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The *Atp1b3* gene for Na,K-ATPase β_3 subunit maps to mouse Chromosome 9, and a related gene, *Atp1b3-rs*, maps to mouse Chromosome 3

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Species: Mouse
Locus name: Na,K-ATPase β_3 subunit gene and Na,K-ATPase β_3 subunit related sequence.
Locus symbols: *Atp1b3* and *Atp1b3-rs*
Map position: *Atp1b3* is located on mouse Chromosome (Chr) 9: *D9Bir12*–(6.38 ± 2.56)–*Ctsh*–(3.19 ± 1.81)–***Atp1b3***–(2.13 ± 1.49)–*Cappa1-ps2*–(1.06 ± 1.06)–*D9Bir13*–(1.06 ± 1.06)–*D9Hun8*–(2.13 ± 1.49)–*D9Mit24,Dag1*–(1.06 ± 1.06)–*CamkI* (Figure 1 A, B). *Atp1b3-rs* is located on mouse Chr 3: *Lxn*–(2.13 ± 1.49)–*D3Bir8,D3Mit22*–(1.06 ± 1.06)–***Atp1b3-rs,****Etfdh,Si-s*–(1.06 ± 1.06)–*Npy2r*–(3.19 ± 1.81)–*Bglap1,D3Bir10* (Fig. 1C, D).
Method of mapping: Both *Atp1b3* and *Atp1b3-rs* were localized by haplotype analysis of 94 progeny from an interspecific backcross, (C57BL/6JEi × SPRET/Ei)F_1 × SPRET/Ei known as Jackson BSS from The Jackson Laboratory [1].
Database deposit information: The scorings are available on the internet at the following address: http://www.jax.org/resources/documents/cmdata/BSS.html.
Molecular reagents: The hybridization probe used for mapping was the 3′UTR of the Na,K-ATPase β_3 subunit cDNA from the *Eco*RI site to the end of the 3′UTR corresponding to nucleotides 1005–1842 [2]. The probe was excised from the cDNA, isolated in a low-melting-point agarose gel, and labeled by the random priming method [3]. The restriction fragments containing the expressed gene were identified by hybridization to a genomic DNA fragment containing only intron sequences of the Na,K-ATPase β_3 subunit gene. The genomic clone was isolated from a mouse strain 129SVJ genomic DNA library and characterized (unpublished data). A 405-bp fragment containing part of intron 6 sequences was excised from the genomic DNA clone, isolated, and labeled by the same methods.
Allele detection: A *Hin*cII polymorphism was detected in mouse genomic DNA between C57BL/6JEi and SPRET/Ei by Southern blot hybridization with the 3′UTR mouse cDNA probe. The most stringent post-hybridization wash was performed at 0.2 × SSC–1% SDS at 65°C for 60 min. The 3′UTR cDNA probe hybridized to 6-kb and 12-kb *Hin*cII fragments in C57BL/6JEi and 3.5-kb and approximately 15-kb *Hin*cII fragments in SPRET/Ei. A mouse genomic DNA probe containing only intron sequences hybridized to the 12-kb *Hin*cII fragment of C57BL/6JEi and 3.5-kb *Hin*cII fragment of SPRET/Ei, indicating that these polymorphic fragments contained the expressed gene. A *Hin*cII-digested panel of 94 progeny DNAs of an interspecific backcross of (C57BL/6JEi ×

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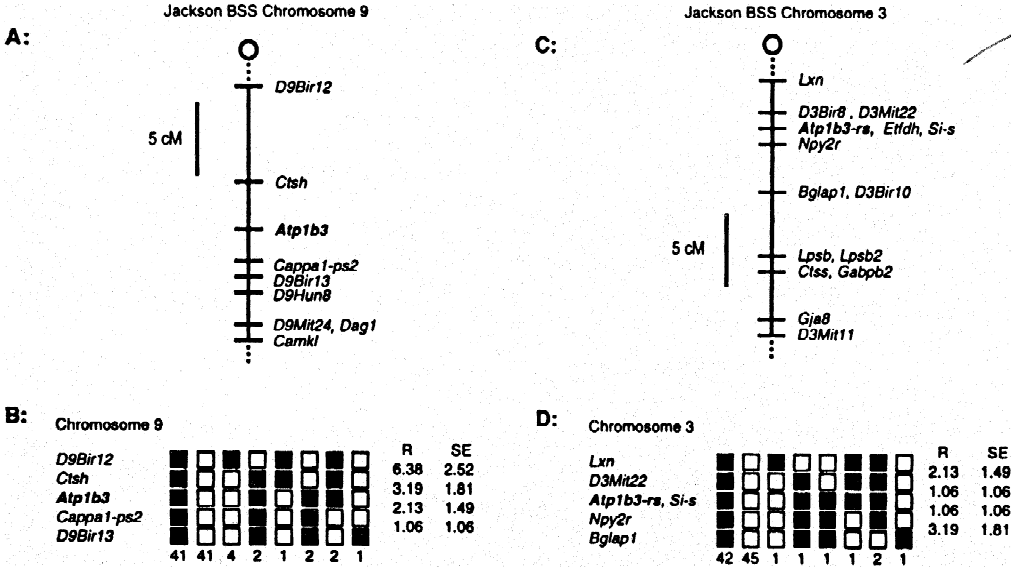

