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Rps4 Maps Near the Inactivation Center on the Mouse X Chromosome

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***RPS4Y*, a Y-linked gene in humans, appears to encode an isoform of ribosomal protein S4. A homologous locus on the human X chromosome, *RPS4X*, lies close to the X-inactivation center but fails to undergo X-inactivation. We have isolated a genomic clone from the mouse *Rps4* locus, the homolog of human *RPS4X*. We derived an intron probe that hybridizes to the functional *Rps4* locus but does not cross-hybridize to related sequences elsewhere in the mouse genome. Genetic mapping utilizing interspecific mouse backcrosses and the intron-specific probe demonstrates that *Rps4* maps close to the *Phka* locus on the mouse X chromosome and in the vicinity of the X-inactivation center. The gene order *Ccg-1–Rps4/Phka–Xist–Pgk-1* is conserved between mouse and human.** © 1992 Academic Press, Inc.

INTRODUCTION

The *RPS4Y* gene is located on the distal short arm of the human Y chromosome and encodes an isoform of ribosomal protein S4, which is a component of the small subunit of the mammalian ribosome (Fisher *et al.*, 1990). A closely related gene, *RPS4X*, is found on the proximal long arm of the human X chromosome (Fisher *et al.*, 1990). It has been postulated that Turner syndrome is the result of monosomy for a gene or genes common to the X and Y chromosomes (Ferguson-Smith, 1965), but nothing is known about the nature of these "Turner" genes or the proteins they might encode. Haploinsufficiency of the *RPS4* genes may play a role in the Turner syndrome phenotype (Fisher *et al.*, 1990). *RPS4X* is one of the few genes on the human X chromosome that are known to escape inactivation. Interestingly, *RPS4X* maps to Xq13, the same band as the putative human X-inactivation center (Fisher *et al.*, 1990).

A mouse cDNA (pDP1340) homologous to human *RPS4X* has been cloned and its nucleotide sequence determined (Zinn *et al.*, 1991). The nucleotide sequence of this mouse cDNA predicts a protein whose amino acid sequence is identical to that encoded by human *RPS4X*. As we demonstrate here, this mouse cDNA derives from a gene located on the mouse X chromosome. In the absence of any demonstrable homolog on the mouse Y chromosome (Ashworth *et al.*, 1991; A.Z., unpublished data) we refer to the X-linked mouse gene as *Rps4*. Here we report the localization of *Rps4*, the mouse homolog of *RPS4X*, and show that it too maps close to the mouse X-inactivation centre.

MATERIALS AND METHODS

PCR amplification and characterization of Rps4 intron sequence. Conditions for PCR amplication were essentially as previously described (Fisher *et al.*, 1990), except that the annealing temperature was 55°C (with genomic DNA template) or 50°C (with phage DNA template). Probe labeling, hybridization, and washing conditions for the 428-bp *Rps4* intron PCR product and the pDP1340 cDNA insert were as previously described (Fisher *et al.*, 1990). Sequencing was carried out according to the chain termination method using T7 DNA polymerase (see Fisher *et al.*, 1990).

Genetic mapping resources. Cl.8 is a human–mouse somatic cell hybrid containing only the mouse X chromosome (Amar *et al.*, 1985). Progeny DNAs from two interspecific backcrosses between lab mice and *Mus spretus* were also utilized (see also legend to Fig. 4) for genetic mapping. For one backcross, the lab mouse parent was C57BL/10/*mdx*/*mdx* (Cavanna *et al.*, 1988), and F1 females were backcrossed to C57BL/10/*mdx*/*mdx* mice. For the other backcross, the lab mouse parent was an outbred mouse carrying the *Hq* and *Ta* loci, and F1 females were backcrossed to inbred 129 mice (Brockdorff *et al.*, 1987).

