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Autosomal Genes Involved in Mammalian Primary Sex Determination [and Discussion]

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Autosomal genes involved in mammalian primary sex determination

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Beginning with findings made during the late 1950s and early 1960s, evidence continues to accumulate in support of the hypothesis that the mammalian Y chromosome carries a gene that induces the undifferentiated foetal gonad in XY individuals to develop as a testis. Recently a DNA sequence has been isolated from the human Y chromosome that appears to be the hypothesized Y-linked testis-determining gene, and advances have also been made toward identifying genes that interact with the Y-linked testis-determining (*Tdy*) gene to initiate testis formation. These loci have been identified in specific stocks of mice carrying the mutant T^{hp} or T^{Orl} allele at the *T* locus located on chromosome 17, and in crosses involving the transfer of a Y chromosome from two populations of *Mus domesticus* into the genomes of specific inbred strains of mice. The data in both cases support the hypothesis that there are several loci involved in testis determination and that abnormal interaction of these loci disrupts initiation of testis determination, resulting in development of ovarian tissue in XY individuals.

Introduction

Mammalian sex determination is one of the most interesting, yet least understood, developmental systems. The first suggestion that sex determination in mammals was under genetic control different from that in *Drosophila* came from discoveries made in the late 1950s that XO mice (Russell *et al.* 1959) and humans (Ford *et al.* 1959) develop ovaries. This information led to the hypothesis that the mammalian Y chromosome carries at least one gene involved in directing the bipotential foetal gonad to develop as a testis and the functional absence of this gene results in ovarian development (Welshons & Russell 1959). This hypothesis was further strengthened by the findings that XXY mice (Cattanach 1961; Russell & Chu 1961) and humans (Jacobs & Strong 1959) develop testes. In 1966 Jacobs & Ross (see review by Davis 1981) presented evidence that the Y-linked testis-determining locus was located on the short arm of the human Y chromosome, and Singh & Jones (1982) reported that this locus was located near the centromere on the mouse Y chromosome. Recently, Page *et al.* (1987) isolated a coding sequence from the short arm of the human Y chromosome that is absent in sex-reversed XY women and present in sex-reversed XX men, suggesting that this DNA sequence is the Y-linked testis-determining gene. Moreover, this Y-derived sequence is highly conserved in mammals, suggesting that it is involved in an important biological function. Also, recent unpublished work from A. McLaren's and C. E. Bishop's laboratories suggests that the testis-determining (*Tdy*) locus in the mouse is located on the short arm of the Y chromosome, not on the long arm, as previously thought. In summary, it has taken almost 30 years to travel from the observation that the mammalian Y chromosome is involved in testis determination to the accomplishment of obtaining a cloned DNA sequence from the mammalian Y chromosome that is most likely involved in testis determination. In some respects, we have come a long way towards understanding mammalian sex determination, and in other respects, we are just beginning.

What I hope to accomplish in this paper is to give a general review of mutant conditions in the mouse that cause complete or partial primary sex reversal. Specifically, I shall discuss two inherited conditions that involve the interaction of autosomal loci with the Y-linked testis determination (*Tdy*) gene. One of these conditions involves the transfer of a specific *Mus domesticus* Y chromosome onto the C57BL/6J inbred strain background. The other involves the interaction of two different deletions in chromosome (chr) 17 that cause sex reversal in the presence of a normal chr 17 derived from the C57BL/6J inbred strain and a Y chromosome derived from the AKR/J inbred strain. A more complete discussion of these and other conditions that cause sex reversal in the mouse can be found in a recent review of this subject by Eicher & Washburn (1986).

