Linkage of the Steroid Sulfatase Gene to the *Sex-Reversed* Mutation in the Mouse

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ABSTRACT

Dosage studies and the inheritance pattern of the gene for steroid sulfatase (*Sts*) in the mouse have previously provided indirect evidence for a functional *Y*-linked allele which recombines obligatorily with its *X*-linked allele in male meiosis. In this study, we have investigated the linkage relationship of *Sts* and the *sex-reversed* mutation (*Sxr*), a gene which is known to reside in the pairing region of the *Y* chromosome. The results clearly demonstrate that *Sxr* and *Sts* are linked in a region of obligatory recombination and *Sts* maps proximal to *Sxr* with most recombinants occurring proximal to the two genes.

OVER 50 yr ago, KOLLER and DARLINGTON (1934) proposed on the basis of cytological observations that *X-Y* pairing and recombination were necessary for normal meiotic disjunction in mammals. HALDANE (1936) then predicted that genes in the pairing region would exhibit partial sex linkage due to the presence of alleles on the *X* and *Y* chromosomes which recombine. The *X-Y* pairing observations of KOLLER and DARLINGTON have been repeatedly confirmed over the years, but until recently, there was no genetic evidence for recombination between the *X* and *Y* mammalian chromosomes. In 1982, SINGH and JONES using molecular hybridization, demonstrated that the murine sex-reversed mutation (*Sxr*) (CATTANACH, POLLARD and HAWKES 1971), formerly thought to be autosomal, was sex-linked and showed obligatory recombination between the *X* and *Y* chromosomes (SINGH and JONES 1982). *Sxr* is a duplication of the testis determining gene(s) that have been translocated to the distal pairing tip of the *Y* chromosome. The gene then undergoes obligatory recombination producing offspring with the *Sxr* gene on the *Y* chromosome (carrier males) or on the *X* chromosome (sterile *XX* males) (SINGH and JONES 1982; EVANS, BURTENSHAW and CATTANACH 1982). The inheritance pattern of *Sxr* in this position is indistinguishable from autosomal inheritance (pseudoautosomal inheritance) (SINGH and JONES 1982; EVANS, BURTENSHAW and CATTANACH 1982). Any other gene in this region would also show pseudoautosomal inheritance and in the absence of a molecular probe, it would not be possible to distinguish such a gene from an autosomal one by conventional genetic analysis. We therefore took advantage of the fertile murine *XO* to design a simple mating scheme to detect such genes. A male carrying a recessive marker, thought to be autosomal but unmapped, is crossed to a normal *XO* female. If the gene is autosomal, all offspring from the cross will be normal, whereas if it is *X*-linked, the *XO* progeny will be mutant and the *XX* and *XY* progeny normal. In the case of *X* linkage, the apparent autosomal inheritance is explained by postulating a *Y*-linked gene that undergoes obligatory meiotic recombination with its *X*-linked allele. Using the above mating scheme and an expression variant for the steroid sulfatase gene, we recently showed that *Sts* appears to be located in the *X-Y* pairing region (KEITGES *et al.* 1985). In this report, we present definitive evidence for the presence of *Sts* in the *Y* segment of the pairing region by demonstrating its linkage to *Sxr*. Our analysis permits us to order the *Sxr* and *Sts* genes in the pairing region and leads to an interesting comparison of the recently described analogous human *X-Y* pairing region (ROUYER *et al.* 1986; SIMMLER *et al.* 1985; COOKE, BROWN and RAPPOLD 1985).

MATERIALS AND METHODS

Crosses: An *Sxr* carrier male with normal STS activity (STS^+) was crossed to an STS deficient (STS^-) tabby (*Ta*) *XO* female. The *X*-linked tabby (*Ta*) coat color marker is used to distinguish hemi- or homozygous mice from heterozygous or wild-type mice. Four types of offspring are produced with regards to coat color and sexual phenotype; *XX Ta*/+ females, *XO* + females, *XX Ta*/+ males, and *XY Ta* males. *XX Ta*/+ males were sex-reversed which was confirmed by examination of internal sex organs. The *XY Ta* males consisted of *Sxr* carrier and noncarrier males. *Sxr* carrier male offspring were distinguished by progeny testing with either *Ta*/*O* or *Ta*/+ females. Males which did not produce an *XX* male after two–three litters (approximately 20 offspring) were considered *Sxr* noncarriers; conversely males which produced one *XX* male were considered *Sxr* carriers.

