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Genetics

Y chromosome short arm–*Sxr* recombination in X*Sxr*/Y males causes deletion of *Rbm* and XY female sex reversal

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ABSTRACT **We earlier described three lines of sex-reversed XY female mice deleted for sequences believed close to the testes-determining gene (*Sry*) on the Y chromosome short arm (Yp). The original sex-reversed females appeared among the offspring of XY males that carried the Yp duplication *Sxr* on their X chromosome. Earlier cytogenetic observations had suggested that the deletions resulted from asymmetrical meiotic recombination between the Y and the homologous *Sxr* region, but no direct evidence for this hypothesis was available. We have now analyzed the offspring of X*Sxr*/Y males carrying an evolutionarily divergent *Mus musculus domesticus* Y chromosome, which permits detection and characterization of such recombination events. This analysis has enabled the derivation of a recombination map of Yp and *Sxr*, also demonstrating the orientation of Yp with respect to the Y centromere. The mapping data have established that *Rbm*, the murine homologue of a gene family cloned from the human Y chromosome, lies between *Sry* and the centromere. Analysis of two additional XY female lines shows that asymmetrical Yp–*Sxr* recombination leading to XY female sex reversal results in deletion of *Rbm* sequences. The deletions bring *Sry* closer to Y centromere, consistent with the hypothesis that position-effect inactivation of *Sry* is the basis for the sex reversal.**

The mouse mutation sex reversed (*Sxr*) arose through a duplication of the Y chromosome short arm (Yp), including the sex-determining gene *Sry* and transposition to the pseudoautosomal region at the end of the Y long arm (1–3). In addition to *Sry*, *Sxr* contains all of the Y chromosome genes necessary for spermatogenesis up to the round spermatid stage (4) and all other known Yp genes including *Zfy1*, *Zfy2*, *Ube1y1*, and *Smcy* (5). Pseudoautosomal crossing-over in carrier (X/Y*Sxr*) males transfers *Sxr* to the X chromosome, causing sex reversal of the X/X*Sxr* progeny (6).

Normally *Sxr* is transmitted only through X/Y*Sxr* males, as X/X*Sxr* males are sterile. However, a nonrandom X-inactivation pattern can be brought about in X/X*Sxr* animals using the T16H translocation [T(X;16)16H]. In some X(T16H)/X*Sxr* individuals the *Sxr* region is inactivated with the result that female development occurs (7, 8). These females transmit *Sxr* to a proportion of their XY male offspring, resulting in males that carry *Sxr* on the X chromosome. Cytogenetic studies on such X*Sxr*/Y males have indicated that, in addition to normal pseudoautosomal pairing, Yp–*Sxr* pairing and exchange occurs. Occasionally this homologous pairing appeared asymmetrical (9).

Three sex-reversed XY females which were found among the offspring of X*Sxr*/Y males were suspected of being derived from asymmetrical Yp–*Sxr* recombination, causing a deletion of *Sry* and perhaps other Yp genes. Accordingly, the chromosomes were named Y^{d1}, Y^{d2}, and Y^{d3}. Contrary to the initial expectation, no evidence of *Sry* deletion could be found. Indeed, the 36-kb region containing the *Sry* structural gene proved to be intact, and all other single-copy Yp markers tested were present on all three Y^d chromosomes (10). The only evidence of chromosomal deletion was a reduction in the copy number of a Y-linked repetitive element, Sx1 (11, 12). Most of the Sx1 sequences were deleted in Y^{d1}, whereas in Y^{d2} and Y^{d3} there was a less extreme reduction (10).

In this communication, we provide direct evidence of Yp–*Sxr* meiotic exchange in X*Sxr*/Y males by exploiting DNA variants between the *Mus musculus musculus*-derived *Sxr* and a *Mus musculus domesticus*-derived Y. This approach has enabled the definition of a map of the Yp/*Sxr* region and the orientation of this map with respect to the centromere. In addition, we have placed another gene (family), *Rbm*, the murine homologue of the human Y-linked gene family RBM (formerly YRRM; ref. 13), between *Sry* and the centromere. We demonstrate that asymmetrical Yp–*Sxr* recombination results in the deletion of *Rbm* sequences and is associated with *Sry* repression in genital ridge tissue, the probable cause of XY female sex reversal.