Mus domesticus Y chromosome placed on the C57BL/6J inbred strain background

In 1982 my laboratory reported that the transfer of the Y chromosome originally derived from a male *Mus domesticus* onto the C57BL/6J inbred strain caused XY mice to develop as females with two ovaries, or as hermaphrodites (an individual with both types of gonadal tissue) with an ovary accompanied by an ovotestis (a gonad containing ovarian and testicular tissue) or with two ovotestes. Our initial analysis indicated that the condition was Y-linked because only males transmitted the sex reversal trait to their offspring (Eicher *et al.* 1982). This inherited sex reversal was first identified in offspring derived during the course of transferring an unrelated dominant mutation, detected in a (C57BL/6J × POS A)F1 male, to the C57BL/6J inbred strain background. (The POS A was a partly inbred stock of mice that had been produced by sib matings derived from the mating of an NMRI female to a *Mus domesticus* male descended from mice captured in the Val Poschiavo, Switzerland.)

During the course of experiments designed to unravel the mode of inheritance of the above sex reversal, we were also transferring a Robertsonian translocation, originally identified in *Mus domesticus* mice trapped in Alpie Orobie, near Bergamo, northern Italy, to the C57BL/6J inbred strain background. After a few generations of backcrosses, we noted that the sex ratio was disturbed in favour of females and that overt hermaphrodites were present within litters derived from translocation carrier males. Analysis of this sex reversal condition also indicated that it was Y-linked. The simplest idea to account for the simultaneous occurrence of two Y-linked sex reversal conditions within a single colony of mice was that the Y chromosome derived from *Mus domesticus* mice trapped in the Val Poschiavo (hereafter designated Y^{POS} for Poschiavo) was 'identical' to the Y chromosome derived from *Mus domesticus* mice captured in Alpie Orobie (hereafter Y^{ORB} for Orobie). We further postulated that these two Y chromosomes carried an allele at the *Tdy* locus that was different from that carried by the C57BL/6J strain, and that the observed sex reversal was caused when the *Tdy* allele on the Y^{POS} or Y^{ORB} chromosomes was present with homozygous C57BL/6J-derived autosomal loci. (For purposes of further discussion, the *Tdy* allele derived from the Y^{POS} and Y^{ORB} chromosomes is designated Tdy^{do}, where '*do*' represents *Mus domesticus* and that from the C57BL/6J strain is designated Tdy^{b}.) The possibility that autosomal genes were involved in this inherited sex reversal was further strengthened by our finding that transfer of the Y^{POS} chromosome to the BALB/cBy, C58/J, or DBA/2J inbred strains did not cause either partial or complete sex reversal of XY individuals. In addition the normal interaction of the Y^{POS} chromosome on the BALB/cBy,

C58/J, and DBA/2J inbred backgrounds suggested that polymorphism for one or more of these autosomal loci existed among inbred strains of laboratory mice.

We decided to test our hypothesis as follows (Eicher & Washburn 1983). We had available a set of inbred strains that were each derived from strict sib matings of offspring obtained from mating an NMRI female to a male descendant of the *Mus domesticus* mice trapped in the Val Poschiavo. If our hypothesis was correct, each of these strains contained the Y^{POS} chromosome, thus the Tdy^{do} allele, and the transfer of this Y chromosome to the C57BL/6J strain background would cause sex reversal of XY offspring. We mated males from three of these strains (RB347BNR/Ei, RB156BNR/Ei, and RB16BNR/Ei) to C57BL/6J females and analysed foetuses at 14–16 days of development for gonad morphology and sex chromosome complement. This time of development was chosen for analysis because well-spread chromosomal preparations are easily obtained from liver and a small amount of one type of gonadal tissue within a majority of the other type is easy to ascertain (Eicher *et al.* 1980). For each cross, the sex ratio was normal and all F1 XY individuals contained two normal appearing testes.

We then mated F1 male offspring obtained from each of the crosses noted above to C57BL/6J females and, as before, analysed foetuses at 14–16 days of development. In addition we analysed foetuses produced from mating C57BL/6J females to F1 males that were derived from mating a POS A strain male to a C57BL/6J female. (The origin of the POS A strain is identical to that of the other three strains being analysed.) A total of 185 backcross foetuses from all crosses were successfully analysed, with the number of XY foetuses in each of the four crosses ranging from 19 to 97. The results indicated that for each backcross, half of the XY foetuses developed some ovarian tissue. We concluded that the C57BL/6J inbred strain contains an allele at an autosomal locus, designated testis-determining autosomal-1 (*Tda-1*), that results in ovarian tissue in XY individuals when this allele is homozygous and present with the Y-linked Tdy^{do} allele. (The *Tda-1* allele carried by C57BL/6J is designated $Tda\text{-}1^{b}$ and that carried by *Mus domesticus* is designated $Tda\text{-}1^{do}$).