Hybridization and washing conditions. DNAs from the somatic cell hybrid Cl.8, human DNA, C57BL/10, *M. spretus*, and a selection of mice from the interspecific backcrosses were all digested with restriction endonucleases according to manufacturer's instructions. The *Xist* probe is a 2.7-kb *Eco*RI insert (Brockdorff *et al.*, 1991a) and was kindly provided by Dr. S. Rastan. Details of all other probes listed on the genetic map (see Fig. 3) are to be found in Brockdorff *et al.* (1991c). Hybridizations for the 368-bp *Rps4* intron PCR product and *Xist* probe (both ^{32}P labeled) were carried out for 16 h at 65°C in 1% SDS, 6× SSC, 10% dextran sulfate, 10 m*M* Tris, pH 8.0, 1 m*M* EDTA, pH 8.0, 1× Denhardt's solution, and 10 μg/ml denatured sheared salmon sperm DNA. Labeled probe was used at 1×10^6 cpm/ml of hybridization mix. When the short *Rps4* intron probe was used, filters were washed in 3× SSC, 0.1% SDS at 65°C for 1 h before being exposed to autoradiography film for 14 days with an intensifying screen.

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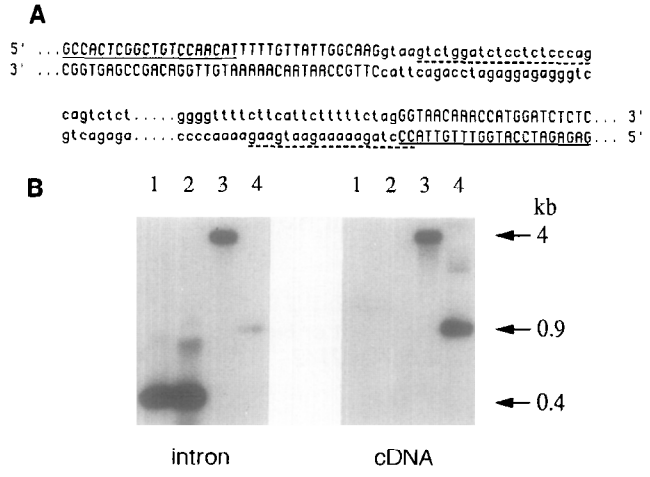

FIG. 1. Identification of the mouse *Rps4* gene. (**A**) Sequence of mouse *Rps4* gene (λFBV41) near 3′ end of the coding region. Uppercase, exon; lowercase, intron. Sequences of oligonucleotide primer pairs used in PCR amplification are underlined (solid and dashed lines). Sequence shown corresponds to nucleotides 655 to 712 in the *Rps4* open reading frame (Zinn *et al.*, 1991) interrupted by a 370-bp intervening sequence between nucleotides 690 and 691. The nucleotide sequence of the region encompassing the intron is available in the GenBank database under Accession No. M77296. (**B**) Southern blots using the 428-bp genomic PCR product (intron, **left**) or pDP1340 cDNA insert (**right**) as hybridization probes. Lanes: **1**, product of PCR with exon primers and genomic DNA template; **2**, product of PCR with same primers and phage λFVB41 DNA template; **3**, *Sal*I digest of λFVB41 DNA; **4**, pDP1340 cDNA insert.

RESULTS

Cloning the Mouse Rps4 Genomic Locus

In mammals, it is generally the case that each ribosomal protein is encoded by a single functional gene from which a large number of processed pseudogenes have derived (Monk *et al.*, 1981). Indeed, cloning of the human genomic locus had been complicated by the existence of multiple cross-hybridizing sequences, presumably processed pseudogenes. These difficulties had been circumvented by PCR amplification of an intron specific to the functional gene (Fisher *et al.*, 1990). We employed the same strategy to isolate the functional locus in mouse. Oligonucleotide primers flanking a potential splice site conserved in human *RPS4X* and *RPS4Y* (Fisher *et al.*, 1990) were used to PCR-amplify a 428-bp intron-containing fragment from mouse genomic DNA (Fig. 1A, solid lines).

This PCR product was used to screen a mouse genomic phage library, and one positive clone, λFVB41, was isolated. Three lines of evidence established that λFVB41 derives from the functional intron-bearing *Rps4* genomic locus rather than from a processed pseudogene. First, the phage and genomic DNAs yielded products of the same length when used as templates for PCR amplification of the intron-containing fragment (Fig. 1B, lanes 1 and 2). Second, λFVB41 contains a 4.0-kb *Sal*I fragment that hybridized strongly to both the intron-containing product and the cDNA insert, which lacks intron sequences (Fig. 1B, lane 3). Third, partial DNA sequencing of the *Sal*I fragment confirmed that λFVB41 contains the pDP1340 mouse *Rps4* cDNA coding sequences interrupted by multiple introns, including a 370-bp intron at the predicted site (Fig. 1A).

Chromosomal Location of the Mouse Rps4 Gene

We then determined the chromosomal location of the mouse *Rps4* gene. Because the 428-bp PCR product contains not only an intron but also 58 bp from the flanking exons, it hybridizes weakly to the cDNA insert (Fig. 1B, lane 4). To prepare an intron-specific probe that does not detect coding sequences including pseudogene sequences, a second set of primers (Fig. 1A, dashed lines) was used to amplify a 368-bp sequence that contains most of the intron but only 2 bp of exon sequence. Hybridization of the intron probe to *Taq*I digests of mouse DNA (Fig. 2) gave a single band of 4.0 kb; no hybridization to *Taq*I-digested human DNA was observed. However, a 4.0-kb band was identified in the human–mouse hybrid Cl.8 (Amar *et al.*, 1985) that contains only the mouse X chromosome, demonstrating that the mouse *Rps4* gene resides on the X chromosome. Finally, the intron-specific probe shows the expected dosage of signal when hybridized to digests of male and female DNA (data not shown).

Genetic Mapping of the Mouse Rps4 Gene

To compare the map position of *Rps4* on the mouse X chromosome to its homolog on the human X, the intron probe was analyzed through progeny derived from two interspecific backcrosses between lab mice and *M. spretus* that have been extensively analyzed for a large number of X-chromosome markers (Brockdorff *et al.*, 1987, 1991c; Cavanna *et al.*, 1988; Keer *et al.*, 1990; and see legend to Fig. 3). Hybridization of the intron probe to *Dra*I digests of mouse DNAs (see Fig. 3) detected a band of 3.8 kb in lab mice and a band of 2.8 kb in *M. spretus*. Backcross progeny used to follow the segregation of the

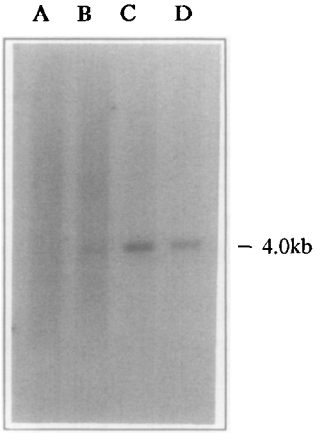

FIG. 2. The mouse homolog to the *RPS4X* gene maps to the X chromosome. A 368-bp intron probe from mouse *Rps4* (and generated by PCR—see Fig. 1) was hybridized to *Taq*I digests of (**A**) human DNA; (**B**) Cl.8 DNA—a human/mouse hybrid containing only the mouse X chromosome; (**C**) C57BL/10 DNA; and (**D**); *M. spretus* DNA.

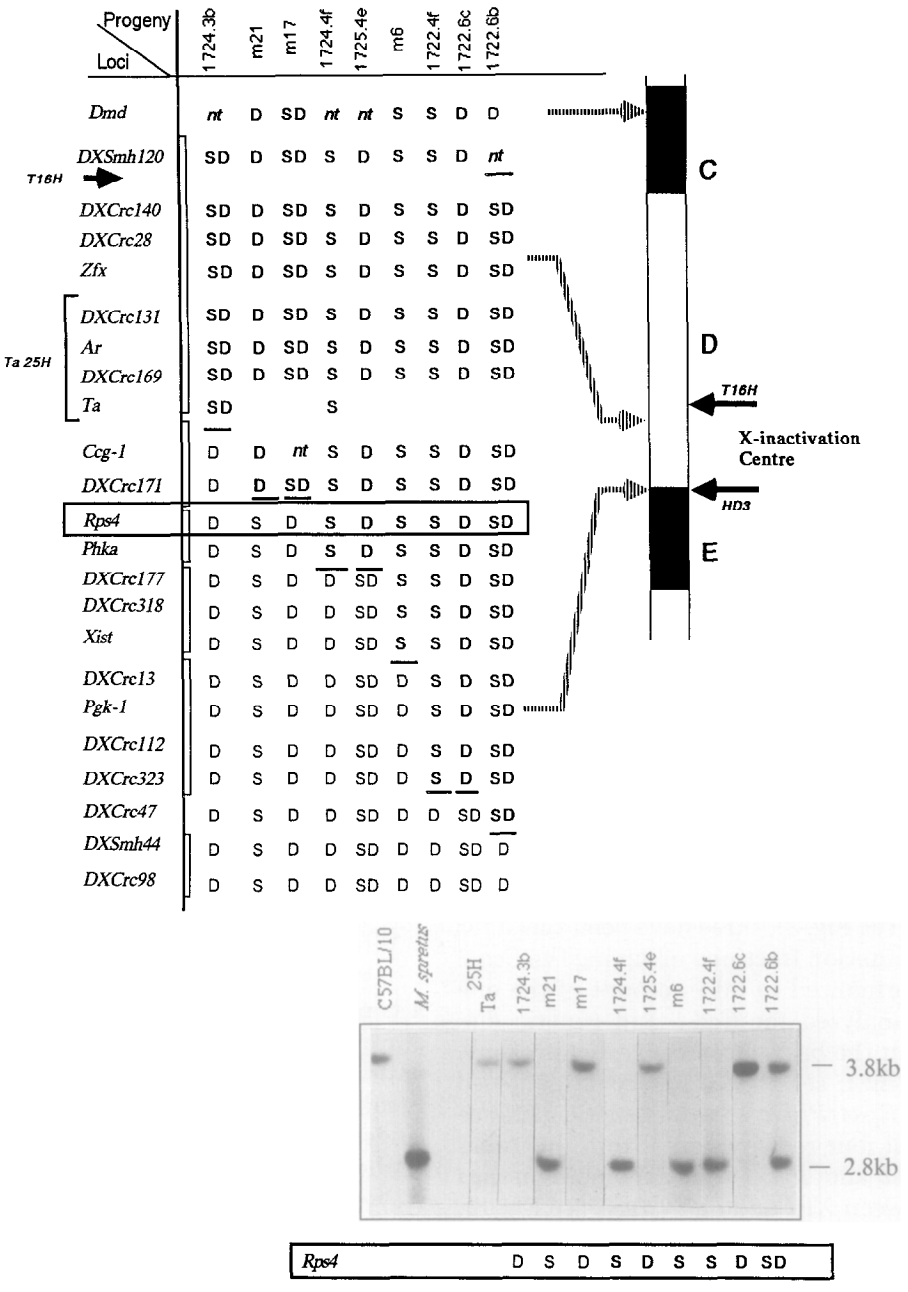

FIG. 3. Pedigree analysis and mapping of the mouse *Rps4* locus. Mice derived from two lab mice/*M. spretus* interspecific backcrosses and carrying recombination breakpoints in the vicinity of the *Ccg-1* and *Pgk-1* loci have been analyzed with a variety of probes from the central span of the mouse X chromosome. In one backcross (mice, m-) the lab mouse parent was C57BL/10/*mdx*/*mdx*. For the other backcross (mice, 17-) the lab mouse parent was an outbred mouse carrying the *Hq* and *Ta* loci, and F1 females were backcrossed to 129 inbred mice (only mice 1724.3b and 1724.4f segregate for the *Ta* locus). Individual backcross progeny mice were scored for the segregation of *M. spretus* (S) and lab mouse (D) RFLVs (nt, not tested) and locus order was determined by minimizing the number of crossovers. The lower panel depicts hybridization of the 368-bp intron probe from mouse *Rps4* to *Dra*I digests of C57BL/10, *M. spretus*, male Ta^{25H}, and a variety of backcross progeny mice. The 368-bp intron probe utilized for mapping *Rps4* was generated by PCR (see Materials and Methods). Details of all other probes on the genetic map are to be found in Brockdorff *et al.* (1991c). Brackets indicate loci that are not separable by recombination events; for example, the loci *Rps4* and *Phka* cosegregate. The mouse X-inactivation center lies distal to *T16H* and proximal to the *HD3* breakpoint (Rastan and Brown, 1990).

lab mice and *M. spretus* RFLVs (restriction fragment length variants) demonstrated recombination breakpoints in the central span of the mouse X chromosome and in the region of the *Ar* and *Pgk-1* loci. Figure 3 demonstrates the segregation of the *Rps4* RFLV in nine backcross progeny with key recombination breakpoints in the vicinity of these loci. Haplotype analysis indicates that *Rps4* cosegregates with *Phka* and lies between the *Ccg-1* and the *Pgk-1* loci. For example, mice m21 and m17 demonstrate that *Rps4* lies distal to both *Ccg-1* and *DXCrc171*. Furthermore, mice 1724.4f, 1725.4e, and m6 demonstrate that *Rps4* maps proximal to the *Pgk-1* locus. A number of other mice, such as 1724.3b and 1722.4f, with more distant proximal and distal breakpoints confirm the location of *Rps4* to the *Ccg-1* to *Pgk-1* region of the mouse X chromosome. In addition, we hybridized the intron probe to a *Dra*I digest of DNA from male mice carrying the large Ta^{25H} deletion which lies

proximal to *Ccg-1* and encompasses the *DXCrc131, Ar, DXCrc169,* and *Ta* loci (Brockdorff *et al.,* 1991b). A 3.8-kb *Dra*I band is present in Ta^{25H} mice, demonstrating, as would be expected from its genetic position distal to *Ccg-1,* that *Rps4* lies outside the Ta^{25H} deletion. Furthermore, this panel of recombinant mice has also been analyzed for the mouse homolog to the *Xist* locus (Borsani *et al.,* 1991; Brockdorff *et al.,* 1991a; and see Fig. 3). The *Xist* probe detects a 2.9-kb *Taq*I band in lab mice and 5.1- and 3.5-kb *Taq*I bands in *M. spretus* (Brockdorff *et al.,* 1991a). The *Xist* probe was hybridized to *Taq*I digests of backcross progeny DNAs (data not shown), and mice were scored according to the lab mice/*M. spretus* RFLV. Haplotype analysis places *Rps4* proximal to *Xist* (mice 1724.4f and 1725.4e, Fig. 3). Thus, gene order determined across this region is *Ccg-1–Rps4/Phka–Xist–Pgk-1.*

We have estimated the genetic distances for the intervals surrounding the *Rps4* and *Xist* loci. As indicated above, recombinants in the *Ccg-1* to *Pgk-1* interval were identified from two crosses. In the case of mice derived from a backcross of C57BL/10/*mdx/mdx* mice to *M. spretus,* recombinants in the *Ccg-1* to *Pgk-1* region were identified from the genetic analysis of 64 backcross progeny in total (Cavanna *et al.,* 1988). Recombinants from this cross were used to separate and order, first, the *Ccg-1* and *Rps4/Phka* loci and, second, the *Xist* and *Pgk-1* loci (see Fig. 3). Of four recombinants identified (m13, m21, m17, and m6; see Fig. 3), three have been typed for *Ccg-1;* thus, recombination fractions involving the *Ccg-1* locus have been normalized to take account of the one recombinant not analyzed for *Ccg-1* and genetic distances calculated on the basis of 48 backcross progeny. One recombinant (m21; see Fig. 3) has been identified between *Ccg-1* and *Rps4/Phka,* giving a genetic distance of 2.1 ± 2.1 cM. All four recombinants from this cross were typed for *Xist* and *Pgk-1;* one recombinant has been identified between *Xist* and *Pgk-1* (m6; see Fig. 3), giving a genetic distance of 1.6 ± 1.6 cM. In the case of mice derived from an interspecific backcross segregating the *Hq* and *Ta* mutations (see Materials and Methods and legend to Fig. 3), recombinants in the *Ccg-1* to *Pgk-1* interval were identified from the detailed genetic analysis of 82 mice in total. Two recombinants were identified in the *Rps4/Phka* to *Xist* interval (1724.4f and 1725.4e; see Fig. 3), giving a genetic distance of 2.4 ± 1.7 cM. In summary, gene order and distance are *Ccg-1*–(2.1 ± 2.1 cM)–*Rps4/Phka*–(2.4 ± 1.7 cM)–*Xist*–(1.6 ± 1.6 cM)–*Pgk-1.*