MATERIALS AND METHODS

Mouse Strains and Crosses. Inbred AKR strain males provided the *M. musculus domesticus* Y (Y^{dom}). X*Sxr*/Y^{dom} males were generated as described (9, 10). Heterozygous T(X;16)16H females carrying the X-linked marker gene tabby (*Ta*) and *Sxr* on their normal X [+(T16H)/*Ta Sxr*] were crossed with AKR males, and hemizygous *Ta* sons carrying *Sxr* (*Ta Sxr*/Y^{dom}) were then crossed to wild-type 3H1 (C3H/HeH × 101/H F_1 hybrid) females. XX and X/X*Sxr* offspring were therefore phenotypically *Ta*/+, whereas the XY and X/Y*Sxr* males were wild type. Any wild-type females were either XO or sex-reversed XY females. These females were distinguished on the basis of C-banded mitotic preparations from cultured lymphocytes by using standard methods. Evidence of Yp–*Sxr* recombination in the XY females was then sought by typing for Y-linked loci by Southern blotting.

One-hundred ninety-two offspring of two X*Sxr*/Y^{dom} males were screened for Yp–*Sxr* recombination events by Southern blot analysis of *Taq* I-digested tail tip DNA using a *Zfy1* cDNA probe. To test for animals resulting from both pseudoautosomal and Yp–*Sxr* recombination, all offspring with an apparent X/Y*Sxr* genotype were bred for one generation, and three of the male offspring from each were typed for *Zfy* variants and the Y-linked repetitive element pY353/B.

Southern Blot Analysis. Southern blotting and hybridizations (except for Sx1 hybridizations) were carried out by using

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Abbreviations: Yp, Y chromosome short arm; RT–PCR, reverse transcription–PCR; dpc, days postcoitum; Y^{dom}, *Mus musculus domesticus* Y.

standard methods (10). Sx1 hybridizations were performed overnight at 68°C in 7% SDS/0.5 M $NaHPO_4$, pH 7.2/1 mM EDTA/1% bovine serum albumin, followed by washing twice for 30 min at 68°C in 2× standard saline citrate/0.1% SDS and once for 30 min at 68°C in 0.1× standard saline citrate/0.1% SDS.

Zfy1 and *Zfy2* were detected by using a *Zfy1* cDNA clone (14); *Sry* was detected by using PCR product 2.1 amplified by using primer pair Y11A and Y11B (10); *Smcy* was detected by using a 0.5-kb *Eco*RI + *Xba* I fragment of cDNA clone pcMY5 (15); the Y-linked repetitive sequence Sx1 was detected by using the probe pSx1 (10, 12); mouse *Rbm*, the homologue of the human RBM gene family, was detected by using two genomic probes LSM2 and LSM15, 0.4-kb *Mbo* I subclones from a λ phage clone positive for human cDNA clone MK5 (13), and the PCR product H114–H118, derived from reverse transcription (RT)–PCR; the repetitive element pY353/B was detected by using the original genomic probe (16).

RT–PCR Analysis. Total RNA samples were prepared either from pools of genital ridges or from adult testes using RNAzolB (Biogenesis, Bournemouth, U.K.). DNA samples from the same individuals were genotyped on the basis of *Taq* I variants at the *Zfy1* and *Zfy2* loci. RNA samples were subjected to RQ1 DNase treatment (Promega) before reverse transcription using Superscript II (Life Technologies, Grand Island, NY). Standard PCR conditions were 94°C for 1 min and either 20 or 30 cycles of 92°C for 30 sec, 55°C for 30 sec, and 75°C for 90 sec, followed by 75°C for 5 min using an MJ Research (Watertown, MA) PTC-100-60 thermal cycler. PCR buffers contained 50 mM KCl, 10 mM Tris (pH 8.4), 0.1% Nonidet P-40, and 1.5 mM $MgCl_2$, 400 nM (each) primer, 200 μM (each) dNTP, and 1 unit of *Taq* polymerase (Applied Biosystems) per 20-μl reaction. Primer pairs were Y11A and Y11B for *Sry* (10), HPRTA and HPRTB for *Hprt* (10), S2Y and S3Y for *Smcy* (15), and PGK1A (5′-CACGCTTCAAAAGCG-CACGTCT-3′) and PGK1B (5′-CTTGAGGGCAGCAG-TACGGAAT-3′) for *Pgk1*.

