Duplication, Deletion, and Polymorphism in the Sex-Determining Region of the Mouse Y Chromosome

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The *ZFY* gene in the sex-determining region of the human Y chromosome encodes a "zinc-finger" protein that may be the testis-determining factor, *TDF*. Although the Y chromosomes of most placental mammals carry a single homolog of *ZFY*, the mouse Y chromosome has two homologs, both in the sex-determining (*Sxr*) region. *Zfy-1* alone may suffice to determine maleness; *Zfy-2* is dispensable, as it was deleted in an *Sxr* variant that retains sex-determining function but has lost other genes. Both loci mapped near the centromere of the mouse Y chromosome. The Y chromosomes of the subspecies *Mus musculus musculus* and *M. m. domesticus* were distinguishable by a *Zfy-1* restriction fragment polymorphism, which can be used to study their differing interactions with autosomal sex-determining genes.

THE SEX OF A HUMAN OR MOUSE embryo is normally determined by one or more genes on the Y chromosome (*1*). In the presence of this gene or genes, the bipotential gonads develop as testes, and male differentiation ensues. The absence of this gene or genes results in the development of ovaries and a female phenotype. Studies in the human have localized an essential portion of this testis-determining factor (*TDF*) to a 140-kb region on the short arm of the Y chromosome (*2*). This region harbors a gene encoding a protein with multiple "zinc-finger" domains (*2, 3*). This gene may prove to be *TDF*. In the absence of more direct evidence of sex-determining function, the gene will be referred to as *ZFY* [zinc-finger protein on Y (*4*)]. Homologs of human *ZFY* are found on the Y (and X) chromosomes of a wide range of placental mammals (*2*). The mouse, because of its suitability for transgenic manipulation, would be one of the best mammals in which to investigate *ZFY* function.

In contrast to other placental mammals examined, including humans, gorillas, chimpanzees, rabbits, dogs, cattle, horses, and goats (*2, 5*), the mouse has two homologs of *ZFY* on its Y chromosome. When clone pDP1171 (which contains a 5-kb mouse genomic Eco RI fragment homologous to human *ZFY*) is hybridized to mouse DNAs, it detects two male-specific Eco RI fragments, 5 and 11 kb in length (Fig. 1A). (The homolog on the mouse X chromosome, in addition to a homolog on a mouse autosome, are detected by pDP1171 only under hybridization conditions of reduced stringency. Human *ZFY* clone pDP1007 detects the same mouse X-specific, Y-specific, and autosomal fragments.) The existence of two pDP1171-homologous loci on the mouse Y chromosome is confirmed by analysis of genomic DNAs digested with seven other restriction endonucleases. These two loci are found in both *Mus musculus* (the house mouse) and *Mus spretus* (the western Mediterranean short-tailed mouse) (Fig. 1A). We conclude that an intrachromosomal duplication occurred prior to the divergence of the two species, 2 to 6 million years ago (*6*). Since the mouse Y-linked loci are closely related, as judged by DNA hybridization, to the human *ZFY* gene, we shall refer to the locus of the 11-kb Eco RI fragment as *Zfy-1* and the locus of the 5-kb Eco RI fragment as *Zfy-2*.

Are both *Zfy-1* and *Zfy-2* required for testis determination in mice? Our studies suggest that *Zfy-1* alone may be sufficient. We focused on *Sxr*, *Sxr'*, and *Sxr''*, three related transpositions of the sex-determining region of the mouse Y chromosome. *Sxr* is a second, functional copy of the sex-determining region, transposed to the region of the Y chromosome that regularly undergoes meiotic exchange with the X chromosome (*7, 8*). Both XY *Sxr* and XX *Sxr* embryos develop as males, with testes (*9*). *Sxr* also carries a gene involved in spermatogenesis (*10*) and encodes or controls the expression of H-Y (*11*), a male-specific minor histocompatibility antigen (*12*). *Sxr'*, a variant that arose from *Sxr*, retains the sex-determining function but has lost both the spermatogenesis function (*10*) and H-Y antigen expression (*13*)—a finding that has been used to argue against the hypothesis (*14*) that H-Y antigen determines sex. *Sxr''* is a derivative of *Sxr'* that has regained H-Y antigen expression (*15*).

