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High rate of recombination and double crossovers in the mouse pseudoautosomal region during male meiosis

(sex chromosomes/steroid sulfatase/Mov-15/transgenic mice/crossover interference)

PHILIPPE SORIANO*†¶, ELISABETH A. KEITGES‡, DANIEL F. SCHORDERET‡, KLAUS HARBERS§, STANLEY M. GARTLER‡, AND RUDOLF JAENISCH*†

*Whitehead Institute for Biomedical Research, Nine Cambridge Center, Cambridge, MA 02142; †Department of Biology, Massachusetts Institute of Technology, Cambridge, MA 02139; ‡Departments of Medicine and Genetics, and Center for Inherited Diseases, SK-50, University of Washington, Seattle, WA 98195; and §Heinrich-Pette-Institut für Experimentelle Virologie und Immunologie an der Universität Hamburg, Martinistrasse 52, 2000 Hamburg 20, Federal Republic of Germany

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ABSTRACT **The recombination rate in meiosis between the mouse X and Y chromosomes was analyzed. Mice heterozygous at two pseudoautosomal alleles, the steroid sulfatase gene and the *Mov-15* provirus marker, were crossed. The provirus in the *Mov-15* transgenic mouse strain had been previously shown to be carried in the pseudoautosomal region of the sex chromosomes. Recombination frequencies were shown to be 7-fold higher in this region in male meiosis than in female meiosis. Three-point crosses indicated the occurrence in male meiosis of double recombination events in the pseudoautosomal region, with little or no crossover interference, which is in marked contrast to observations made on the similar region of the human sex chromosomes. This result is contrary to a previous model, which predicted a single crossover event in male meiotic pairing of mammalian sex chromosomes.**

Pairing of mammalian X and Y chromosomes occurs during meiosis in a short region located near the telomeres. Alleles in this region do not show strict sex-linked inheritance because they can undergo reciprocal exchange between the sex chromosomes and have therefore been termed pseudoautosomal (1, 2). A gradient of sex linkage and a higher frequency of recombination during male meiosis than in female meiosis have been established in the human pseudoautosomal region (3). To explain the lack of sex linkage of distal markers, a single obligatory crossover has been postulated to occur in this region (2, 3). Similar studies in the mouse have not yet been performed because few pseudoautosomal markers are available. In a previous study, information on recombination in the mouse pseudoautosomal region was obtained by investigating the linkage between the mouse steroid sulfatase gene (*Sts*), a pseudoautosomal marker that does not show sex linkage and therefore should be located in the distal pairing region (4), and the sex-reversed mutation (*Sxr*; see refs. 5 and 6; Fig. 1). The two markers were shown to be linked, and one double recombinant between these two markers and sexual phenotype was identified among 59 progeny tested (7). This suggested that the single obligatory crossover model may not be applicable to the mouse sex chromosomes. However, because *Sxr* is a translocation to the telomere of the part of the Y chromosome bearing *Tdy*, the testis-determining gene (for review, see ref. 8), these crosses could not examine recombination in the pseudoautosomal region of the normal Y chromosome. Moreover, in these crosses, recombination between *Sts* and *Sxr* in female meiosis could not be analyzed because XX animals carrying the *Sxr* mutation were sterile males.

To further study recombination in the mouse pseudoautosomal region, we have examined the linkage between *Sts* and another pseudoautosomal marker, the *Mov-15* provirus carried in a transgenic mouse strain (9). We have previously shown that the *Mov-15* provirus recombines with *Tdy* in 10–20% of male meioses. We describe here linkage analysis between *Mov-15*, *Sts*, and *Tdy*. Our results show that the frequency of recombination between *Sts* and *Mov-15* is 7-fold higher during male meiosis than during female meiosis, that there is a gradient of sex linkage in the mouse pseudoautosomal region, and that, in contrast to the human sex chromosomes, double recombination events take place with little or no crossover interference.