For analysis of transcription in genital ridge samples, XXY^d females were mated to XY^{dom} males, and the resulting embryos were dissected for genital ridges at 11.5 days postcoitum (dpc). Preliminary experiments showed that 20 cycles of PCR followed by Southern blotting and visualization with ^{32}P-labeled probes resulted in an approximately quantitative detection of different ratios of the relevant transcripts. Reverse-transcribed RNA samples derived from a pool of four XX, XXY^d, and XY embryos were subjected to 20 cycles of PCR, followed by electrophoresis on 3% agarose and Southern blotting. *Pgk1* primers were used as a control in conjunction with *Sry* primers, whereas *Hprt* primers were used as a control in conjunction with *Smcy* primers. The PCR products were visualized by hybridization with the relevant cDNA probes or PCR products labeled with ^{32}P.

To assess *Sry* transcription from adult testes RT–PCR was performed on XY, XY^{dom}, XYY^{d1}, and XYY^{d2} adult testes RNA using *Sry* primers Y11A and Y11B. After amplification, an aliquot of the PCR product was digested with *Mbo* I (Life Technologies) and electrophoresed on a 3% gel, before blotting and hybridization with an *Sry* probe. Other PCR products were cloned into the *Eco*RV site of pBluescript (17), and individual clones were subjected to PCR analysis with primers Y11A and Y11B as above before digestion with *Mbo* I and electrophoresis on 3% agarose gels.

RESULTS

Breeding Performance of $XSxr/Y^{dom}$ Males. A total of 1009 progeny were generated from the cross of *Ta* Sxr/Y^{dom} males with wild-type females. These consisted of 170 *Ta*/+ XX females, 262 *Ta*/+ X/X*Sxr* males, 567 non-*Ta* XY and X/Y*Sxr* males, and 9 non-*Ta* XO or XY females. There was also one exceptional individual, a hemizygous *Ta* male, which proved on later analysis to be X*Sxr*/O and so had not inherited a maternal X. Among the total progeny a divergence from a 1:1 ratio of pseudoautosomal recombinant X/X females relative to non-recombinant X/X*Sxr* males (170:262) was evident, as in previous studies (9, 10), although this was somewhat less extreme (1:1.5, as opposed to 1:4 in ref. 10), perhaps reflecting the different origin of the Y chromosome. Among the nine non-*Ta* females, three were chromosomally XO, two were not classified for karyotype, and four were the sex-reversed XY females specifically sought. Breeding lines were successfully established for two of these presumptive deleted Y chromosomes and named Y^{d5} and Y^{d6}.

XY Female Sex Reversal Derives from Yp–*Sxr* Meiotic Exchange. The Y chromosome content of Y^{d5} and Y^{d6} was analyzed by using three Yp markers, *Sry*, *Zfy1*, and *Smcy*. Both Y^d chromosomes were found to carry *musculus*, rather than the original *domesticus* variants at all three Yp loci tested (Fig. 1), clearly establishing that Yp–*Sxr* recombination had occurred and suggesting that Yp–*Sxr* recombination is causally related to XY female sex reversal. In common with the previous Y^d lines, Y^{d5} and Y^{d6} have intact Yp chromosome arms, but these derive, at least in part, from *Sxr*.

A Recombination Map of Yp and *Sxr*. To detect and characterize Yp–*Sxr* recombination in X*Sxr*/Y males, 192 offspring from the cross described above were screened using the *Zfy1* cDNA probe to detect variants at both the *Zfy1* and *Zfy2* loci. The animals consisted of 39 XX females, 51 X/X*Sxr* males, 57 XY males, 43 X/Y*Sxr* males, 1 XO female, and 1 X*Sxr*/O male. Five XY males and two X/X*Sxr* males carried recombination events between Yp and *Sxr*, as established by *musculus Zfy* variants in XY animals and *domesticus Zfy* variants in X/X*Sxr* animals. These recombinants were then typed for *Sry*, *Zfy*, *Smcy*, and *Sx1* (Fig. 2), further characterizing the recombination events. The same recombinant animals were also typed with probes derived from *Rbm* (Fig. 2), the mouse homologue of the gene family implicated in azoospermia in man (13) that maps to Yp in the mouse (S.K.M., P.S.B. and H.J.C., unpublished observation).