We also mated a pure *Mus domesticus* male (derived from mice trapped in the Val Poschiavo) from the inbred strain ZALENDE/Ei to a C57BL/6J female and analysed F1 foetal offspring at 14–16 days of development. All XY mice contained two normal testes. Reciprocal (ZALENDE/Ei female × C57BL/6J male)F1 XY foetal mice were also analysed and found to be normal males. We then backcrossed (C57BL/6J × ZALENDE/Ei)F1 males to C57BL/6J females and found, as expected, that half of the backcross XY offspring developed some ovarian tissue. This finding was in agreement with the results obtained using the inbred strains derived from the C57BL/6J and *Mus domesticus* genomes and provided additional support for our suggestion that the C57BL/6J autosomal $Tda\text{-}1^{b}$ allele causes ovarian tissue development when present in the homozygous state with the Tdy^{do} allele.

Comparison of the data from the above crosses suggests that genes other than *Tda-1* also play a role in causing sex reversal of C57BL/6J-Y^{POS} XY mice. For example, all XY mice of the C57BL/6J-Y^{POS} strain develop either as females with two ovaries or as hermaphrodites, half of which have two ovotestes and half have an ovary and an ovotestis. No C57BL/6J-Y^{POS} XY mouse develops even a single testis. This result is in contrast to what was observed in first backcross XY offspring produced in matings involving C57BL/6J females mated to the F1 males obtained from mating C57BL/6J females to males of the POS A strain or one of the other three similar strains. In these cases, although half of the backcross XY mice developed ovarian

tissue, the ovarian tissue was usually present in an ovotestis and more often accompanied by a testis or another ovotestis than an ovary. In terms of the actual types of gonads recorded, out of 102 backcross XY foetuses analysed, 6 developed two ovaries; 5 developed an ovary and an ovotestis; 63 developed two ovotestes; and 28 developed an ovotestis and a testis. To explain these contrasting results we have suggested that there are other C57BL/6J-derived alleles at autosomal loci that, when present in the homozygous state in a *Tda-1*^{b}/*Tda-1*^{b} XY^{POS} individual, increase the probability that ovarian tissue will develop.

The question arises as to why all XY mice of the C57BL/6J-Y^{POS} strain do not develop exclusively ovarian tissue. To perpetuate the C57BL/6J-Y^{POS} strain, we must breed mice from this strain that will transmit a Y^{POS} chromosome. As previously noted, all of the XY mice in this strain are either females or hermaphrodites. With the exception of one XY female, all XY females tested from this strain thus far have proven sterile (Eicher *et al.* 1983). Therefore we must use hermaphrodites that have developed a sufficient amount of testicular tissue to masculinize their external genitalia and at least one side of their internal reproductive tract completely, to transmit the Y^{POS} chromosome to the next generation. If, in the C57BL/6J-Y^{POS} strain, there still exists one autosomal gene that in the heterozygous state allows some testicular tissue to develop and in the homozygous state (homozygous for the C57BL/6J-derived allele) causes exclusively ovarian tissue to develop, by definition this gene is kept in a forced heterozygous state to perpetuate the C57BL/6J-Y^{POS} strain. Proof for this idea would come from finding that C57BL/6J-Y^{POS} XY females differ from XY hermaphrodites at an autosomal locus.

