

characteristic for the human and rat 2X isoform [10,11]. Thus, the clone isolated can be considered as the porcine 2X isoform according to the human classification of MYH isoforms [12].

The porcine pAZMY9 clone was hybridized to GTG-banded pig metaphase spreads. In 16 of the 18 metaphases examined, distinct fluorescent signals were found on both chromatids of each of the two homologs of Chr. 12 at the qter region (Fig. 1a). This high efficiency may be caused by clustering of skeletal myosin heavy chain genes [1], as reported for other species [5,7]. From the GTG-banded images it could be deduced that the signals were located at band q1.4-q1.5 (Fig. 1b). The physical mapping of the porcine *MYH1* locus to Chr 12 is consistent with the previously noticed highly conserved synteny between human Chr 17 and pig Chr 12 [13], which was also demonstrated by ZOO-FISH [14,15].

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The gene encoding PRBP, the mouse homolog of human TRBP, maps to distal Chromosome 15

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Species: Mouse

Locus name: Protamine RNA binding protein

Locus symbol: *Prbp*

Map position: *Prbp* is on mouse Chromosome (Chr) 15 and cosegregates with *D15Mit16*, *D15Wsu77e*, *D15Wsu97e*, *D15Xrf232*

and *D15Hun17* (Fig. 1). A *Prbp* pseudogene maps to Chr 6, band B.

Methods of mapping: The *Prbp* gene was mapped by fluorescence in situ hybridization (FISH) and by typing 96 animals from The Jackson Laboratory BSS interspecific [(C57BL/6J × SPRET/Ei)F₁ × SPRET/Ei] backcross panel [1]. A *Prbp* pseudogene was mapped by FISH.

Database deposit information: The typing data have been deposited in the Mouse Genome Database (accession No. MGD-MRK-37240) and may be obtained through the World Wide Web (<http://www.jax.org/resources/documents/cmdata>).

Molecular reagents used for mapping: The *Prbp* FISH mapping was performed with a 16.1-kb genomic fragment containing exons 1-9. PCR primers for the *Prbp* gene were 5' aagctgctgccctgttcctact 3' (exon 4) and 5' ctcagcaatgcccctgtgat 3' (intron 4). PCR conditions were: 96°C 1 min, and then 40 cycles of 96° 30 s, 58° 30 s, 72° 1 min, followed by a final extension at 72° for 4 min. The *Prbp* pseudogene FISH mapping was performed with an 11.9-kb intronless genomic fragment that contains exons 2-9 but is missing exons 1 and 5.

Method of allele detection: A 300-bp PCR product is generated from DNA of both mouse strains. A single base pair polymorphism (G to A substitution) in intron 4 of the SPRET/Ei allele destroys an *AatII* site. Cleavage of the C57Bl/6J PCR fragment with *AatII* generates restriction fragments of 250 bp and 50 bp.

Previously identified homolog: Human TAR RNA binding protein (TRBP) [2].

Discussion: The mouse protamine 1 gene (*Prm-1*) encodes an arginine-rich protein involved in nuclear condensation during spermatogenesis. The *Prm-1* gene is transcribed in haploid round spermatids, and its mRNA is translationally repressed for up to a week before it is eventually translated in elongating spermatids [3]. *Prbp* encodes a 43-kDa, double-stranded RNA binding protein that we have suggested functions in the translational control of *Prm-1* mRNA [4]. PRBP protein is most abundant in the cytoplasm of round spermatids, the site of storage of the *Prm-1* mRNA, and is reduced in elongating spermatids, the site of *Prm-1* mRNA trans-

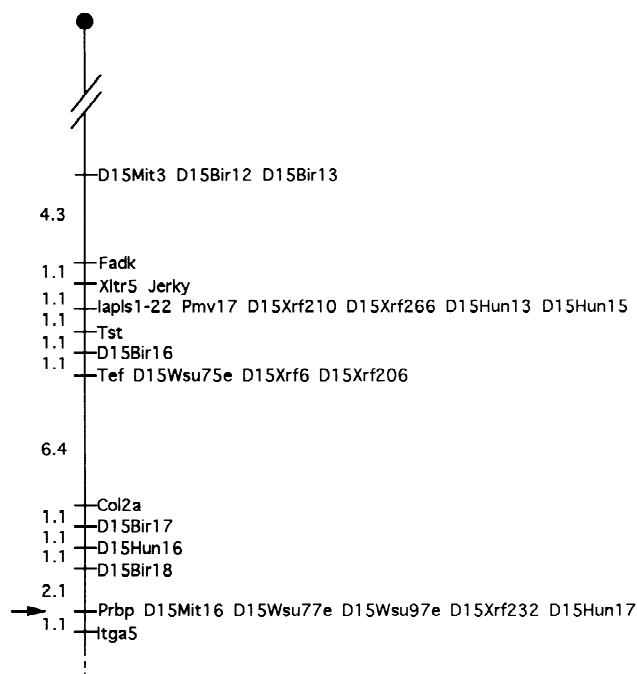


Fig. 1. Map position of the *Prbp* gene. A partial map of the mouse Chr 15. Map distances (cM) are indicated at the left. The map location of the *Prbp* gene is indicated by an arrow. The map information was provided by The Jackson Lab Backcross Mapping Resource.