Hybridization with pDP1171 detects differences among *Sxr*, *Sxr'*, and *Sxr''*. Although *Zfy-1* is present in all three, *Zfy-2* is present in *Sxr*, absent in *Sxr'*, and regained in *Sxr''* (Fig. 1A). We infer that *Sxr'* is a partially deleted form of *Sxr*, lacking *Zfy-2* as well as other genes (Fig. 1B). Since XX *Sxr'* mice develop as males, we conclude that *Zfy-1* and *Zfy-2* are not *both* necessary for testis determination. *Zfy-1* alone may be sufficient for testis determination. *Zfy-2* may encode a functionally redundant testis-deter-

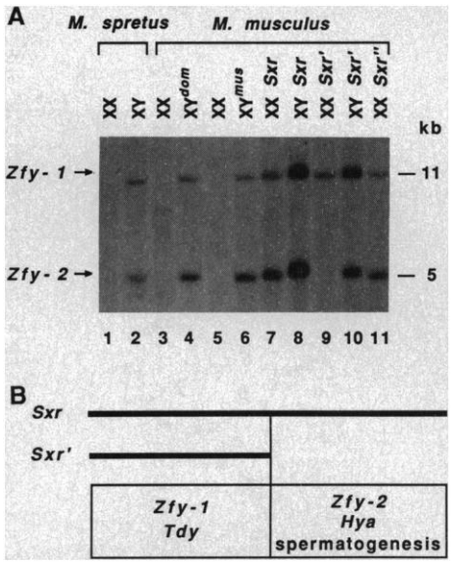

Fig. 1. (**A**) Hybridization of Eco RI–digested mouse DNAs with probe pDP1171, a mouse *Zfy-2* clone (*27*). *Mus spretus* and *Mus musculus* are species of mice. The strain backgrounds of the *M. musculus* animals are as follows: FVB/N (lanes 3 and 4), BALB/c (lanes 5 and 6), and C57BL/6Mcl (lanes 7 through 11). Y^{dom} and Y^{mus} indicate Y chromosomes of, respectively, *M. m. domesticus* and *M. m. musculus* origin as determined by the use of restriction fragment polymorphisms (*19–23*). (The Y chromosomes in lanes 8 and 10 are also of *M. m musculus* origin.) The presence of the 11- and 5-kb Eco RI fragments in XX *Sxr* and XX *Sxr''* demonstrates that both *Zfy-1* and *Zfy-2* are contained within *Sxr* (*2*) and *Sxr''*. As expected, both fragments hybridize at twice the intensity in XY *Sxr* as compared with either XX *Sxr* or XY. (Lane 8 is slightly overloaded.) In contrast, only the 11-kb Eco RI fragment is detected in XX *Sxr'*, while the 5-kb fragment is absent. Thus, *Zfy-1* is present but *Zfy-2* is absent in *Sxr'*. In keeping with this interpretation, there appear to be two copies of *Zfy-1* but only a single copy of *Zfy-2* in XY *Sxr'*. The patterns of hybridization seen in the XX *Sxr*, XY *Sxr*, XX *Sxr'*, and XY *Sxr'* mice have been reproduced in three additional animals of each genotype (not shown). The XX *Sxr''* animal in lane 11 is mouse 719 described by McLaren *et al.* (*15*). (**B**) A two-interval deletion map of the *Sxr* region of the mouse Y chromosome. The black bars indicate the portions present in *Sxr* and *Sxr'*. The order of the two intervals with respect to the centromere is not known. *Zfy-1* and *Zfy-2*, homologs of *ZFY*, the human Y-linked zinc-finger protein gene (*2, 4*); *Tdy*, Y-linked testis determinant (*18*); *Hya*, Y-linked histocompatibility antigen.