Although we have made a concerted effort to locate the chromosomal position of the *Tda-1* gene, we have been unsuccessful. Our initial approach to locate the chromosomal position of *Tda-1* was to utilize a set of recombinant inbred (RI) strains produced by Benjamin Taylor of The Jackson Laboratory. This is the BXD RI strain set, which has as progenitors the C57BL/6J (B) and DBA/2J (D) strains. Our experimental design involved mating C57BL/6J-Y^{POS} hermaphrodites to females of each of the BXD RI strains and analysing foetuses at 14–16 days of development for sex chromosome complement and gonad morphology. We chose the BXD set of RI strains because, as previously stated, the Y^{POS} chromosome does not cause sex reversal when placed on the DBA/2J inbred strain background, suggesting that DBA/2J carries an allele at the *Tda-1* locus that is different from the allele carried by C57BL/6J and interacts normally with the Y-linked *Tdy*^{do} locus. In addition, a sufficient number of BXD RI strains are available for analysis so that evidence for co-segregation of *Tda-1* with another locus would be strong evidence for the linkage of these two loci.

As expected, we recovered XY foetuses containing ovarian tissue in matings involving half of the BXD RI strains, again suggesting that C57BL/6J and DBA/2J carry different alleles at the *Tda-1* locus. Unfortunately, none of the potential linkages were confirmed when we attempted to verify the suggested chromosomal locations directly by analysing foetuses produced by mating (C57BL/6J × DBA/2J)F1 females to C57BL/6J-Y^{POS} hermaphrodites. In summary, although the chromosomal location of *Tda-1* remains unknown.

We have, however, been successful in locating the chromosomal position of another autosomal gene that interacts abnormally with the *Tdy*^{do} allele to cause sex reversal of XY mice. This finding was made during the course of trying to map the *Tda-1* gene by using another set of RI strains produced from the progenitor strains NZB/BLNJ (N) and SM/J (SM). We chose this RI strain set, designated NXSM, because hermaphrodites are produced

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by mating NZB/BLNJ females to C57BL/6J-Y^{POS} hermaphrodites whereas only normal XY males are produced if SM/J females are used. Analysis of the data indicates that a locus on chr 12 causes ovarian tissue to be formed when the NZB/BLNJ-derived allele for this gene is present in the F1 XY offspring. Because the autosomal sex-determining gene that we were attempting to map by using the BXD RI strains is not located on chr 12 (E. M. Eicher, L. L. Washburn & B. K. Lee, unpublished data), we conclude that the *Tda-1* locus we were trying to map by using the BXD RI strains is not the same locus that we have located on chr 12 with the NXSM RI strains. We have designated this locus on chr 12 testis-determining autosomal-2 (*Tda-2*).

In conclusion, what began as a simple story of Y-linked sex reversal has become increasingly complicated. Experiments are underway to analyse foetuses produced from mating C57BL/6J-Y^{POS} hermaphrodites to (NZB/BLNJ × SM/J)F1 females to confirm the mapping of one autosomal locus to chr 12; to (SM/J × C57BL/6J)F1 females to determine whether this chr 12 locus also segregates in this cross; and to (NZB/BLNJ × C57BL/6J)F1 females to determine whether all XY foetuses develop ovarian tissue.

Sex reversal caused by the T^{hp} and T^{Orl} deletions

The hairpin tail (T^{hp}) mutation, discovered by Dickie (1965) in an AKR/J male, was shown by Johnson (1974, 1975) to be an allele at the brachyury (T) locus, which is located in the proximal region of chr 17. As is the case for other T alleles, the presence of T^{hp} with a wild-type allele (+) causes a shortening of the tail and when present with most t haplotypes causes a tail-less condition. Of special interest was Johnson's findings that the $T^{hp}/+$ condition is lethal if offspring with this genotype inherit the T^{hp} allele from a female, but viable if they inherit T^{hp} from a male. Of importance to the effect of T^{hp} on primary sex determination is the fact that T^{hp} can only be transmitted through males. Thus, because T^{hp} occurred in the AKR/J inbred strain, all stocks of mice carrying T^{hp} contain an AKR/J-derived Y chromosome. Finally, T^{hp} is known to involve a deletion that includes the quaking (*qk*) locus (Bennett 1975) as well as several DNA sequences (Mann *et al.* 1986; see review by Silver 1985).

