Rps4 Maps Near the Inactivation Center on the Mouse X Chromosome


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INTRODUCTION

The RPS4Y gene is located on the distal short arm of the human Y chromosome and encodes an isoform of ribosomal protein S4. A closely related gene, RPS4X, is found on the proximal long arm of the human X chromosome (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. Partially sufficient 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 In the absence of any demonstrable homolog on the mouse Y chromosome (Ashworth et al., 1991; A.Z., unpublished data) we refer to the X-linked mouse gene as Rps4.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 amplification 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 DNA 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. C1.8 is a human–mouse somatic cell hybrid containing only the mouse X chromosome (Amar et al., 1985). Genes 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., 1991) 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. (1991a). Hybridizations for the 368-bp Rps4 intron PCR product and Xist probe (both 32P labeled) were carried out for 16 h at 65°C in 1% SDS, 6X SSC, 10% dextran sulfate, 10 mM Tris, pH 8.0, 1 mM EDTA, pH 8.0, 1× Denhardt’s solution, and 10 μg/ml denatured sheared salmon sperm DNA. Labeled probe was used at 1 × 106 cpm/ml of hybridization mix. When the short Rps4 intron probe was used, filters were washed in 3X 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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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 SalI 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 SalI 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 TaqI digests of mouse DNA (Fig. 2) gave a single band of 4.0 kb; no hybridization to TaqI-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 DraI 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. 1. Identification of the mouse Rps4 gene. (A) Sequence of mouse Rps4 gene (λFVB41) near 3' end of the coding region. Upper-case, exon; lower-case, 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 XFVB41 DNA template; 3, SalI digest of XFVB41 DNA; 4, pDP1340 cDNA insert.

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 TaqI digests of (A) human DNA; (B) C57BL/10 DNA; (C) a human/mouse hybrid containing only the mouse X chromosome; (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 To 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 DraI digests of C57BL/10, M. spretus, male T16H, 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. (1991). 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 DXCrcl171. 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 DraI digest of DNA from male mice carrying the large T16H deletion which lies...
proximal to Ccg-1 and encompasses the DXCre131, Ar, DXCre169, and Ta loci (Brockdorff et al., 1991b). A 3.5-kb DraI band is present in Ta<sup>29</sup>H mice, demonstrating, as would be expected from its genetic position distal to Ccg-1, that Rps4 lies outside the Ta<sup>29</sup>H 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 TaqI band in lab mice and 5.1- and 3.5-kb TaqI bands in M. spreitus (Brockdorff et al., 1991a). The Xist probe was hybridized to TaqI digests of backcross progeny DNAs (data not shown), and mice were scored according to the lab mice/M. spreitus 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. spreitus, 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—(9.1 ± 9.1 cm)—Rps4/Phka—(2.4 ± 1.7 cm)—Xist—(1.6 ± 1.6 cm)—Pgk-1.

**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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