Of the seven recombinants, four (317.1m, 317.5e, 317.5g, 317B.4h) appeared to derive from simple exchanges between Yp and *Sxr*, such that *musculus* loci had been substituted for *domesticus* on the Y, or vice versa on *Sxr* (see Figs. 2 and 3). For offspring 316.1h and 317B.4f both *musculus* and *domesticus* variants for *Rbm* and Sx1 were detected (Figs. 2 and 3). These chromosomes could have resulted from asymmetric recombination events, which had duplicated both Sx1 and *Rbm*. An alternative explanation is that the Sx1 and *Rbm* sequences are present in more than one copy on the mouse Y chromosome and are interspersed. The latter interpretation agrees with the observation that Sx1 repetitive elements are present in three independent genomic clones containing *Rbm* sequences (S.K.M. and P.S.B., unpublished observation). The seventh recombinant (317B.4k) is clearly the result of asym-

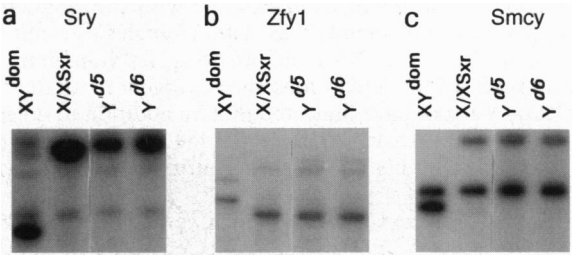

FIG. 1. Southern blot analysis of the Yp loci of Y^{d5} and Y^{d6}. Genomic DNA from XXY^d females and relevant controls (strain AKR XY^{dom} male and X/X*Sxr* male) digested with either *Taq* I (*a* and *b*) or Pvu II (*c*) and probed with Sry PCR product 2.1 (*a*), Zfyl cDNA (*b*), and Smcy cDNA (*c*).

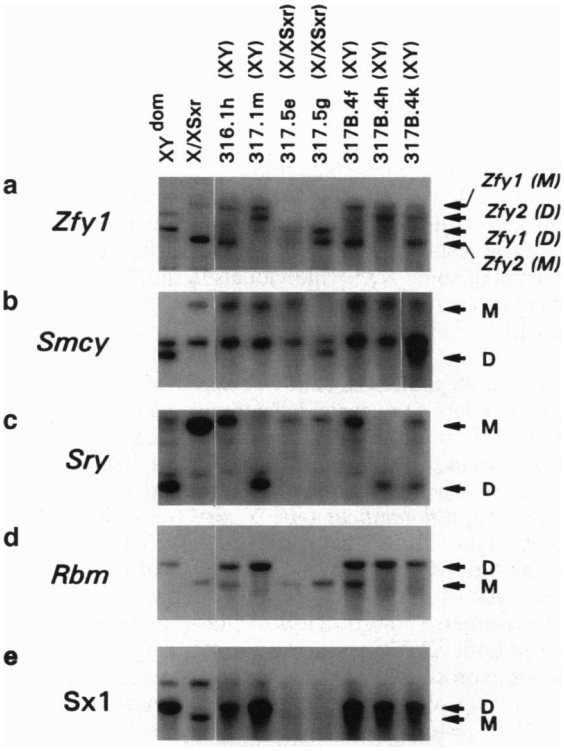

FIG. 2. Southern blot analysis of recombinants from X*Sxr*/Y^{dom} males. Genomic DNA of the Yp–*Sxr* recombinant males digested with either *Taq* I (*a*, *c*–*e*) or *Pvu* II (*b*) and probed with Zfyl cDNA (*a*), Smcy cDNA (*b*), *Sry* PCR product 2.1 (*c*), *Rbm* probe LSM2 (*d*), and repeat sequence pSx1 (*e*). In recombinant 317B.4k the *musculus Smcy* band is faint; therefore this track has been printed darker to clearly establish band presence. The faint bands detected by the *Sry* probe in certain tracks are due to cross-hybridization with *Sox* genes. The scoring of animal 317.1m as *domesticus* alone for Sx1 is clear from the original autoradiograph. M, *musculus*; D, *domesticus*.

metrical exchange, as it possessed both *musculus* and *domesticus* variants of the *Sry*, *Zfy2*, and *Smcy* loci, but *musculus* alone at *Zfy1* and *domesticus* alone at *Rbm* and Sx1 (Fig. 3). This male has since transmitted all of the observed Yp variants to his male offspring, indicating that his Yp carries a tandem duplication (see Fig. 6*c*) and that he is not some form of X/Y*Sxr*. Excluding the latter animal, the recombination events described show that regular homologous exchange can occur along the length Yp and *Sxr*, and by minimizing the number of