During the course of transferring T^{hp} onto the C57BL/6J inbred strain background, we noticed that the sex ratio of litters was skewed in favour of females and a number of $T^{hp}/+$ offspring were hermaphrodites (Washburn & Eicher 1983). A closer examination revealed that some $T^{hp}/+$ females were XY and all of the $T^{hp}/+$ hermaphrodites were XY. To investigate the inheritance of this sex reversal condition further, we mated C57BL/6J females to $T^{hp}/+$ males from the C57BL/6J-T^{hp} strain and analysed foetuses for sex chromosome complement, gonad morphology, and tail phenotype. Among a total of 95 foetuses analysed, 23 foetuses were XX, +/+ and 20 were XX, $T^{hp}/+$; all 43 of these foetuses had two normal ovaries. Of the 25 XY, +/+ foetuses analysed, all had two testes. The 27 XY, $T^{hp}/+$ foetuses, however, were different from expected: 15 had two ovaries, 7 had an ovary accompanied by an ovotestis, and 5 contained two ovotestes. We concluded from this data that on the C57BL/6J inbred strain background all XY, $T^{hp}/+$ offspring develop either exclusively ovarian tissue (completely sex reversed) or some ovarian tissue (partly sex reversed). We also determined that when C57BL/6J-$T^{hp}/+$ hermaphrodites are mated to C3H/HeJ females, all XY, $T^{hp}/+$ offspring develop as normal males.

From the above results we postulated that the inherited effect of T^{hp} on gonad determination

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was due to either (*a*) a pleiotropic effect of T^{hp}; (*b*) a mutation in a locus closely linked to T^{hp}, or (*c*) the effect of a locus on the normal chr 17 situated *trans* to the genetic deletion involving T^{hp}. For all of these possibilities, however, the presence of a chr 17 derived from the C57BL/6J inbred strain appeared essential for sex reversal to occur in XY, $T^{hp}/+$ mice, because if T^{hp} is placed onto another inbred strain background, such as C3H/HeJ, XY, $T^{hp}/+$ mice develop as normal males. We designated the inherited sex reversal associated with T^{hp} as T-associated sex reversal (*Tas*).

Through our work with the sex reversal that resulted from the transfer of the Y^{POS} chromosome to the C57BL/6J inbred strain background, we were aware that the transfer of a Y chromosome from one genetic background to another could affect testis determination. For this reason, we decided to analyse the *Tas* locus further by using another dominant mutation at the *T* locus that also involves a deletion of chr 17 including the *qk* locus (Moutier 1973*b*; Erickson *et al.* 1978) and loci identified by DNA sequences (Hermann *et al.* 1986; Sarvetnick *et al.* 1986; Mann *et al.* 1986; and review by Silver 1985). This mutation, designated T-Orleans (T^{Orl}) (Moutier 1973*a*), can, however, be transmitted to live offspring regardless of the sex of the $T^{Orl}/+$ parent. Thus, we could use T^{Orl} to investigate simultaneously (*a*) whether the presence of the deletion in the region of chr 17 common to T^{Orl} and T^{hp} caused sex reversal when accompanied by a normal chr 17 derived from the C57BL/6J inbred strain, and (*b*) whether substitution of the C57BL/6J Y chromosome for the AKR/J Y chromosome caused normal testis development in XY, $T^{Orl}/+$ mice. I shall present some of the results of these studies. The full study will be published elsewhere (Washburn & Eicher 1988).