MATERIALS AND METHODS

Mice and Assays. C3H/An steroid sulfatase-negative animals and *Mov-15* animals (BALB/c background) have been described (4, 9). Steroid sulfatase activity was determined on ear punches as described (4) using [^{3}H]estrone sulfate or [^{3}H]dihydroepiandrosterone sulfate as substrates and standardizing for the amount of protein. Because the presence of the provirus has been shown to lead in all cases to the establishment of viremia (9), offspring from viremic fathers could be screened by radioimmunoassay for the viral antigen p30. Because maternal transmission of the provirus by milk can lead to the establishment of viremia, offspring resulting from viremic mothers were genotyped by blot analysis of tail DNA using a viral-specific probe to reveal hybridization bands diagnostic for the *Mov-15* provirus (Fig. 2; ref. 9). All offspring found to be recombinants were confirmed by two independent assays for steroid sulfatase activity and for the presence of the provirus.

RESULTS AND DISCUSSION

The genetic crosses are outlined in Fig. 3. Females homozygous for the *Mov-15* proviral insertion were crossed with steroid sulfatase and provirus negative (*Sts*$^-$; *Mov*$^-$) animals. Resulting F_1 offspring were further crossed with (*Sts*$^-$; *Mov*$^-$) animals. Male and female offspring of the F_2 generation were typed for steroid sulfatase activity and for the presence of the provirus as described in *Materials and Methods*. Analysis of the results obtained by crossing female F_1 animals provided information on recombination during female meiosis between the two pseudoautosomal markers *Mov-15* and *Sts*. Analysis of the results obtained by crossing the male F_1 progeny provided information on recombination between three markers, *Sts*, *Mov-15*, and *Tdy*, which is

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¶Present address: Institute for Molecular Genetics, Baylor College of Medicine, Houston, TX 77030.

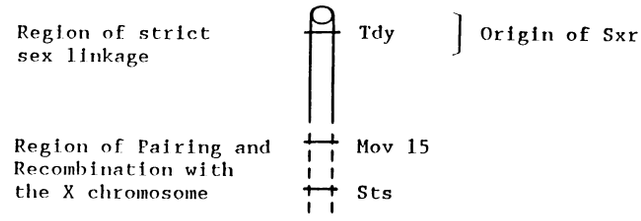

FIG. 1. Diagram of the mouse Y chromosome. The positions of the three markers used in this study—*Tdy*, *Mov-15*, and *Sts*—are indicated. The drawing is not to scale.

carried on the Y chromosome, constituting a three-point cross (Fig. 3).

The results of these crosses are presented in Table 1. In male meiosis, recombination between *Sts* and *Tdy* was 42%, confirming previous results that showed the absence of significant sex linkage for this marker (4). Because the *Mov-15* provirus was inherited in the males of the F_1 generation from their mothers, the provirus must be located on the X chromosome. Analysis of the progeny of F_1 males showed that 20% of the offspring were either males containing the proviral genome, or females without the proviral genome, indicating recombination between the *Mov-15* marker and *Tdy*. This recombination rate was similar to previous results (9). The recombination frequency between the two pseudoautosomal loci, *Sts* and *Mov-15*, was found to be 27%. In female meiosis, recombination between these two markers was found to be a factor of 7 lower (4%). Three double recombinants were also detected among the 127 offspring tested. Such offspring would be expected to be *Sts*-negative males carrying the *Mov-15* provirus or *Sts*-positive females not carrying the provirus (see Fig. 3). One such male and two such females were observed.

A significantly higher frequency of recombination during male meiosis than during female meiosis has also been documented in the human pseudoautosomal region, which is located on the distal short arm of the sex chromosomes (3). Koller and Darlington had already postulated many years ago (10), on the basis of cytological observations, that chiasma formation and subsequent recombination were necessary for normal disjunction of chromosome pairs, including the X and the Y chromosome. The high frequency of recombination in the pseudoautosomal region that we have observed during male meiosis is consistent with this model. While recombination between the X and the Y chromosomes during male meiosis can take place only in the pairing region, recombination between the X chromosomes in female meiosis could occur over the entire length of the X chromosome. One may expect therefore that the recombination rate in the pseudoautosomal region should be higher in male meiosis than in female meiosis.