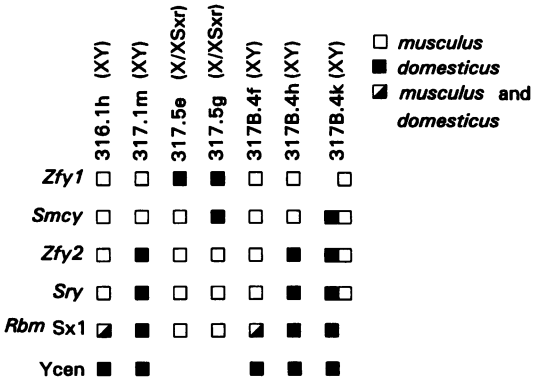

FIG. 3. Order of loci in Yp and *Sxr* inferred from characterization of the Yp–*Sxr* recombinant males. Locus names are at left, and the Yp–*Sxr* recombinant animals are listed at top. Order of loci has been inferred by minimizing number of double recombinants except for the order of *Sry* and *Zfy2*, which was established from a preliminary experiment (S.H.L. and B.M.C., unpublished observations) and published sources (5, 10). Ycen, Y centromere.

double recombinants, the order of loci can be concluded to be *Zfy1-Smcy-Zfy2-Sry-Rbm*/Sx1-centromere. The order is consistent with the consensus map of Yp (5), but orientation with respect to the centromere is now provided.

Because Sx1 is known to be deleted on Y^{d1}, Y^{d2}, and Y^{d3} chromosomes and *Rbm* maps to the same region, it seemed likely that *Rbm* sequences might also be deleted. This hypothesis was therefore investigated.

Rbm Deletion Is Common to All of Y^d Strains. DNA from the XY^d females and relevant controls were hybridized with three probes for mouse *Rbm*. Overall, a reduction in intensity of the bands and/or the loss of specific bands on the Y^d chromosomes relative to the controls demonstrated that *Rbm* was partially deleted in all Y^d lines (Fig. 4). The variable copy number of *Rbm* on the Y^d chromosomes shows that RBM is a multiple-copy gene family on the mouse Y chromosome, as on the human Y, contrary to initial indications (18). The lowest copy number of *Rbm* was found in Y^{d1} and Y^{d6}, whereas the other lines were less extensively deleted. For Y^{d5}, derived from a Y^{dom}, detection of both *musculus* and *domesticus* variants using one of the probes (Fig. 4*c*) shows that the site of recombination between Yp and *Sxr* must lie between two *Rbm* sequences. It may, therefore, be concluded that the recombination events that led to the Y^d chromosomes resulted in the effective deletion of *Rbm* genes and associated Sx1 sequences and, hence, were asymmetrical. From the map position of *Rbm*, these deletions occur proximal to *Sry*, bringing this locus closer to the Y centromere.

Sry Is Repressed in Gonadal Ridges of XXY^d Embryos. To determine whether *Sry* repression relates to and correlates with the extent of *Rbm* sequence deletion, semiquantitative

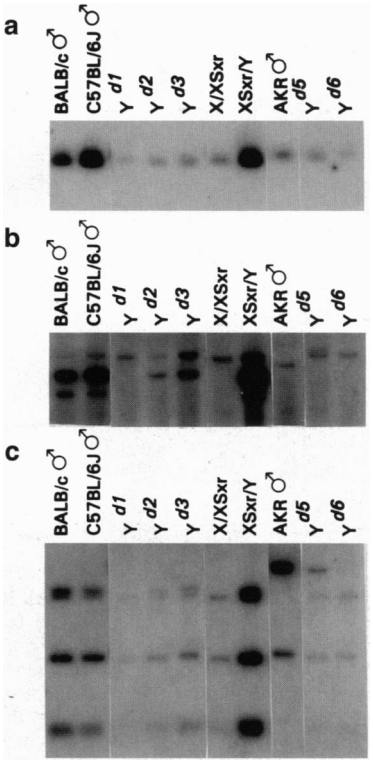

FIG. 4. Southern blot analysis of genomic DNA from the carrier XXY^d females with the relevant controls hybridized with three *Rbm* probes. Y^{d1} is derived from a BALB/c Y chromosome, Y^{d2} and Y^{d3} are derived from C57BL/6J, and Y^{d5} and Y^{d6} are derived from AKR (XY^{dom}). The *Rbm* probes and restriction digests used were probe LSM15 on an *Eco*RI and *Pst* I double digest (*a*), probe LSM2 on an *Acc* I digest (*b*), and PCR product H114–H118 on an *Acc* I digest (*c*). Reprobing filters with a Y-linked control probe showed that loadings per track were equivalent.

RT–PCR analyses were performed on gonadal ridges at 11.5 dpc, the critical period for *Sry* expression and testis determination. The level of Sry transcript in XXY^d embryos was greatly reduced relative to XY embryos, as compared to control *Pgk1* expression (Fig. 5*a*). To ascertain whether *Rbm* deletion influences other Yp genes, *Smcy*, a Y-linked gene mapping between the two *Zfy* loci (15) was similarly investigated. Fig. 5*b* shows that *Smcy* expression in the XXY^d class of offspring in 11.5-dpc genital ridge samples relative to *Hprt* was approximately equivalent to the XY class. Therefore, the mechanism of *Sry* repression does not affect this more distal locus.

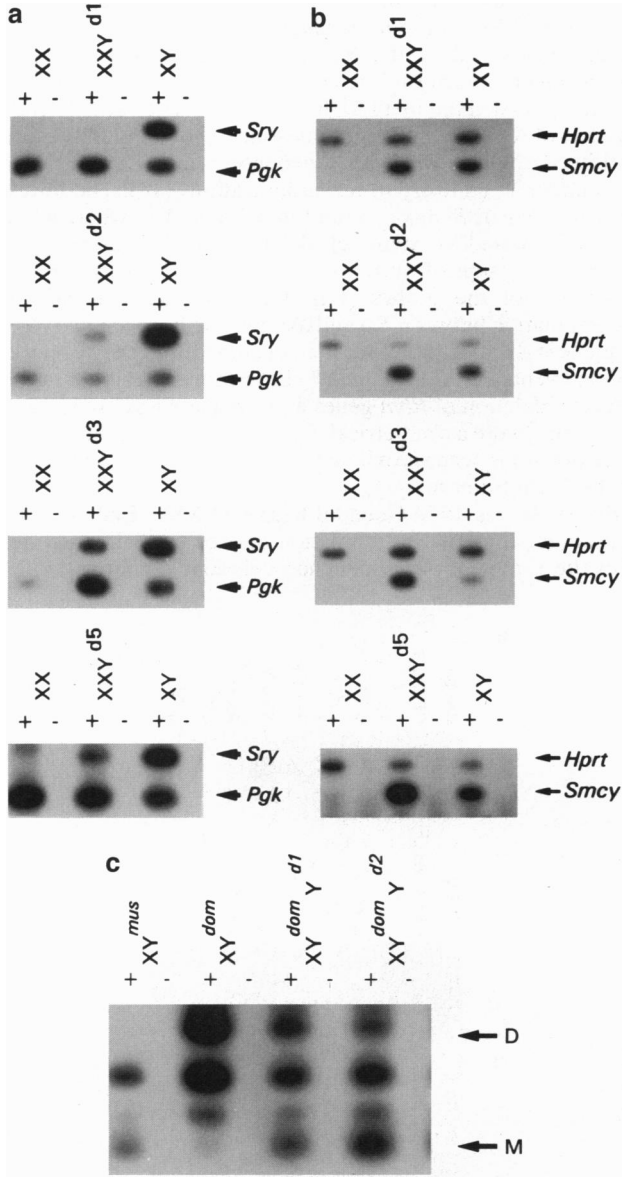

FIG. 5. Analysis of expression using RT–PCR. All RT–PCR reactions are shown with (+) or without (−) reverse transcription to control for genomic DNA contamination. (*a*) Semi-quantitative RT–PCR analysis of *Sry* and *Pgk1* expression in 11.5-dpc genital ridge tissue from XX, XXY^d, and XY embryos. The band of slightly higher molecular weight than *Sry* in the XX embryo pool of the Y^{d5} samples is artifactual. (*b*) Semiquantitative RT–PCR analysis of *Smcy* and *Hprt* expression in 11.5-dpc genital ridge tissue of XX, XXY^d, and XY embryos. Variation in the ratio of *Hprt* to *Smcy* between different XY embryo pools is caused by the relative efficiencies of probe radiolabeling in the various experiments, which were conducted at different times; each panel should, therefore, be considered separately. (*c*) Analysis of *Sry* expression in XYY^d adult testes. RT–PCR reactions using *Sry* primers were digested with *Mbo* I, blotted, and probed with the *Sry* PCR product 2.1, distinguishing the *musculus*—i.e., Y^d-derived transcript—from that of *domesticus* (Y^{dom}) origin, labeled M and D accordingly.

Although this technique allows only approximate quantification, the *Sry* repression was clearly greatest in Y^{d1}, the strain with the most extensive deletion of *Rbm* sequences, and less in Y^{d2}, Y^{d3}, and Y^{d5}, where *Sry* transcription can be observed, although at a lower level than in XY embryos (Fig. 5*a*). We were unsuccessful in obtaining a complete expression profile for Y^{d6}. The residual *Sry* expression results in the incomplete sex reversal of some XXY^d individuals in the Y^d stocks, which also roughly correlates with *Rbm* deletion (10% hermaphroditism with Y^{d2} and Y^{d3}, 2% with Y^{d5}, and 0% with either Y^{d1} or Y^{d6}).

***Sry* Is Not Repressed in Adult Testes.** The other major site of *Sry* expression is the germ-cell compartment of adult testes; however, this transcript is spliced between 5′ donor and 3′ acceptor sites to produce a circular transcript (19). Adult testis expression of *Sry* was investigated for Y^{d1} and Y^{d2} using XYY^d males bearing a *domesticus*-type Y. Sry transcripts from the *domesticus*-type Y are distinguishable from the Y^d-derived (*musculus* type) transcript by the absence of one *Mbo* I site (20). Analysis of *Mbo* I-digested RT–PCR products amplified using *Sry* primers showed that *musculus*-type transcripts were present in both XYY^{d1} and XYY^{d2} testes (Fig. 5*c*), indicating that *Sry* is expressed from the Y^d chromosome. To quantitate this expression, undigested RT–PCR products from XYY^{d1} and XYY^{d2} testes was cloned into a plasmid vector, and individual clones were analyzed for the presence of the *Mbo* I site in the insert. Six out of 17 clones from XYY^{d1} testes RT–PCR and 7 out of 16 from XYY^{d2} RT–PCR were of *musculus* type (data not shown), indicating that *Sry* expression from the Y^d chromosome is not suppressed in adult testes.

DISCUSSION

The data presented here demonstrate that Yp may undergo homologous pairing and exchange with its *Sxr* derivative during meiosis in X*Sxr*/Y males. The recombination appears to be regular in some instances, such that the recombinants are not detectably unbalanced and have a single copy of each known gene on Yp and *Sxr*. This result indicates that the appropriate mechanisms for exchange are still operative, even though the Yp region has not undergone pairing or recombination for a significant period of evolutionary time.

Yp–*Sxr* recombination in X*Sxr*/Y males has been postulated to explain the unexpectedly low frequency of pseudoautosomal recombinants among their offspring (9). In the present study, 7 Yp–*Sxr* recombinants out of 192 offspring were retrieved, giving a frequency of 4%. This percentage is slightly lower than the estimate of 10% calculated from the skewed segregation of *Sxr* (170 XX:262 X/X*Sxr*). The difference may be due to recombination events occurring distal to *Zfy1*, the most distal known locus in the *Sxr* region, that would not be detected in this study.

None of the five Yp–Sxr recombinant XY animals carried *Sxr*, indicating that pseudoautosomal exchange involving the same chromatids had not occurred in these meioses. From this it may be inferred that Yp–*Sxr* pairing and exchange is sufficient for germ-cell survival, and pseudoautosomal exchange is not an absolute requirement. However, it is possible that pseudoautosomal pairing occurs, but without exchange, to fulfill a pairing requirement for germ-cell survival (4). However, this interpretation is not supported by the relatively infrequent occurrence of both Yp–*Sxr* and pseudoautosomal pairing in the same cells, observed in pachytene spermatocyte studies of X*Sxr*/Y mice (21).

In addition to regular homologous exchange, two types of irregular Yp–*Sxr* recombination were found in the present study: the recombination leading to deletion of sequences in

the sex-reversed XY^d females (Fig. 6*b*) and that leading to a tandem duplication of Yp in one recombinant male (Fig. 6*c*). In the former case, a deletion event including members of a gene family, *Rbm*, adjacent to *Sry* on the mouse Y chromosome is associated with the down-regulation of *Sry* transcription and associated XY female sex reversal. In humans there has been one example of an SRY-positive XY female with a deletion outside of the sex-determining region of the Y chromosome (22), and deletion of SRY regulatory elements was postulated. A role for *Rbm*, Sx1, or associated sequences in the regulation of *Sry* seems unlikely, as X/X*Sxr* animals only have a few such sequences and yet develop as males; however, we cannot rule this possibility out. The simplest explanation is that deletion of *Rbm* and associated sequences causes the sex reversal in a secondary way, by decreasing the distance between *Sry* and a repressive chromatin domain, as has been suggested (10). This domain could represent the Y centromere or could be associated with the normal regulation of *Rbm*. The latter concept would be consistent with *Sry* repression at 11.5 dpc, when *Rbm* is not expressed at significant levels, and with the normal expression of *Sry* in adult testes, where *Rbm* is abundantly expressed (data not shown). A position effect of this type would explain the approximate correlation between extent of *Rbm*/Sx1 deletion, the level of *Sry* expression in the gonadal ridges, and the incomplete sex reversal of some XXY animals in the Y^d lines.

The Y chromosome of the AKR strain carries fewer *Rbm* sequences than that of the SWR strain (S.H.L., unpublished observations), even though both are of *domesticus* origin. This result could relate to the finding that the AKR Y may bring about XY sex reversal when introduced into the C57BL/6 inbred strain background, whereas the latter does not (20). Because we have demonstrated that the level of *Sry* expression in 11.5-dpc genital ridge tissue correlates with number of *Rbm* sequences, the variation in the degree of sex reversal between different *domesticus* Y chromosomes in the C57BL/6 system may be explicable in terms of *Rbm* copy number.

The second example of asymmetric Yp–*Sxr* recombination was found in a male with an apparent duplication of Yp. This Y resulted from an exchange at a site between the *Smcy* and *Zfy1* loci on Yp and a site proximal to *Sry* on *Sxr* (Fig. 6*c*). This exchange could have been mediated by recombination between Sx1 repeats because some members of this repetitive element also map more distally in *Sxr* (Sx1 band D; refs. 2 and 12). This recombination may have the same basis as the postulated aberrant recombination between *Zfy* loci, which generated a deletion variant of *Sxr* (23, 24).

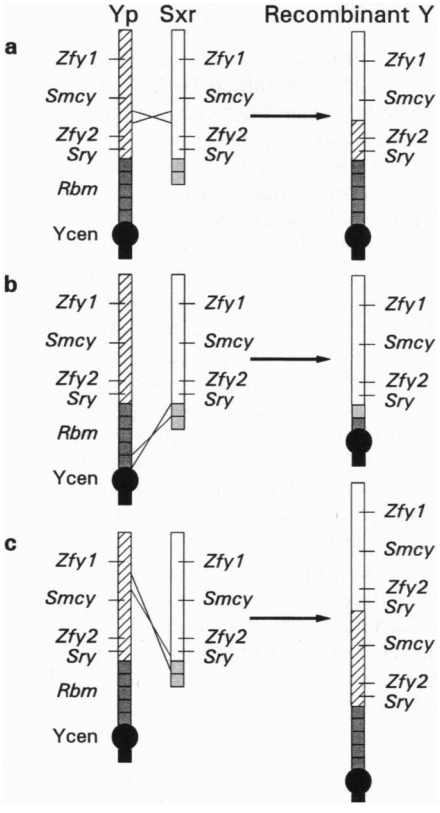

FIG. 6. Three types of Yp–*Sxr* exchange. (*a*) Regular recombination with exchange point between *Zfy2* and *Smcy*, such as found in XY male recombinants 317.1m and 317B.4h. (*b*) Irregular recombination between *Rbm* loci, leading to generation of a Y^d chromosome. (*c*) Irregular recombination with exchange point lying between *Smcy* and *Zfy1* on Yp and proximal to *Sry* in *Sxr*, such as found in XY male recombinant 317B.4k.

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