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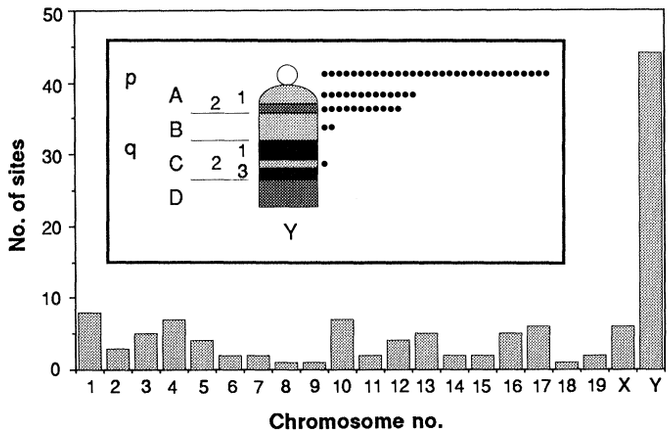

Fig. 2. In situ hybridization of pDP1055, a mouse *Zfy-1* clone, to mouse chromosomes (*28*). Histogram summarizing the sites of hybridization in the 95 metaphase cells scored. Thirty-six percent of the hybridization sites (44 of 121 sites) were on the Y chromosome. Multiple autoradiographic grains were found at the site of hybridization on many Y chromosomes. (Inset) Distribution of grains on a diagram of the Y chromosome. Chromosome band numbers are indicated at the left; p and q indicate the short and long arms, respectively. The mouse insert of plasmid pDP1055 is a genomic 2.0-kb Hind III fragment from within the 11-kb Eco RI fragment at *Zfy-1*. When hybridized to gel transfers of Eco RI–digested mouse genomic DNAs, pDP1055 hybridizes strongly to the same male-specific 5- and 11-kb fragments as seen with pDP1171 (Fig. 1A).

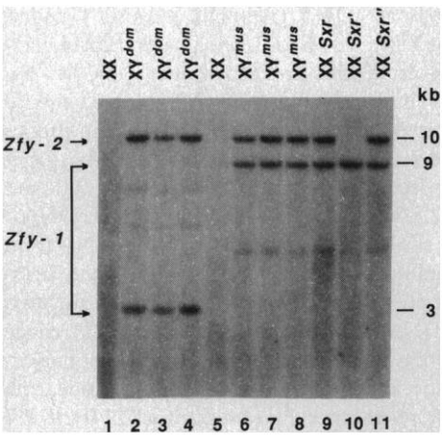

Fig. 3. A Taq I restriction fragment polymorphism at *Zfy-1* that distinguishes Y chromosomes of *M. m. musculus* and *M. m. domesticus* origin. Genomic DNAs were digested with Taq I and hybridized with probe pDP1171. The mice are of the following strains: FVB/N (lanes 1 and 2), MA/MyJ (lane 3), BUB/BnJ (lane 4), BALB/c (lanes 5 and 6), AEJ/GnLe (lane 7), BDP/J (lane 8), and C57BL/6Mcl (lanes 9 through 11).

Table 1. Y-chromosomes of 34 inbred strains of mice classified on the basis of the Taq I restriction fragment polymorphism at *Zfy-1*. Male genomic DNAs were hybridized with probe pDP1171. Each sample was characterized by either the *M. m. musculus* or the *M. m. domesticus* pattern shown in Fig. 3.

M. m. musculus type		*M. m. domesticus* type
A/J	NZW/LacJ	AKR/J
AEJ/GnLe	P/J	BUB/BnJ
BALB/c	RIIIS/J	FUB/N
BDP/J	SB/Le	IS/CamEi
CBA/FaCam	SEA/GnJ	Ma/MyJ
CE/J	SEC/1ReJ	PL/J
C57BL/6J	SF/CamEi	RF/J
DA/HuSn	SK/CamEi	SJL/J
DBA/2J	SM/J	St/bJ
I/LnJ	WB/ReJ	SWR/J
LP/J	YBR/Ei	SWV
NZB/BINJ		

mining gene product, carry out a function unrelated to sex determination, or be a pseudogene of *Zfy-1*. These findings also suggest that the absence of H-Y antigen expression in XX *Sxr'* mice is due not to an immunologic artifact (*16*) but instead to the actual deletion of one or more genes encoding or controlling expression of the antigen (Fig. 1B). Accordingly, these results reinforce the evidence (*13, 17*) that H-Y transplantation antigen does not function in gonadal sex determination.