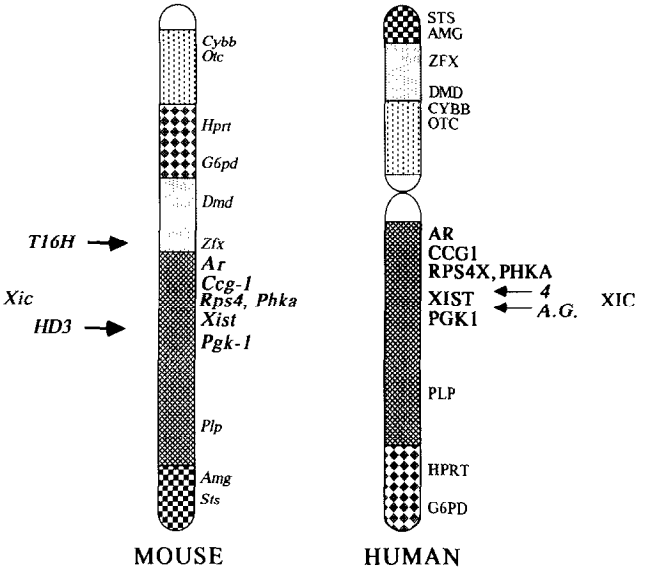

FIG. 4. Comparative maps of the mouse and human X chromosomes. The relationship between the various linkage groups conserved between the mouse and the human X chromosomes is indicated by different shading patterns along with the relative positions of the human *RPS4X* and mouse *Rps4* loci. The approximate positions of the human (*XIC*) and mouse (*Xic*) X-inactivation centers are also included. Two hybrids, 4 and A.G. (Brown *et al.,* 1991b), delineate the X-inactivation center and *XIST* on the human X chromosome; *RPS4X* maps proximal to hybrid 4 (Fisher *et al.,* 1990). In the mouse, the X-inactivation center lies distal to *T16H* and proximal to the *HD3* breakpoint (Rastan and Brown, 1990). *Xist* maps into this region of the mouse X chromosome (Borsani *et al.,* 1991; Brockdorff *et al.,* 1991a).

DISCUSSION

The human *RPS4X* gene and its mouse homolog, *Rps4,* map to the large conserved *AR* to *PLP* linkage group on the mouse and human X chromosomes (see Fig. 4). The proximal end of this linkage group contains the X-inactivation centre that maps close to the *Pgk-1* locus in mouse (Keer *et al.,* 1990; Rastan and Brown, 1990) as in human (Brown and Willard, 1989). Recently, a transcript *XIST,* transcribed only on the inactive X chromosome, has been shown to map into the X-inactivation centre region in human Xq13, as defined by breakpoints characterized in two key somatic cell hybrids carrying inactivated chromosomes—4 and A.G. (Brown *et al.,* 1991a,b; and see Fig. 4). Analysis of *RPS4X* in these hybrids carrying breakpoints in the vicinity of the X-inactivation centre indicates that *RPS4X* maps proximal but close to *XIST* (Fisher *et al.,* 1990; and see Fig. 4). Analysis of a variety of somatic cell hybrids carrying breakpoints along the proximal long arm of the human X chromosome indicates a gene order of *AR–CCG1–RPS4X/PHKA–XIST–PGK1* (Brown *et al.,* 1989a,b; Mandel *et al.,* 1989). In mouse, a locus homologous to human *XIST*—*Xist*—has been shown to cosegregate with *Phka* (Borsani *et al.,* 1991; Brockdorff *et al.,* 1991a) and to map into the region of the mouse X-inactivation centre. The genetic data presented here on the mouse X chromosome for the mapping of *Rps4* and *Xist* demonstrate that gene order has been conserved across the X-inactivation centre region of mouse and human chromosomes. It is clear that the *RPS4X* gene maps close to the X-inactivation centre in both mouse and human. In human, despite its proximity to the X-inactivation centre, *RPS4X* escapes X-inactivation (Fisher *et al.,* 1990). However, in the mouse, it has recently been found that the *Rps4* locus is inactivated (Ashworth *et al.,* 1991; Zinn *et al.,* 1991). The conserva-

tion of gene order in the vicinity of the mouse and human homologs suggests that the species difference with regard to inactivation of *RPS4X/Rps4* cannot be readily explained by a change in physical relationship to the X-inactivation center.

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