Two *Sxr* carrier males (tabby and heterozygous for STS activity) resulting from the first cross were test crossed to STS^- C3H/An females (parental cross Figure 1A). The

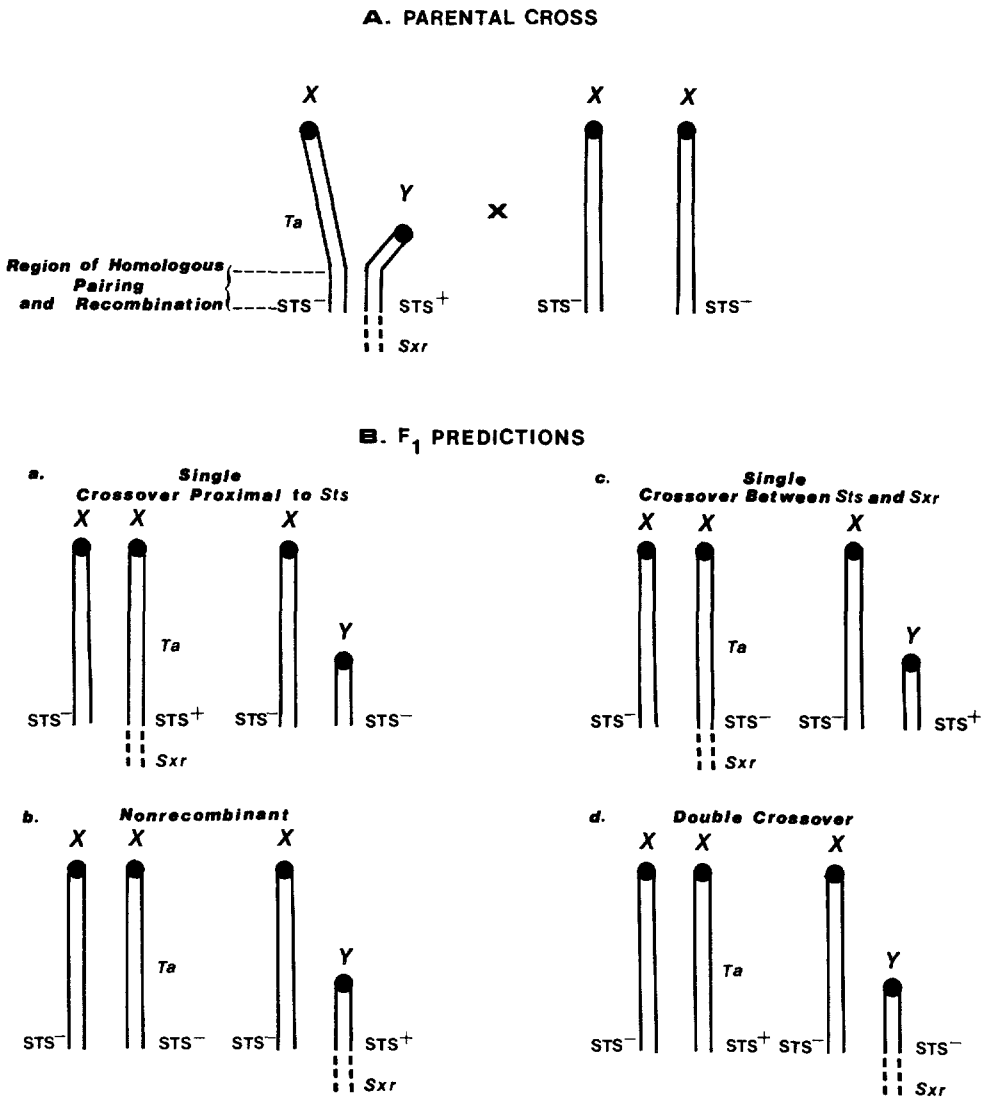

FIGURE 1. The region of synapsis in the mouse is up to 90% of the *Y* and 30–35% of the *X* chromosome (TRES 1977). The extent of the region of homologous pairing and recombination has yet to be measured, but is certainly smaller. A, Two STS$^+$ tabby *Sxr* carrier male offspring were mated with STS$^-$ C3H/An females. B, Several predictions as to the F_1 offspring from this cross can be made. a, A single crossover proximal to *Sts* would produce STS$^+$ *XX Sxr* males and STS$^-$ *XY* males. b, A nonrecombinant class. c, A crossover between *Sts* and *Sxr* would produce STS$^-$ *XX Sxr* males, or STS$^+$ *XY* males. d, A double crossover would produce STS$^+$ *XX* females and STS$^-$ *XY Sxr* carrier males.

offspring were again classified by sex and the presence of the *Sxr* mutation as described above.