On the human Y chromosome, *ZFY* lies extremely close to the pseudoautosomal region (*2*), where X-Y recombination normally occurs during male meiosis. By in situ hybridization, we determined that both *Zfy-1* and *Zfy-2* map near the centromere of the mouse Y chromosome, far from the region of frequent X-Y recombination (Fig 2). Close proximity of *ZFY* homologs to the pseudoautosomal region is evidently not a constant feature of mammalian Y chromosomes. Since comparable numbers of grains are found on the short arm and on the most proximal portion of the long arm (bands A1 and A2), it is not possible to conclude whether *Zfy-1* and *Zfy-2* map to the short or long arm, or whether they map to opposite arms. Like *Zfy-1* and *Zfy-2*, certain repetitive sequences present in *Sxr* are also found near the centromere of the normal mouse Y chromosome (*7*), suggesting that *Sxr* represents a duplication of a single contiguous block of the mouse Y chromosome.

Y chromosomes of *Mus musculus musculus* and *M. m. domesticus* origin differ functionally: unlike *M. m. musculus* Y chromosomes, *M. m. domesticus* Y chromosomes appear to be ineffective in inducing testis differentiation on certain genetic backgrounds (*18*). This inability may be due to allelic differences in the Y-chromosomal testis-determining genes of the two subspecies. We have found a Y-linked restriction fragment polymorphism that should be of use in examining this phenomenon. Although both *M. m. musculus* and *M. m. domesticus* Y chromosomes carry the *Zfy-1/Zfy-2* duplication (Fig. 1A), they are distinguished by a Taq I restriction fragment polymorphism (Fig 3). Mice with an *M. m. musculus* Y chromosome have 9- and 10-kb Taq I fragment, while mice with an *M. m. musculus* Y chromosome have 9- and 10-kb Taq fragments while mice with an *M. m. domesticus* Y chromosome have 3- and 10-kb Taq I fragments. Since the invariant 10-kb Taq I fragment is absent in *Sxr'*, it must derive from *Zfy-2*. The polymorphic fragments must derive from *Zfy-1*. In a survey of males of 34 inbred mouse strains, only the two hybridization patterns shown in Fig. 3 were found. Our classification of each of these Y chromosomes as being of either the *M. m. musculus* or *M. m. domesticus* type (Table 1) agrees completely with groupings based on other Y-linked restriction fragment polymorphisms, none of which map to *Sxr* (*19–25*). Judging by the *Zfy-1* polymorphism, *Sxr, Sxr'*, and *Sxr''* are all of *M. m. musculus* origin (Fig. 3). This polymorphism could be used to screen for a derivative of *Sxr* that has acquired the *M. m. domesticus* allele of the sex-determining region [by the sort of recombination event that presumably caused the reversion of *Sxr'* that produced *Sxr''* (*15*)]. The availability of *Sxr* variants of *M. m. domesticus* and *M. m. musculus* origin would permit one to more directly examine the interactions of Y-chromosomal with autosomal or X-chromosomal sex-determining genes.

Using a Y-specific repetitive DNA probe, other workers have also found that *Sxr'* is a partially deleted form of *Sxr* (*26*). In situ hybridization reveals that certain other sequences present in *Sxr* are also found near the centromere of the Y, perhaps on the short arm.

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27. Genomic DNAs were digested with Eco RI, separated by electrophoresis in 0.8% agarose, transferred to nylon membrane, and hybridized with the mouse genomic insert of plasmid pDP1171 as previously described (*2*).
28. Mouse (DBA/2J) chromosomes from cultured spleen lymphocytes were hybridized with ^{3}H-labeled pDP1055 at 42°C in 50% formamide, 2× standard saline citrate (SSC), 10× Denhardt's, 0.01% denatured salmon sperm DNA, and 10% dextran sulfate. Slides were washed three times for 2 min each at 39°C in 50% formamide, 2× SSC, exposed for 18 days, and stained for Q banding. The slides were examined by fluorescence microscopy to identify chromosomes and by transmitted light to localize autoradiographic grains.
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Chromosome Mapping and Expression of a Putative Testis-Determining Gene in Mouse

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Isolation and mapping of a mouse complementary DNA sequence (mouse Y-finger) encoding a multiple, potential zinc-binding, finger protein homologous to the candidate human testis-determining factor gene is reported. Four similar sequences were identified in Hind III–digested mouse genomic DNA. Two (7.2 and 2.0 kb) were mapped to the Y chromosome. Only the 2.0-kb fragment, however, was correlated with testis determination. Polymerase chain reaction analysis suggests both Y loci are transcribed in adult testes. A 3.6-kb fragment was mapped to the X chromosome between the T16H and T6R1 translocation breakpoints, and a fourth (6.0 kb) was mapped to chromosome 10. Hence, mYfin sequences have been duplicated several times in the mouse, although they are not duplicated in humans.