The steroid sulfatase locus has been shown to exhibit no sex-linkage (4), whereas the *Mov-15* marker recombined with a frequency of 10–20% with sex. Because of this varying degree of sex linkage, recombination in the pseudoautosomal region must not always occur at the same point but rather at different points in different meioses. The gradient of sex

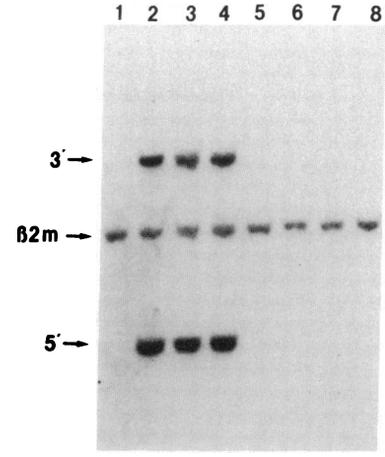

FIG. 2. DNA blot analysis of *Mov-15* offspring. Genomic DNA was isolated from the terminal third of tails, digested with *Kpn* I, and blot hybridized with a viral-specific probe as described (9). Animals 2–4 were positive for the *Mov-15* provirus, as shown by the presence of viral-specific bands representing the 3′ and 5′ ends of the viral genome. β2m is a β_2-microglobulin probe (a gift of Mike Gilman), which serves as an internal control for the amount of DNA loaded in each lane.

linkage observed in the pseudoautosomal region must correlate with the linear order of loci within this region. This is consistent with the following linear order: *Sts*, *Mov-15*, *Tdy*, and centromere. If genetic distances are proportional to physical distances, our results would assign the *Mov-15* locus to the more proximal part and *Sts* to a distal part of the pseudoautosomal region.

To account for the absence of sex linkage of distal pseudoautosomal markers, it has been postulated that a single obligatory crossover must take place in this region during male meiosis (2). Support for this model was provided by a previous study of the human pseudoautosomal region, where no double crossovers were observed among three different pseudoautosomal loci (3). This result, however, may have been at the limit of statistical significance, since only 2.4 such events could have been expected on the basis of the linkage between the pseudoautosomal markers involved. In another study, however, no double crossover events have been detected in 143 informative meioses, where 6 or 7 should have been observed in the absence of crossover interference (D. Page, personal communication). These results suggest that double crossover events occur very infrequently if at all in the human pseudoautosomal region. Our observation of 3 double recombinants among 127 F_2 mice is therefore in striking contrast with the observations in the human genome and supports the previous study, which had demonstrated a double crossover between *Sxr* and *Sts* (7). In addition, this observation is in disagreement with the single obligatory crossover model for the mouse genome.

Our results demonstrate that the mouse and human pseudoautosomal region exhibit varying degrees of crossover interference. In the mouse, the number of double recombi-

Table 1. Recombination between pseudoautosomal loci

Meiosis	Non-recombinants	Recombinants between			Double recombinants	Total
		Sts/Mov	*Mov/Tdy*	*Sts/Tdy*		
Male	70 (55.1%)	34 (26.8%)	26 (20.4%)	54 (42.5%)	3 (2.4%)	127
Female	100 (97.1%)	4 (3.9%)	—	—	—	103

Females homozygous for the *Mov-15* provirus insertion were mated with *Sts* males. Resulting F_1 offspring were further mated with *Sts* animals. Recombinants between *Sts/Mov* and *Mov/Tdy* include double recombinants. Double recombinants were one *Sts*-negative male containing the provirus, and two *Sts*-positive females not containing the provirus (see Fig. 2).