Before initiating the experiments involving T^{Orl}, we transferred T^{Orl} onto the C57BL/6J inbred strain background. In addition, we constructed a C57BL/6J strain of mouse that contained an AKR/J Y chromosome (strain designated C57BL/6J.AKR/J-Y, abbreviated B6.AKR-Y). In one experiment we mated C57BL/6J-$T^{Orl}/+$ females to B6.AKR-Y males and, as before, analysed foetuses at 14–16 days of development for gonad morphology, sex chromosome complement, and tail phenotype. Of the 13 $T^{Orl}/+$ foetuses analysed, all contained two ovaries. Although 7 of these females were chromosomally XX, 6 were XY, thus completely sex-reversed. In another experiment, we mated C57BL/6J-T^{Orl} females to C57BL/6J males and analysed foetuses as before. Among the 22 $T^{Orl}/+$ foetuses recovered, 11 were XX and contained two ovaries, and 11 were XY and contained two testes.

From the results presented above, together with additional supporting evidence not presented in this review, we conclude that T^{hp} and T^{Orl} cause sex reversal of XY individuals when placed on a C57BL/6J inbred strain background, provided that the AKR/J Y chromosome is also present. These data also indicate that the *Tas* locus is located in the region of chr 17 common to T^{hp} and T^{Orl}. Finally, these results suggest that the *Tdy* allele carried by the AKR/J Y chromosome differs from the *Tdy* allele carried by the C57BL/6J Y chromosome.

Experiments are underway to define more precisely the position of *Tas* on chr 17. We are utilizing DNA probes that both identify loci deleted from the T^{hp} and T^{Orl} deletions and identify restriction fragment length polymorphisms (RFLPs) between C3H/HeJ and C57BL/6J. Specifically, we shall determine whether foetuses that are produced by mating C57BL/6J-$T^{hp}/+$ males to (C3H/HeJ × C57BL/6J)F1 females inherit a C3H/HeJ- or C57BL/6J-derived allele from their F1 parent. The sex chromosome complement, gonad morphology and T^{hp} phenotype of each foetus will also be determined. A similar experiment will also be done by

using foetuses derived from the mating of C57BL/6J-T^{Orl}/+ females to (C3H/HeJ × B6.AKR-Y)F1 males. If we successfully position *Tas* between two closely linked DNA fragments, experiments will be initiated to clone the *Tas* locus.

General discussion

A model to account for the findings summarized in this review was first published in a paper by Eicher & Washburn (1983) and later expanded in a review published in 1986. We hypothesized that in mammals two primary sex determination pathways are available to a foetus: the ovary determination pathway and the testis determination pathway. In place at the time this decision is made are the primordia for both the male and female internal duct systems. Once gonadal sex is determined, barring secondary complications, the internal and external genitalia develop concordantly with the sex of the gonads.

We suggested that mammals evolved a genetic control mechanism that guarantees that an individual will utilize either the ovary or testis determination pathways. That is, each individual develops either ovaries or testes, but not both. This dichotomy was successfully accomplished when the first gene in the testis determination (TD) pathway, *Tdy*, became located on the chromosome that evolved into the Y chromosome, the only chromosome unique to males. We suggested that, to guarantee further that XY individuals develop only testes, the *Tdy* gene functions significantly earlier in development than the first gene in the ovarian determination (OD) pathway (gene symbolized *Od*). We also suggested that testis-only development in XY males is further guaranteed by inactivation of the *Od* locus by the *Tdy* gene product or another gene in the TD pathway. The outcome of this genetic control mechanism is that the presence of the *Tdy* locus in XY individuals guarantees development of only testicular tissue, and the functional absence of the *Tdy* locus, as is the case in XX individuals, guarantees that the *Od* locus initiates development of ovarian tissue.

We think that the mouse autosomal loci we have identified, *Tas*, *Tda-1* and *Tda-2*, are some of the loci constituting the TD pathway. We suggest that within a species all of the loci in the TD pathway are selected to function in a coordinated and sequential manner, with the *Tdy* locus functioning significantly earlier than the *Od* gene so as to 'lock in' the TD pathway before the OD pathway is initiated.

