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An Interstitial Deletion in Mouse Y Chromosomal DNA Created a Transcribed *Zfy* Fusion Gene

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The small portion of the mouse Y chromosome retained in the Sxr^a transposition is thought to carry at least five genes including, as demonstrated here, the entirety of the zinc-finger genes *Zfy-1* and *Zfy-2*. Sxr^b, a derivative of Sxr^a, was previously thought to retain *Zfy-1* but to be deleted for *Zfy-2*. Here we show that Sxr^b differs from Sxr^a as the result of unequal crossing-over between *Zfy-1* and *Zfy-2*. This unequal crossing-over created a transcribed *Zfy-2/1* fusion gene and an interstitial deletion. Our data and previous results together suggest that this deletion encompassed the 3′ portion of *Zfy-2*, the histocompatibility gene *Hya*, the spermatogenesis factor *Spy*, and the 5′ portion of *Zfy-1*. We suggest that not only *Zfy* but also other neighboring genes such as *Spy* and *Hya* may exist in two copies on the Y as the result of a large tandem duplication during rodent evolution.

INTRODUCTION

In mammals, the Y is the chromosome least accessible to classical genetic study. Most of the chromosome does not undergo meiotic recombination, and Y-linked genes cannot be readily identified, distinguished one from another, or ordered by meiotic linkage analysis. As a result, characterization of the Y chromosome relies heavily upon deletions, translocations, and the cloning of genes. The most studied region of the mouse Y is that contained in the Sxr^a transposition, which constitutes roughly a 10th of the chromosome (Cattanach *et al.*, 1971; Evans *et al.*, 1982). The Sxr^a region is thought to carry at least five genes: *Sry* (also known as *Tdy*) which determines gonadal sex and encodes a putative DNA-binding protein (Gubbay *et al.*, 1990a; Koopman *et al.*, 1991); the homologs *Zfy-1* and *Zfy-2*, each encoding a protein with an acidic domain and 13 zinc fingers, probably functioning as sequence-specific activators of transcription (Page *et al.*, 1987; Ashworth *et al.*, 1989; Mardon *et al.*, 1989, 1990); *Hya*, which encodes or regulates H-Y, a minor histocompatibility antigen (Simpson *et al.*, 1981); and *Spy*, which enhances the survival and proliferation of spermatogonia (Burgoyne *et al.*, 1986; Sutcliffe and Burgoyne, 1989). *Hya* and *Spy* have not been cloned.

Little is known about the arrangement of these five genes. What information does exist derives mainly from studies of Sxr^b, a deleted derivative of Sxr^a. Sxr^a and Sxr^b were originally known as *Sxr* and *Sxr′*, respectively (McLaren *et al.*, 1988). Sxr^b appeared to have lost *Hya*, *Spy*, and *Zfy-2* (McLaren *et al.*, 1984; Burgoyne *et al.*, 1986; Roberts *et al.*, 1988; Mardon *et al.*, 1989; Nagamine *et al.*, 1989; Sutcliffe and Burgoyne, 1989), suggesting that these three genes were physically separable from both *Sry* and *Zfy-1*, which Sxr^b retains. Thus, *Zfy-2* might be near or even identical to *Hya* or *Spy*. We now describe in greater detail the Sxr^b deletion, the nature of which forces a reassessment of the arrangement and possible identity of genes in this intensively studied region of the Y chromosome.

MATERIALS AND METHODS

Cosmid Walk and Characterization of Zfy-1 and Zfy-2 Gene Structure

A library of *Mbo*I partially digested FVB/N male mouse genomic DNA (XY^{dom}, see below) was constructed in the cosmid vector c2RB (Bates and Swift, 1983). *Zfy-1* and *Zfy-2* cosmid clones were identified by hybridization to *Zfy-2* cDNA pDP1122 (Mardon and Page, 1989). Contigs were assembled and assigned to *Zfy-1* or *Zfy-2* by restriction mapping and hybridization with gene-specific oligonucleotides. Exons were identified and characterized using oligonucleotides (ranging in length from 17 to 24 nucleotides) synthesized on the basis of known cDNA sequences. These oligonucleotides were numbered according to the most 5′ nucleotide's position with

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respect to the translation start in, for *Zfy-1*, cDNA p955 (Ashworth *et al.*, 1989) or, for *Zfy-2*, cDNA pDP1122. Cloned *Zfy-1* exons depicted in Fig. 1 (from 5′ to 3′) are detected by the following oligonucleotides: −155 and −44; 116 and 403; 680; 778; 999; 1028 and 1144; 1210 and 2539. *Zfy-2* exons depicted in Fig. 1 are detected by the following oligonucleotides: −318 and −135; −68 and −44; −8 and 42; 116 and 680; 778; 999; 1028 and 1144; 1210 and 2451.

DNA Probes and Blot Hybridization

Probes A through E derive from subclones of the *Zfy-2* cosmids. In the case of probes A through D, the subclones consist of genomic *Eco*RI fragments ligated into the *Eco*RI site of pBluescript. Probe A is a 1.3-kb *Hin*dIII fragment isolated from the 8.2-kb *Eco*RI insert of plasmid pEMS283. Probe B is the 0.8-kb *Eco*RI insert of pEMS612. Probe C is a 0.8-kb *Hin*dIII/*Taq*I fragment isolated from the 1.5-kb *Eco*RI insert of pEMS616. Probe D is a 0.4-kb *Eco*RI/*Hin*dIII fragment isolated from the 1.9-kb *Eco*RI insert of pEMS602.2. Probe E is the insert of plasmid pDP1350, a 0.34-kb *Dra*I fragment subcloned into the *Sma*I site of pBluescript.

Murine Y chromosomes of *Mus musculus domesticus* (Y^{dom}) and *M. m. musculus* (Y^{mus}) origin differ at numerous restriction sites (Lamar and Palmer, 1984; Bishop *et al.*, 1985; Nishioka and Lamothe, 1986). Since the cosmids used in this study derive from a Y^{dom} chromosome (strain FVB/N), while *Sxr* arose from a Y^{mus} chromosome (Mardon *et al.*, 1989), we have generally chosen probe and restriction enzyme combinations for which there is no Y^{dom}/Y^{mus} polymorphism.

Genomic DNAs were digested with restriction enzyme, separated by electrophoresis in 0.8% agarose, and transferred to nylon membranes. Probes were labeled by random-primed synthesis using [α-^{32}P]-dCTP. Membranes were prehybridized and hybridized in 50% formamide, 5× SSC, 5× Denhardt's solution (10% Ficoll 400, 10% polyvinylpyrrolidone, 10% bovine serum albumin), 1% SDS, 50 mg/ml sheared salmon sperm DNA; prehybridization also included 50 m*M* $NaPO_4$, pH 6.6; hybridization also included 20 m*M* $NaPO_4$, pH 6.6, 8.75% dextran sulfate. Membranes were then washed three times for 15 min in 0.1× SSC, 0.1% SDS, prior to exposure to X-ray film. For probe A, hybridization was at 47°C, washing at 65°C. For probes B through E, hybridization was at 42°C, washing at 56°C. Probe E was preannealed by adding sheared genomic DNA, boiling, and incubating at 65°C for 2 h prior to hybridization.

Oligonucleotides were end-labeled using T4 polynucleotide kinase and [γ-^{32}P]-ATP. Membranes were prehybridized and hybridized in 6× SSC, 5× Denhardt's solution, 0.5% SDS, 0.5% $Na_4P_2O_7$; prehybridization also included 100 mg/ml sheared salmon sperm DNA. Membranes were then washed three times for 20 min at 42°C in 6× SSC, 0.1% SDS, prior to exposure to X-ray film. When necessary, wash temperatures were increased in 5°C steps until gene specific hybridization was achieved.

Reverse Transcription–Polymerase Chain Reaction (RT–PCR)

Testicular poly(A)$^+$ RNAs were reverse-transcribed with oligo(dT) and amplified with *Taq*I polymerase for 35 cycles: 94°C for 1 min, 65°C for 1 min, 72°C for 2 min. PCR primers were as follows: *Zfy-1* 5′, GCATCAAACGGTCGTTACTCAT; *Zfy-1* 3′, TATCTGAAGTGTCAGCTGTTAT; *Zfy-2* 5′, GTTGCTGTTGTGGTTCTCGTA; and *Zfy-2* 3′, TATCTGAAATGTCGGCTGTCAA. PCR products were separated by electrophoresis in 2% agarose (NuSieve 3:1; FMC, Rockland, ME), stained with ethidium bromide, transferred to a nylon membrane, and hybridized with an oligonucleotide (CATCTTCATCCATGGCCTT) common to *Zfy-1* and *Zfy-2*. Gene-specific oligonucleotides were also used to confirmed the gene assignment of the PCR products (data not shown).

RESULTS

The Zfy-1 and Zfy-2 Genes

The zinc-finger domains of the mouse *Zfy* genomic loci have been scrutinized previously (Page *et al.*, 1987; Kalikin *et al.*, 1989; Mardon *et al.*, 1989; Nagamine *et al.*, 1989; Gubbay *et al.*, 1990b). To extend these studies, we cloned, restriction mapped, and positioned exons within larger portions of the *Zfy-1* and *Zfy-2* genes (Fig. 1). The nucleotide sequences of the two genes appeared to be very similar throughout; even most intron and flanking probes cross-hybridized to both loci on genomic DNA blots (data not shown). These findings confirm that the existence of two *Zfy* loci is the result of an intrachromosomal duplication during rodent evolution (Mardon *et al.*, 1989). All available evidence indicates conservation of coding exon boundaries among the mouse *Zfy-1*, *Zfy-2*, and *Zfx* genes and the human homologs *ZFY* and *ZFX* (Page *et al.*, 1987; Schneider-Gädicke *et al.*, 1989a,b; Ashworth *et al.*, 1990; Mardon *et al.*, 1990; S.-W. Luoh and D. C. Page, unpublished results). The highly acidic amino-terminal halves of these proteins are encoded by at least six and probably seven exons, while the zinc fingers are encoded by the most 3′ exon (Fig. 1). Previous studies of these genes in Sxr^a and Sxr^b have focused on the zinc-finger exons of *Zfy-1* and *Zfy-2*. While both are present in Sxr^a, in Sxr^b the

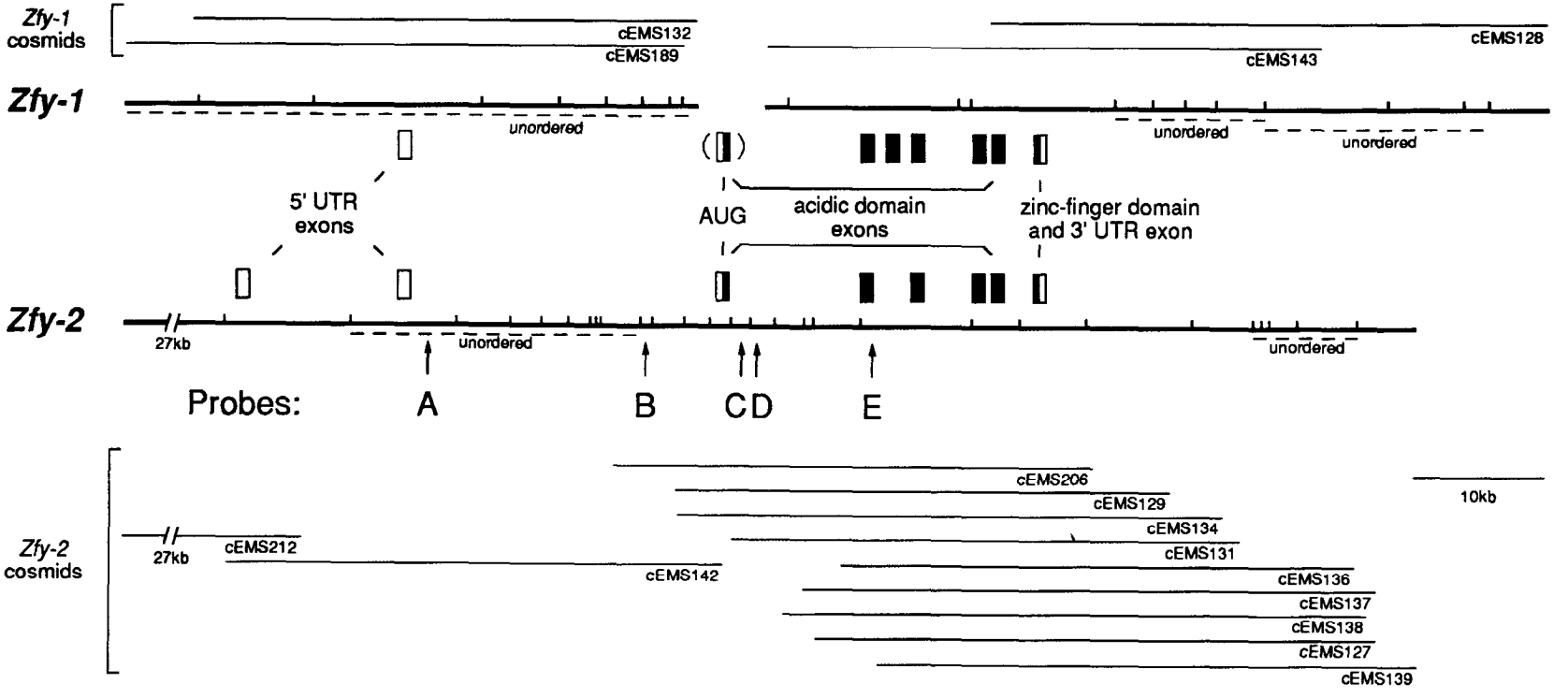

FIG. 1. Cloning of *Zfy-1* and *Zfy-2* genes and location of probes A through E. One hundred kilobases has been cloned at the *Zfy-1* locus and 125 kb at *Zfy-2*. The *Zfy-1* and *Zfy-2* contigs do not overlap. *Eco*RI sites are depicted by short vertical lines; regions in which fragments have not been ordered are indicated. Exons were detected by oligonucleotide hybridization (see Materials and Methods) and are depicted as open (noncoding) or closed (coding) rectangles (not to scale). Additional exons may yet be detected; the numbers of coding exons in *Zfy-1* and *Zfy-2* are not necessarily different. The first coding exon of *Zfy-1* (bracketed) remains uncloned. Hybridization probes A through E derive from *Zfy-2*.

zinc-finger exon of *Zfy-1* is retained and the zinc-finger exon of *Zfy-2* is deleted (Mardon *et al.*, 1989; Nagamine *et al.*, 1989).

Deletion of 5′ Portion of Zfy-1 and 3′ Portion of Zfy-2

We used hybridization probes A through E (Fig. 1) to further characterize the Sxr^a and Sxr^b transpositions. The probes, which derive from *Zfy-2*, cross-hybridized to *Zfy-1* but not to the more distantly related homologs *Zfx* (on the X chromosome) and *Zfa* (on chromosome 10). For each probe, restriction fragment differences distinguishing *Zfy-1* and *Zfy-2* were identified; fragments were assigned to one gene or the other by comparison with cosmids analyzed on the same gels (Fig. 2). When hybridized to DNAs from XX mice carrying Sxr^a, each of the five probes detected the *Zfy-1* and *Zfy-2* bands observed in normal XY males. Identical results were obtained with XX mice carrying yet another related transposition, Sxr^e, a revertant of Sxr^b (McLaren *et al.*, 1988; Sxr^e was originally known as Sxr^{719}). These results map the entirety of both *Zfy* genes to Sxr^a and add to the evidence that Sxr^a and Sxr^e have similar DNA contents (Roberts *et al.*, 1988; Mardon *et al.*, 1989; Nagamine *et al.*, 1989).

In contrast, XX mice carrying Sxr^b exhibited only a subset of the *Zfy-1* and *Zfy-2* fragments. Results obtained with E, the most 3′ of the new probes, were in keeping with previous studies of the zinc-finger exons: the *Zfy-1* fragment was present and the *Zfy-2* fragment was absent in Sxr^b (Fig. 2A). Surprisingly, the opposite results were obtained with probes A and B, located further 5′: *Zfy-1* fragments were absent and *Zfy-2* fragments were present in Sxr^b (Fig. 2A). Thus, Sxr^b retains the 5′ portion of *Zfy-2* as well as the 3′ portion of *Zfy-1*. In all, we have identified at least 100 kb that is deleted in Sxr^b.

Homologous Recombination Created the Deletion and Zfy-2/1 Fusion Gene

These findings could be explained by two noncontiguous deletions, one involving *Zfy-1* and a second involving *Zfy-2*. To the contrary, results with probes C and D (Fig. 2B) indicate that a single deletion in Sxr^b simultaneously affected both genes. When hybridized to *Taq*I-digested DNAs, probe C detects only a *Zfy-2* fragment in Sxr^b, matching previous results with probes A and B. When hybridized to *Eco*RI-digested DNAs, probe D detects only a *Zfy-1* fragment in Sxr^b, matching results with probe E and the zinc-finger probe. When hybridized to DNAs digested with *Bgl*II (or *Eco*RV; not shown), probes C and D detect the same fragment in Sxr^b, a fragment not present in Sxr^a. No other probes are known to reveal novel fragments in Sxr^b (Roberts *et al.*, 1988; Mardon *et al.*, 1989; Nagamine *et al.*, 1989; Fig. 2A and our unpublished results). We conclude that a single deletion has fused the 5′ portion of *Zfy-2* to the 3′ portion of *Zfy-1*, creating a junction detected by probes C and D. Thus, in Sxr^a (and in the normal Y chromosome), *Zfy-2* must be 5′ of *Zfy-1*, with the two genes having the same 5′-to-3′ orientation.

All available evidence is consistent with the Sxr^b deletion having resulted from homologous recombination between the *Zfy* genes. One deletion endpoint falls within a 1.5-kb region in *Zfy-2* that includes probes C and D, while the other falls within the homologous region in *Zfy-1* (Figs. 1 and 2B and data not shown). As these endpoints fall between the first two coding exons of the *Zfy* genes, the resulting locus comprises the 5′ portion of *Zfy-2* (including the first coding exon) and the remaining coding exons of *Zfy-1* (Fig. 3A). We refer to this fusion locus as *Zfy-2/1*.

The Zfy-2/1 Fusion Locus Is Transcribed

We then used RT–PCR analysis to explore the possibility that this locus might be expressed and, if expressed, to confirm the structure of the fusion gene. *Zfy-1* and *Zfy-2* are abundantly transcribed in the adult testes of normal XY males (Koopman *et al.*, 1989; Mardon and Page, 1989; Nagamine *et al.*, 1990). However, the testes of XXSxr^a and XXSxr^b males develop abnormally (Cattanach *et al.*, 1971; McLaren *et al.*, 1984) due to the presence of two X chromosomes (Russell and Chu, 1961) and the absence of much of the Y chromosome (Burgoyne *et al.*, 1986). We therefore decided to look for *Zfy-2/1* transcription in the testis of XYSxr^a and XYSxr^b mice. RT–PCR assays were carried out using primers from exons on both sides of the junction (Fig. 3A). Using either *Zfy-1* primers or *Zfy-2* primers, transcripts were detected in XY, XYSxr^a, and XYSxr^b, as expected in the presence of an intact Y chromosome (Fig. 3B, rows 1 to 4). In the critical experiment, the 5′ primer from *Zfy-2* was used in conjunction with the 3′ primer from *Zfy-1*. This primer pair would detect transcripts from *Zfy-2/1*, present only in mice carrying Sxr^b. As predicted, a product of appropriate size was detected in XYSxr^b but not in XY or XYSxr^a (Fig. 3B, rows 5 and 6). We conclude that *Zfy-2/1* is transcribed. The RT–PCR results also confirmed that the structure of the fusion gene is as predicted.

DISCUSSION

Zfy-2/1 Fusion Gene

The *Zfy-2/1* fusion derived from two genes which, though closely related, encode slightly different proteins and have overlapping but distinct patterns of expression (Ashworth *et al.*, 1989; Koopman *et al.*,

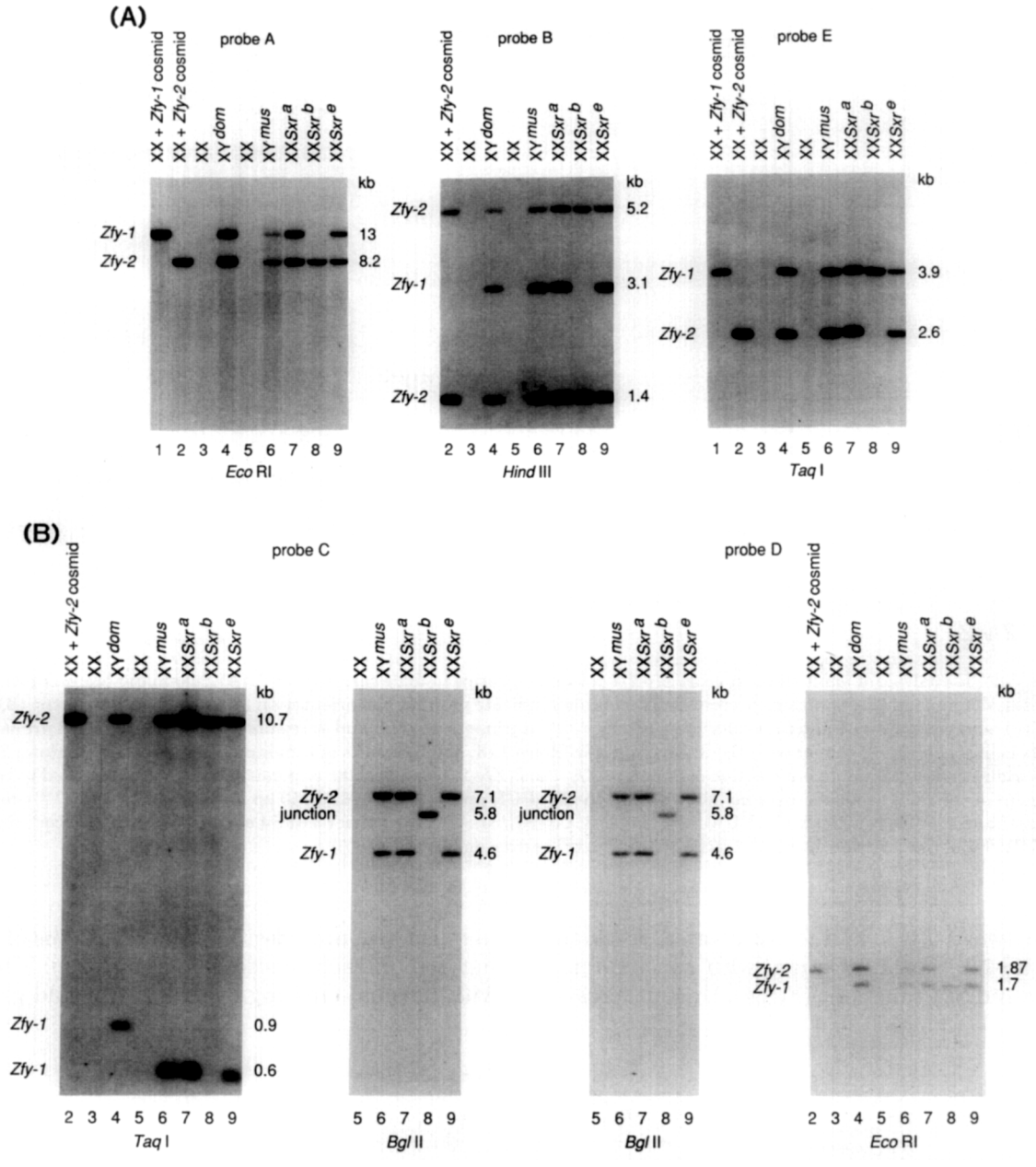

FIG. 2. Characterization of Sxr^a and Sxr^b by hybridization of probes A through E (Fig. 1) to blots of genomic DNAs. (**A**) Deletion of the 5′ end of *Zfy-1* and of the 3′ end of *Zfy-2* in Sxr^b. The *Zfy* gene from which the hybridizing restriction fragments derive are indicated, as are their sizes (in kb). (**B**) A single junction fragment in Sxr^b. The *Zfy-1* fragment detected by probe C is of different size in Y^{dom} and Y^{mus} (see Materials and Methods). For all panels (**A**) and (**B**): lane **1**, FVB/N female with *Zfy-1* cosmid added; **2**, FVB/N female with *Zfy-2* cosmid added; **3**, FVB/N female; **4**, FVB/N male; 5, C57BL/6J-$A^{w\text{-}J}$ female; **6**, C57BL/6J-$A^{w\text{-}J}$ male; 7, C57BL/6J-$A^{w\text{-}J}$ Sxr^a sex-reversed male; **8**, C57BL/6J-$A^{w\text{-}J}$ Sxr^b sex-reversed male; **9**, C57BL/6Mcl Sxr^e sex-reversed male 719.

1989; Mardon and Page, 1989; Nagamine *et al.*, 1990). The predicted fusion protein would most closely resemble Zfy-1, differing at only a few amino acid residues specified by the first coding exon. The promoter of the fusion gene would derive from *Zfy-2*. There is no reported phenotypic difference between XYSxr^a and XYSxr^b animals (McLaren *et al.*, 1984; Sutcliffe and Burgoyne, 1989), suggesting that the *Zfy-2/1* fusion gene does not interfere with the function of the intact *Zfy-1* or *Zfy-2* loci. Though the fusion gene is clearly transcribed, the existence of the predicted fusion protein has yet to be demonstrated.

Ancestral and Contemporary Murine Y Chromosomes

We propose that during rodent evolution, a Y chromosomal segment containing a single *Zfy* gene was duplicated, and that Sxr^b arose when unequal cross-

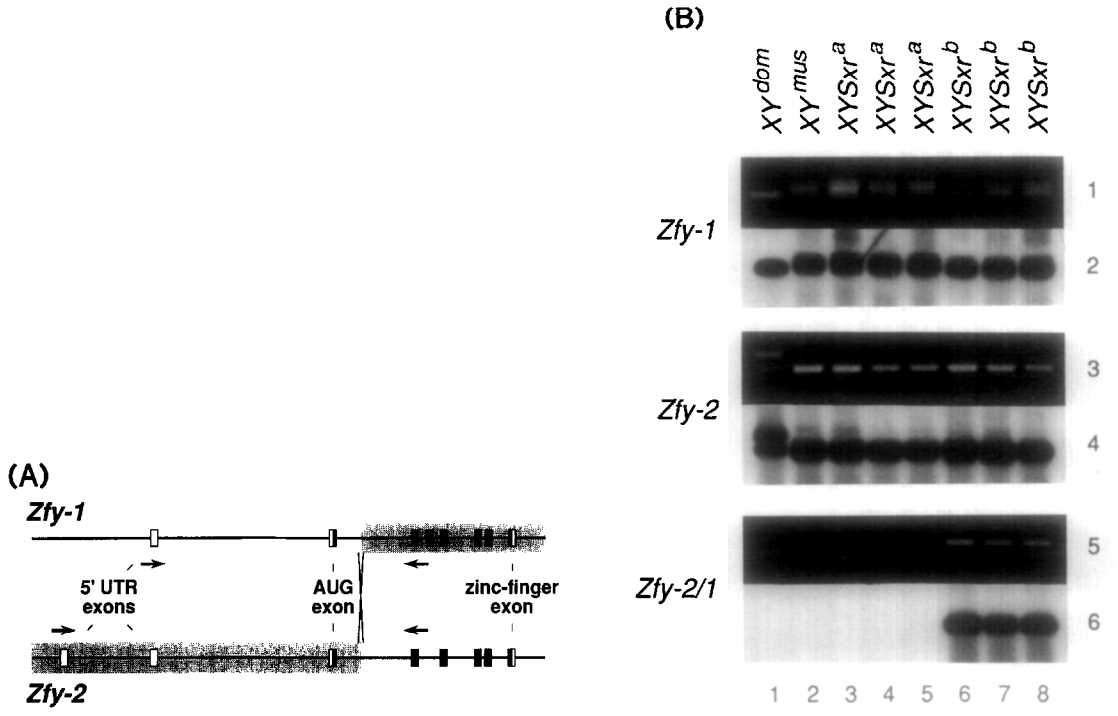

FIG. 3. RT–PCR analysis of adult testes reveals *Zfy-2/1* fusion transcript in mice carrying Sxr^b. (**A**) Schematic representation of *Zfy-1* and *Zfy-2* genes; shading indicates portions present in Sxr^b. Arrows indicate position and orientation (5′ to 3′) of PCR primers. (**B**) RT–PCR analysis of *Zfy* transcripts. Rows **1** and **2**: results using two *Zfy-1* PCR primers. Rows **3** and **4**: results using two *Zfy-2* PCR primers. Rows **5** and **6**: results using the 5′ *Zfy-2* primer with the 3′ *Zfy-1* primer. Rows **1, 3,** and **5**: results of ethidium bromide staining. Rows **2, 4,** and **6**: results of hybridizations with an oligonucleotide common to *Zfy-1* and *Zfy-2*. Gene-specific oligonucleotides were also used to confirm the gene assignment of the PCR products (data not shown). All *Zfy* RT–PCR products are 600 to 750 bp in length; Y^{dom} and Y^{mus} products vary slightly in size. Lane **1**, FVB/N male; **2**, C57BL/6J-$A^{w\text{-}J}$ male; **3**, C57BL/6J-$A^{w\text{-}J}$ Sxr^a carrier male; **4** and **5**, C57BL/6Mcl Sxr^a carrier males; **6** and **8**, C57BL/6Mcl Sxr^b carrier males; **7**, C57BL/6J-$A^{w\text{-}J}$ Sxr^b carrier male.

ing-over between *Zfy-1* and *Zfy-2* created a fusion gene and an interstitial deletion, with concomitant loss of *Hya* and *Spy* functions (Fig. 4). Unequal crossing-over also provides the simplest means of explaining the intrachromosomal duplication. Consistent with unequal crossing-over, which would generate a

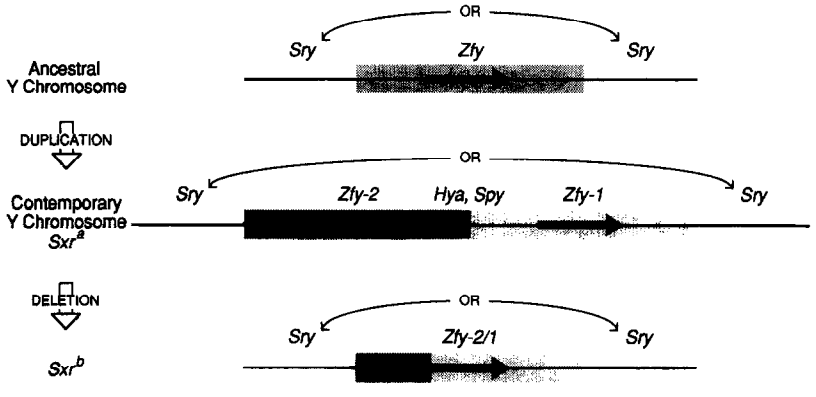

FIG. 4. Schematic model depicting evolution and mutation of a block of mouse Y chromosomal DNA. The single *Zfy* gene on the ancestral Y chromosome was duplicated during rodent evolution, creating *Zfy-1* and *Zfy-2*, both found within Sxr^a. An interstitial deletion created *Zfy-2/1*, the fusion gene in Sxr^b. Black arrows indicate the 5′ to 3′ orientation of the *Zfy* genes. *Hya* and *Spy* may be located anywhere within the deleted region. *Sry* may be located either 5′ or 3′ of the duplicated region. The sizes of the ancestral duplication and the Sxr^b deletion may be similar, but only in the absence of intervening rearrangements. As described in the text, mutations during rodent evolution may have resulted in the presence of some nonduplicated sequences between or near the *Zfy* genes on the contemporary mouse Y chromosome. Sxr^a may or may not contain the entirety of the duplication.

tandem duplication, *Zfy-1* and *Zfy-2* have the same 5′-to-3′ orientation. The animal in which the Sxr^b deletion occurred may have had as many as three copies of the Sxr^a region (McLaren *et al.*, 1984). Thus, it is not known whether multiple copies of Sxr^a actually participated in the recombination event, nor is it known whether the recombination involved sister chromatids. Since the duplication and subsequent deletion had different endpoints, we can be certain that the Sxr^b deletion was not a precise reversion of the tandem duplication. Our data suggest that both the duplication and the subsequent deletion involved at least 100 kb. When considered together with other recent DNA hybridization studies of Sxr^a and Sxr^b (M. J. Mitchell and C. E. Bishop, personal communication), the combined data suggest that both events were more extensive; the deletion measures at least 290 kb, and could be much larger.

If the duplication was tandem, then one would suppose that it involved all material, including any genes, located between the newly duplicated *Zfy* genes. On the contemporary mouse Y chromosome, *Hya* and *Spy* appear to be located between the *Zfy* genes (Fig. 4). Thus, there may be two copies of *Hya* and *Spy* on the contemporary Y chromosome, the second copies being located 5′ or 3′ of the Sxr^b deletion, but nonetheless within the tandem duplication. Since the Sxr^a transposition does not necessarily contain the entirety of the duplication, the predicted second copies of *Hya* and *Spy* need not map within Sxr^a. These second copies could be pseudogenes, but recent independent studies suggest the possible existence of multiple expressed *Hya* genes (Scott *et al.*, 1991). That *Sry* is not duplicated in the contemporary Y chromosome (Gubbay *et al.*, 1990a) suggests that *Sry* maps outside the tandem duplication, either 5′ or 3′ (Fig. 4). These conjectures as to the location of *Sry* and the possible duplication of *Hya* and *Spy* must be tempered by the realization that the intrachromosomal duplication occurred prior to the divergence of *M. musculus* and *M. spretus*, at least two million years ago (Mardon *et al.*, 1989). Subsequent insertions, deletions, or other mutations may have resulted in the presence of some unduplicated sequences near or between the *Zfy* genes on the contemporary mouse Y chromosome. Indeed, apparently nonduplicated sequences have been identified within and between the *Zfy* genes (our unpublished results; M. J. Mitchell and C. E. Bishop, personal communication).

In mammals, creation of expressed fusion genes via homologous recombination between tandemly duplicated segments has been observed previously with hemoglobins (Thompson and Thompson, 1986), visual pigments (Neitz *et al.*, 1989), T-cell receptor (Kotzin *et al.*, 1985), and glucocerebrosidase (Zimran *et al.*, 1990). In these other cases, the unit of tandem duplication (and hence of deletion) ranged in size from 7 to 39 kb and was thought to contain a single gene, with the phenotypic consequences of deletion stemming entirely from alterations in the gene family undergoing fusion. In the present case the tandem duplication and deletion have apparently been much larger. Also, the consequences of this deletion reflect, at least in part, the loss of other genes (*Hya* and possibly, as discussed below, *Spy*) interposed between those undergoing fusion.

Potential Gene Identities

Zfy-1 and *Zfy-2* likely activate transcription of specific target genes (Page *et al.*, 1987; Ashworth *et al.*, 1989; Mardon *et al.*, 1989, 1990), as yet unidentified, but little else is known about their functions. Questions arise as to whether *Zfy-1* or *Zfy-2* might be synonymous with either *Hya* or *Spy*, neither of which has been cloned. *Hya* encodes or regulates a minor histocompatibility antigen (Simpson *et al.*, 1981), and *Spy* functions in spermatogenesis (Burgoyne *et al.*, 1986; Sutcliffe and Burgoyne, 1989). Previous DNA studies of the Sxr^b deletion (Mardon *et al.*, 1989; Nagamine *et al.*, 1989) had implied that *Hya* and *Spy* were genetically separable from *Zfy-1* but possibly synonymous with *Zfy-2*. Since transcription of the *Zfy* genes appears to be testis-specific in the adult mouse (Koopman *et al.*, 1989; Mardon and Page, 1989; Nagamine *et al.*, 1990), both *Zfy* genes are likely distinct from the more widely expressed *Hya* (Johnson *et al.*, 1981). The Sxr^b results reported here raise the possibility, yet to be tested, that *Spy* is synonymous with *Zfy-1*, *Zfy-2*, or both, in which case the *Zfy-2/1* fusion fails to replace the *Spy* function of its progenitor genes. We now have molecular probes which flank the Sxr^b deletion and a map to guide analysis of the multiple biological activities of the Sxr^a region of the murine Y chromosome.

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