STS assay: STS assays were performed on ear punch samples which had been collected and frozen in 110 μl of 0.1 M Tris-HCl (pH 7.2). They were sonicated on ice for 4 sec at 250 W. The assay was performed on 50 μl of extract as previously reported, using [^{3}H]estrone sulfate as the substrate (KEITGES *et al.* 1985). Protein assays were done according to the Lowry method (LOWRY *et al.* 1951). The range of STS$^-$ and STS$^+$ values never overlapped. The mean and standard deviation for STS$^-$ and STS$^+$ mice, respectively, were 5,029 ± 2,827 and 38,326 ± 13,966 cpm.

RESULTS AND DISCUSSION

If *Sts* and *Sxr* are tightly linked, or recombination occurred at a single point proximal to both genes, all of the *Ta*/+ *XX Sxr* males should be STS$^+$ and the normal +/*Y* males should be STS$^-$ [Fig. 1B(a)]. If, however, recombination were to occur over a region with crossing over occurring occasionally between the markers, then recombination between the markers should be observed producing some *Ta*/+ *XX Sxr* males that are STS$^-$ and normal +/*Y* males which are STS$^+$ [Fig. 1B(c)]. The separation of the *Y* chromosome into a homologous and nonhomologous section creates a third marker somewhat like a translocation breakpoint. In effect, we are carrying out a three point cross which permits ordering of the *Sts* and *Sxr* genes based on any recombination occurring between them. Of course, if *Sts* were in fact not sex-linked, but autosomal, then the two markers would exhibit independent assortment.

Eight litters totaling 59 offspring were analyzed (Table 1). Nineteen *Ta*/+ *XX Sxr* males were obtained; 17 were STS$^+$ and 2 were STS$^-$. The remaining males (+/*Y*) (Table 1) include *Sxr* carrier and normal *XY* males which can only be distinguished by progeny testing. Unfortunately not all of the males reached maturity for progeny testing (Table 2 "Untested males").

The data in Table 1 were tested to determine whether *Sts* assorts independently with respect to *Sxr*, and the hypothesis was rejected (Table 1) ($\chi^2_5 = 27.8$, $P < 0.005$). When *Sxr* is transferred to the *X* chro-

TABLE 1

Test of independent assortment of *Sts* and *Sxr*

	Ta/+ *XX* Females		*Ta*/+ *XX Sxr* Males		+/*Y* *XY* Males	
	STS^+	STS^-	STS^+	STS^-	STS^+	STS^-
Observed	0	13	17	2	18	9
Expected	6.5	6.5	9.5	9.5	13.5	13.5
χ^2	6.5	6.5	5.9	5.9	1.5	1.5

χ^2 5 = 27.8.

TABLE 2

Classification of *XY* offspring

Sxr carrier males		*Sxr* noncarrier males		Untested males	
STS^+	STS^-	STS^+	STS^-	STS^+	STS^-
12	1	3	3	3	5

Untested males died before they could be progeny tested.

TABLE 3

Recombination frequencies in *XX* offspring

Ta/*Sts*	*Sts*/*Sxr*	*Ta*/*Sxr*
17/32 = 53.1%	2/32 = 6.2%	19/32 = 59.4%

mosome in male meiosis, *Sts* is also mobilized in 85.5% of *XX* offspring. Male offspring confirm these data and indicate that *Sts* and *Sxr* are very tightly linked and near the distal end of the pairing region. Linkage of these two markers provided direct evidence for the presence of a functional *Y*-linked gene, the first functional mammalian *Y*-linked gene to be mapped to the region of pairing and recombination. The 50% recombination frequency between *Ta*/*Sts* and *Ta*/*Sxr* indicates that recombination in this region is obligatory as was found in the human pseudoautosomal region (ROUYER *et al.* 1986; SIMMLER *et al.* 1985; COOKE, BROWN and RAPPOLD 1985) (Table 3).

In the human pseudoautosomal region, it has been shown that recombination in female meiosis is an order of magnitude lower than male meiosis, presumably due to the absence of obligatory recombination in this region in female meiosis (ROUYER *et al.* 1986). Recombination in female meiosis cannot be measured in the current study since *XX* animals carrying the *Sxr* mutation are sterile males. The question naturally arises as to why the pseudoautosomal region exhibits obligatory recombination in male meiosis, but such is not the case in the same region in female meiosis which must carry the same array of genetic sequences including those on the *Y* pairing region. A simple explanation follows from KOLLER and DARLINGTON's (1934) predictions of more then 50 yr ago that at least one crossover event per homologous pair is necessary for normal disjunction. In female meiosis this crossover can take place over the entire *X*, while in male meiosis, it can only occur in the limited *XY* pairing region.

Recombination was also observed to occur between *Sts* and *Sxr* producing two STS^- *XX Sxr* males, a recombination frequency of 6.2% in *XX* offspring (Table 3), and three STS^+ normal *XY* males (Table 2). Since *Sxr* was mobilized without *Sts*, the order of the genes must be, centromere, *Sts*, and *Sxr* (Fig. 1). If the reverse order is considered, then one has to assume a double crossover event for each of the STS^- *XX Sxr* males and STS^+ normal *XY* males. Using the 6.2% recombination frequency observed in female offspring, the number of STS^+ and STS^- males can be predicted assuming that the two classes, *Sxr* carrier and *XY* normal males, are equal. The expectations are 15.7 STS^+ males and 12.3 STS^- males which, is close to our observations of 18 STS^+ and 9 STS^- males.

Double recombinants between the junction of the pairing region/*Sts*/and *Sxr* should produce STS^+ *XX* females, and STS^- *Sxr* carrier males (Fig. 1B(d)). One STS^- *Sxr* carrier male was found (Table 2). Assuming no crossover interference, one would expect to find 1.8 double crossovers in 59 offspring (0.53 × 0.06 × 59). However, the assumption of no crossover interference is almost certainly incorrect, and fewer double crossovers would be expected. Since the number of offspring in this study is small, further litters will be needed to determine a more precise estimate of the number of double recombinants and rule out the possibility of a gene conversion event.

No double crossovers were found in the human pseudoautosomal region, but only a maximum of 2.4 crossovers between DXYS14, DXY17, and sex phenotype would be expected (ROUYER *et al.* 1986). ROUYER *et al.* (1986) hypothesized that this region of obligatory recombination is restricted to a single crossover. This assumption of a single crossover appears to be an unnecessary and complicating one at this time. There is no apparent selective advantage to mandating a single crossover event in this region. The absence of double crossovers is more likely due to the limited size of the pairing region. The pairing region in humans has been estimated to be on the order of 5000 kb which is less than 1% of the genome (ROUYER *et al.* 1986). Considering the total number of chiasmata observed in humans (HULTEN 1974), it is not likely that double crossovers would occur with any frequency in such a restricted region.

On the basis of *XO* infertility in humans, and fertility in mice, the pairing region has been hypothesized to be smaller in mice (ROUYER *et al.* 1986). The argument is that the region is not dosage compensated (KEITGES and GARTLER 1986; MULLER *et al.* 1980; CHANCE and GARTLER 1983; ROPERS *et al.* 1981; MIGEON *et al.* 1982; GOODFELLOW *et al.* 1984; RACE

and SANGER 1975) and, therefore, a gene in *XO* individuals is underexpressed as compared to *XX* or *XY* offspring (FERGUSON-SMITH 1966; BURGOYNE 1982). If this region is important in normal female development as FERGUSON-SMITH has hypothesized, then a smaller pairing nondosage compensated region in the mouse could be the basis of the difference in *XO* fertility in mice and humans. However, human females with visible *X* chromosome deletions of the distal part of the short arm may have normal fertility (WYSS *et al.* 1982) which is contrary to the importance of this region in normal female development. Finally, the only evidence on the relative genetic size of the mouse and human pairing regions comes from studies of the steroid sulfatase locus. Here we have presented definitive evidence for the presence of the *Sts* gene well within the murine pairing region. Furthermore, the murine *Sts* gene is not subject to *X* inactivation as would be expected from its location inside the pairing region and the presence of a functional *Y*-linked allele (KEITGES and GARTLER 1986; GARTLER and RIVEST 1983). However, the human *Sts* gene is partially inactivated (MULLER *et al.* 1980; CHANCE and GARTLER 1983; ROPERS *et al.* 1981; MIGEON *et al.* 1982) and proximal to the region of recombination. We interpreted these findings to indicate that the human *Sts* gene was formerly in the pairing region, but that evolutionary "shrinkage" of the homologous region has moved it outside that region (KEITGES and GARTLER 1986). Thus, from an evolutionary standpoint, the argument can be made that the murine pairing region is larger than its human counterpart. It does not appear that a sound argument can be made for explaining the *XO* murine, human fertility differences on the basis of a difference in the size of the pairing region.

CONCLUSION

In conclusion, we have provided direct evidence for a functional *Y*-linked murine *Sts* allele which is linked to the *Sxr* mutation, both of which are in the region of obligatory recombination. Recombination appears to occur over the homologous pairing region as opposed to a single point, and multiple crossovers are probably restricted by the limited size of the region.

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