The genomes of the standard inbred strains of mice are a mixture from two different species of mice, *Mus domesticus* and *Mus musculus*, with each inbred strain having a unique set of alleles derived from *M. musculus* or *M. domesticus*. The Y chromosome carried by the C57BL/6J, C3H/HeJ, and DBA/2J strains is of *M. musculus* origin whereas the AKR/J Y chromosome is of *M. domesticus* origin. Interestingly, the Y chromosome carried by C57BL/6J is derived from a different source of *M. musculus* than are the Y chromosomes carried by C3H/HeJ and DBA/2J, which appear to be of identical origin (P. K. Tucker, B. K. Lee & E. M. Eicher, unpublished data). We suggest that the Tdy^{do} allele carried on the Y^{POS} chromosome, derived from *M. domesticus* trapped in Switzerland, functions later in development than does the Tdy^{b} allele carried on the C57BL/6J inbred strain, but not significantly different in time from the *Tdy* allele carried by the C3H/HeJ or DBA/2J Y chromosomes. Thus when the Tdy^{do} allele is transferred to the C57BL/6J inbred strain background, it now interacts with OD loci derived from C57BL/6J, which normally function earlier in time than the same loci derived from the *M. domesticus* genomes (or those carried in the C3H/HeJ or DBA/2J genomes). The

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result is that the OD pathway is set into motion before the TD pathway is 'locked in', causing development of ovarian tissue in XY individuals.

To account for the occurrence of sex reversal in C57BL/6J-T^{hp}/+ and C57BL/6J-T^{Orl}/+ mice carrying an AKR/J Y chromosome, we suggest that the presence of a single copy of a C57BL/6J-derived Tas^b allele, as occurs in the presence of the T^{Orl} or T^{hp} deletions, delays slightly the initiation of the TD pathway. In addition, the *Tdy* allele carried on the AKR/J Y chromosome functions slightly later in development than the *Tdy* allele carried on the C57BL/6J chromosome. Evidence for this suggestion comes from a preliminary observation in our laboratory that testis cord development is delayed at the ends of the foetal testis in B6.AKR-Y mice compared with C57BL/6J mice. In tandem, these two delays allow initiation of the OD pathway and formation of ovarian tisue in XY individuals (Washburn & Eicher 1988).

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Discussion

Ursula Mittwoch (*University College London, U.K.*). I have a question regarding the Y chromosome in the AKR/J strain, which promotes ovarian development when combined with the T^{Orl} allele. In their comparison of testis masses in inbred strains of mice, Shire & Bartke (1972) found males of the AKR/J strain to have the lowest relative testicular mass at eight weeks of age. Is it known whether a Y-linked allele is involved in the causation of this low testis mass?

Reference

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Eva M. Eicher. I do not know. If this is unknown, it is certainly testable.

B. M. Cattanach (*M.R.C. Radiobiology Unit, Didcot, U.K.*). On reviewing the literature on sex reversals a year or so ago I became concerned at the number of factors that appeared capable of reversing sex. Thus there is the information Dr Eicher has provided on the influence of the 'foreign' *domesticus* Y in C57BL/6J mice, the T^{hp} deletion with the AKR chromosome (and the T^{Orl} deletion), the X deletion in wood lemmings, certain autosomal translocations in humans, and perhaps the T16H translocation together with Sxr in the mouse. It seemed to me that perhaps these do not represent autosomal sex-determining genes but may only be non-specific interferences with male development in the presence of the Y. Any delay caused by such genetic changes might override the male-determining influence of the Y.

To test this hypothesis I have introduced the W^{19} deletion of chromosome 5 together with the AKR Y into the C57BL strain and have found from examination of both 15-day foetuses and adults that most W^{19} animals develop as phenotypic females or hermaphrodites, and that such males as appear possess very small testes as adults.

Do I conclude we have discovered by chance a W-associated sex reversal or could it not be that this observation supports the idea that sex-specific genetic events that perhaps delay or retard development may cause XY individuals to develop as females?

Eva M. Eicher. Dr Cattanach's ideas and observations are interesting. What they mean, however, must await further analysis. It is not known that the *Tda-1* locus is not located on mouse chromosome 5 near *W*.

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES