-channel d-c fluorometer-reflectometer with dual Y-shaped light guides in the study of brain energy metabolism. We now describe the use of the four-channel d-c fluorometer-reflectometer to monitor four different organs and also to monitor four different locations on the same organ.

The principles of the single-channel d-c fluorometry are shown schematically in Fig. 1. In the present four-channel fluorometer, this unit was quadrupled, and small variations were made in the light guide, as described below. The light source was a 100-W water- or air-cooled mercury arc having a 366-nm filter in front of it between the fluorometer and the excitation bundle of the fibers. The light guide contains four bundles of excitation fibers split from the light source; another four bundles of fibers transmitting the emitted light form four Y-shaped light guides. We used quartz fibers having a diameter of 2 mm in each common part, as well as plastic ones having a diameter of 0.8 mm in each common part. The emitted light from the tissue

0036-8075/82/0806-0537$01.00/0