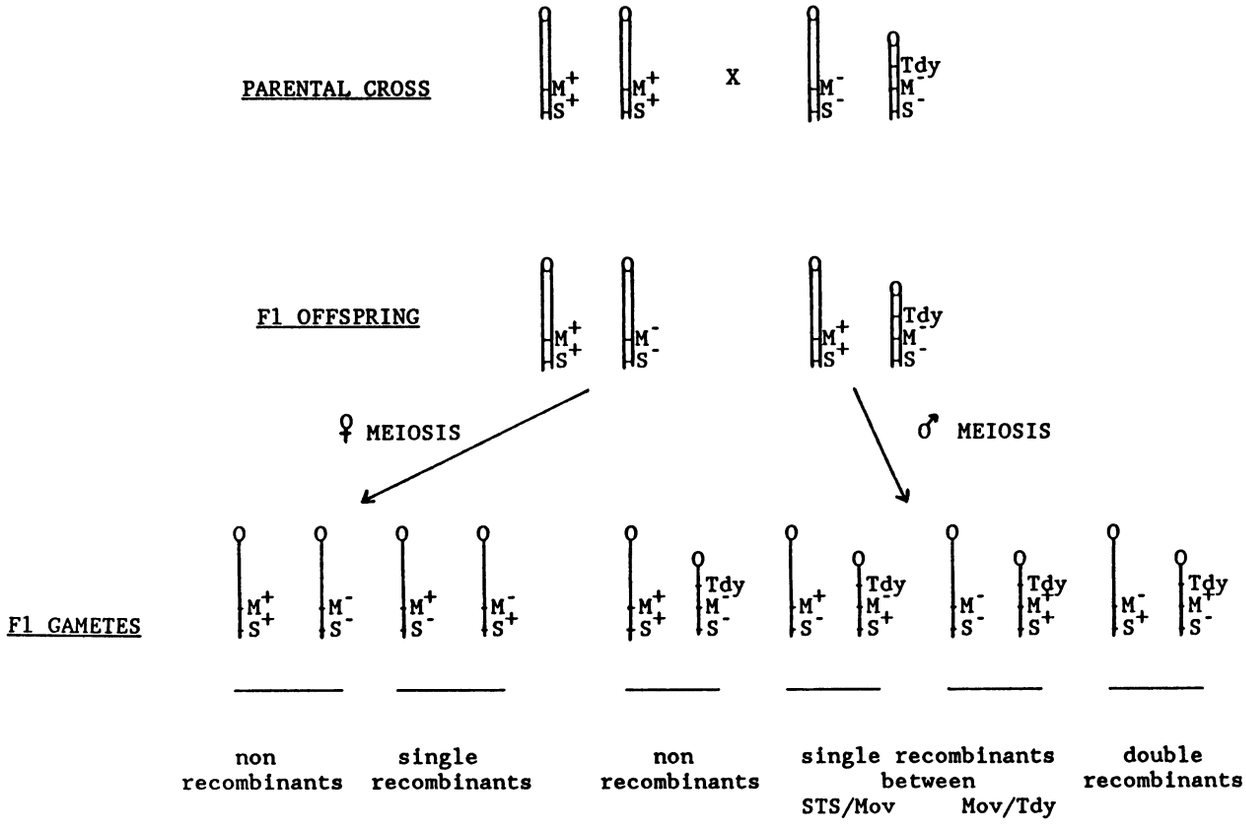

FIG. 3. Recombination in the pseudoautosomal region. Parental cross: Females positive for the *Mov-15* provirus (M^+) and steroid sulfatase activity (S^+) were mated with males negative for the *Mov-15* provirus (M^-) and steroid sulfatase (S^-). The Y chromosome is denoted here by the presence of the testis determining gene, *Tdy*, and is drawn shorter than the X chromosome. F_1 offspring: Both males and females (genotypes shown) were further mated with M^- S^- animals. For the sake of simplicity, only F_1 gametes are presented.

nation events that we have observed (3/127) is not significantly different from the expected number in the absence of crossover interference ($0.27 \times 0.20 \times 127 = 6.9$; $\chi^2_1 = 2.3$), suggesting little or no crossover interference. Without further information about the chromosomal structures of human and mouse pseudoautosomal regions, it is difficult to explain the difference in recombination between the two species. It is possible that the pseudoautosomal region is larger in the mouse than in humans. This might allow for double crossovers if the number of possible crossovers is related to the size of the pseudoautosomal region. This region has been estimated to be ≈5000 kilobases in the human genome (3), a distance that can now be studied by pulse field gel analysis techniques and that may therefore allow the comparison of genetic estimates and physical distances. It is probable that the availability of new pseudoautosomal markers in the two genomes will allow the resolution of these issues. In addition, because these regions are hot spots for recombination in male meiosis, such studies might be particularly useful for the study of the mechanisms of crossover interference in mammalian genomes.

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