IN THE FETAL MOUSE, THE PAIRED GONADAL primordia are capable of developing into either ovaries or testes. If the fetus is of XY karyotype, the bipotential gonads differentiate into testes. The testes in turn secrete testosterone and Mullerian inhibiting substance, which induce development of the male accessory sex ducts and male secondary sexual characteristics. In the absence of a Y chromosome (for example, XX or XO karyotypes), the gonads develop into ovaries (*1*). The gene on the mouse Y chromosome initiating testis differentiation has been designated testis-determining Y (*Tdy*).

Recently Page *et al.* (*2*) isolated a human Y-chromosomal DNA fragment (pDP1007) that had characteristics making it a candidate for the testis-determining factor gene (*TDF*), the human equivalent of *Tdy*. A homologous sequence was also found on the X chromosome. The nucleotide sequence of pDP1007 encoded a protein containing multiple potential zinc-binding fingers. Zinc-finger proteins have been hypothesized to bind to and regulate DNA or RNA in a sequence-specific manner (*3*).

To isolate and characterize sequences in the mouse that are similar to pDP1007, a 36-base oligonucleotide (5′-CTC ATA CTC ACA GAA CTT GCA CTT GTG CAT CTT GTT-3′) deduced from amino acids 115 to 126 of the protein encoded by pDP1007 (*2*) was synthesized. It was used to probe a human Y chromosome–enriched library constructed in λ Charon 21A (*4*). A 1.3-kb Hind III DNA fragment (human Y-finger, hYfin) was isolated that is similar, if not identical, to pDP1007 on the basis of size and amino acid sequence. In addition, Southern blots of Eco RI–digested mouse genomic DNA revealed male-specific bands at 5.1 and 11.2 kb, similar to what was reported for pDP1007.

Using hYfin as a probe to screen a BALB/c adult testis cDNA library constructed in λ gt11 (*5*), we isolated a homologous 1.28-kb partial cDNA fragment (mouse Y-finger, mYfin). The Eco RI insert was subsequently isolated and subcloned into pUC18. On Southern blots of Eco RI–digested mouse genomic DNA, hYfin and mYfin probes detected the same 5.1- and 11.2-kb male-specific fragments and, in addition, 2.0- and 2.1-kb fragments in male and female DNAs.

In Hind III–digested genomic DNA from inbred strains, four DNA fragments hybridized to the mYfin probe in males and two DNA fragments in females (Fig. 1a). Since inbred male and female mice differ only in the presence of the Y chromosome, the fragments present only in males (2.0 and 7.2 kb) were mapped to the Y chromosome. When Southern blots of male mouse genomic DNA digested with Bam HI, Bgl II, Eco RI, Hind III, and Taq I were probed with mYfin, two Y-specific bands of different sizes but of equal hybridization intensities were observed. Our data suggest that either there are two copies of mYfin sequences on the Y chromosome or the cDNA spans an intron harboring these sites.

To determine whether mYfin sequences were closely linked on the Y chromosome, we analyzed the mouse sex-reversed mutation (*Sxr*). Male mice carrying the *Sxr* mutation (XYSxr) have a duplication of a segment of the Y comprising several thousand kilobases in length (*1*). This duplicated (*Sxr*) region, which is located on the telomere of the long arm of their variant Y chromosome, contains *Tdy*, the locus for the male-specific minor transplantation antigen (*H-Ya*) and banded krait minor satellite DNA repeat sequences (*Bkm*) (*1, 6*). Spermatozoa bearing a recombined X chromatid that possesses the *Sxr* region give rise to XXSxr males. When mYfin was used to probe Southern blots of genomic DNA from

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