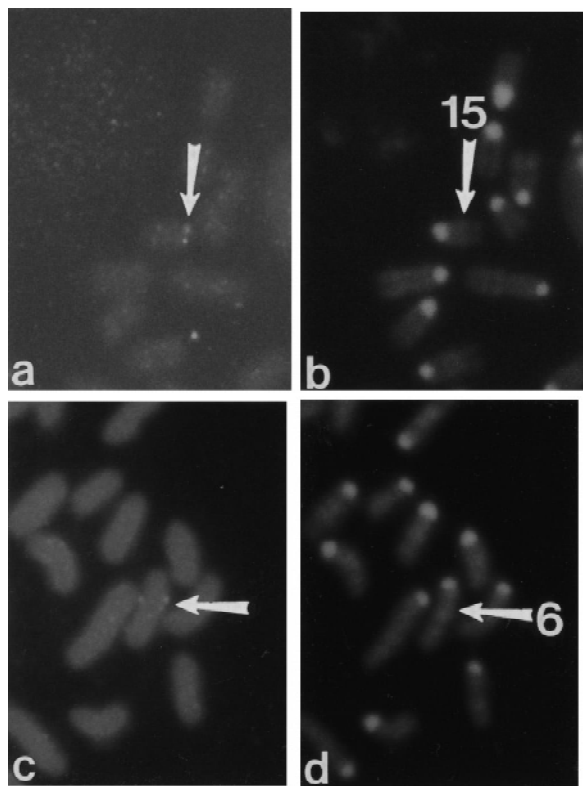


Figure 2. FISH mapping of the *Prbp* gene (a,b) and pseudogene (c,d). (a) Signals on Chr 15; (b) identification of Chr 15 by banding; (c) signals on Chr 6; (d) identification of Chr 6 by banding.

lation. Recombinant PRBP protein represses translation of reporter mRNAs in cell-free translation extracts, although repression is not dependent on the *Prm-1* 3' UTR [4]. Mice that are deficient for *Prbp* are severely growth retarded and usually die within the first few weeks of birth (Zhong et al. unpublished). Males that survive to adulthood are sterile because of defective spermatogenesis.

Using a *Prbp* genomic fragment as a probe, we mapped the *Prbp* gene by FISH. The probe was hybridized to fixed metaphase chromosomes prepared from lymphocytes of a male C57BL/6J mouse. The chromosomes were identified by banding, with an actinomycin D-DAPI stain and previously described methodology [5]. Of 39 cells examined, 28 cells had FISH signals on both chromatids of one Chr 15, and 11 cells had FISH signals on both chromatids of both Chr 15 at region F (Fig. 2a and b). In order to more precisely determine the location of the expressed *Prbp* gene,

we mapped the gene by typing an interspecific backcross DNA panel from The Jackson Laboratory Backcross DNA Panel Mapping Resource. *Prbp* co-segregates with several markers on distal Chr 15 (Fig. 1). In the process of cloning the genomic *Prbp* gene, we isolated and characterized what is likely to be a *Prbp* pseudogene that arose by integration of a reverse transcribed and alternatively spliced RNA (unpublished). We mapped the *Prbp* pseudogene by FISH to Chr 6. Of 32 cells examined, 21 cells showed FISH signals on both chromatids of one Chr 6 at region B, and 11 cells showed FISH signals on both chromatids of both Chr 6 region B (Fig. 2c,d). There was no significant hybridization to other chromosomes.

PRBP shares 93% amino acid identity with the human TAR RNA-Binding Protein (TRBP) [2] and is likely to be its mouse homolog. It has been suggested that TRBP functions in non-infected cells to regulate the activity of the double-stranded, RNA-activated protein kinase PKR, and therefore indirectly to control protein synthesis [6,7]. Kozak and associates mapped the TRBP gene to human Chr 12, and a TRBP-related sequence to human Chr 8 [8]. Using TRBP probes on DNA prepared from mouse and Chinese hamster somatic cell hybrids, they found three TRBP-related sequences on mouse Chrs 6, 7, and 15. Because mouse Chr 15 contains a region syntenic to human Chr 12, they tentatively concluded that the mouse Chr 15 contains the expressed *Prbp* gene. However, owing to the lack of suitable polymorphic loci, they were unable to position the murine homolog of TRBP to a specific location on Chr 15. Our data definitively map the *Prbp* gene. It is likely that the *Prbp* pseudogene that we have mapped corresponds to the TRBP-related sequence on proximal Chr 6.

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