Fig. 1. **A** and **C:** Map figures from The Jackson BSS backcross showing parts of Chr 9 and Chr 3 respectively, and the positions of *Atp1b3* and *Atp1b3-rs* in relation to several previously mapped loci. The maps are depicted with the centromere toward the top. A 5-cM scale bar is shown to the left of the figure. Missing typings were inferred from surrounding data where assignment was unambiguous. Raw data from The Jackson Laboratory were obtained from the World Wide Web address http://www.jax.org/resources/documents/cmdata/BSS.html. **B** and **D:** Haplotype figures from The Jackson BSS backcrossshowing parts of Chr 9 and Chr 3 respectively with loci linked to *Atp1b3* and *Atp1b3-rs*. Loci are listed in order with the most proximal at the top. The black boxes represent the C57BL/6JEi allele, and the white boxes the SPRET/Ei allele. The number of animals with each haplotype is given at the bottom of each column of boxes. The percentage recombination (R) between adjacent loci is given to the right of the figure, with the standard error (SE) for each R. Missing typings were inferred from surrounding data where assignment was unambiguous.

SPRET/Ei)F_1 × SPRET/Ei was probed with the labeled 3′UTR mouse cDNA probe by Southern blot hybridization and then scored for the presence (BS) or absence (SS) of the 12-kb *Atp1b3* and 6-kb *Atp1b3-rs* genes in C57BL/6JEi.

Previously identified homologs: Two loci that flank the *Atp1b3* gene on mouse Chr 9, *Rbp1* [4] and *Trf* [5], have been previously localized to human 3q21-22 and 3q21 respectively. A human sequence-tagged site (STS), SHGC-16162 (GenBank Accession No. G19318), whose sequence is identical to the 3′ end of the human Na,K-ATPase β_3 subunit cDNA [6], was mapped to human Chr 3q21-23 by means of the GB3 radiation hybrid mapping panel (Stanford Human Genome Center, Stanford School of Medicine). This region of human Chr 3 is syntenic to the region of mouse Chr 9 flanked by *Rbp1* and *Trf* that contains *Atp1b3*. Thus, the assignment of the mouse gene designated *Atp1b3* is consistent with the localization of its human homolog.

Discussion: The Na,K-ATPase is an integral membrane protein responsible for active transport of sodium and potassium ions across the cell membrane. This enzyme consists of an α subunit and a β subunit. The α subunit contains the catalytic site for ATP hydrolysis and the binding site for ouabain, a specific inhibitor of Na,K-ATPase. The β subunit is a glycosylated polypeptide of poorly defined function, but it appears to be involved in maturation and membrane localization of the ATPase [7]. We have recently identified a novel mouse β_3 subunit of Na,K-ATPase [2]. The gene for the β_3 subunit is widely expressed. In Northern blot analysis, transcripts were seen in RNA from total mouse embryos, plus a variety of adult mouse tissues including brain, heart, kidney, lung, and spleen. In this experiment, we have localized expressed β_3 subunit gene, *Atp1b3*, to mouse Chr 9, and a related DNA sequence, *Atp1b3-rs*, to mouse Chr 3. Malik et al. [6] have previously reported that *Atp1b3* is located on mouse Chr 7 [6]. They have identified *Msp*I fragments in A/J and C57BL/J6 mice that hybridized with the full-length human Na,K-ATPase β_3 subunit cDNA probe. A 1.5-kb *Msp*I genomic DNA fragment, hybridized with the human cDNA probe only in A/J mouse DNA, was used to map β_3 subunit gene by RFLP analysis. However, these investigators have not shown that this polymorphic *Msp*I fragment contains the expressed β_3 gene. We have used an intron probe to distinguish the expressed gene from the related DNA sequence that hybridized with our 3′UTR cDNA probe. The 6-kb *Hin*cII fragment from C57BL/6JEi, which hybridized to 3′UTR mouse probe but not to the genomic DNA probe, was localized to mouse Chr 3 and designated *Atp1b3-rs* for Na,K-ATPase β_3 subunit related sequence.

Acknowledgments: The authors thank Lucy Rowe and Mary Barter (The Jackson Laboratory, Bar Harbor, Me.) for their assistance with data analysis and the MapManager figures. This work was supported in part by National Institutes of Health grant DC02492 (M.I. Lomax) and Deafness Research Foundation (T.-W. Gong).

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Linkage mapping of *Csrp* to proximal mouse Chromosome 3

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Species: Mouse
Locus name: Cysteine-rich protein
Locus symbol: *Csrp*
Map position: *D3Bir1*–(1.15 ± 1.14)–[*Il7*–(0.00 ± 0.00)–*D3Mit60*–(0.00 ± 0.00)–*Csrp*]–(1.06 ± 1.06)–*Agtr1b*–(7.45 ± 7.45 ± 2.71)–*Il2*
Method of mapping: Haplotype and linkage analysis of the 94-individual N_2 progeny of The Jackson Laboratory BSS interspecific backcross panel [(C57BL/6JEi × SPRET/Ei) F_1 × SPRET/Ei] [1] with the marker CA19.9, corresponding to nt 122–607 of the *Csrp* transcript [N. Hashimoto, unpublished].
Database deposit information: GenBank accession #D88793
Molecular reagents used for mapping: cDNA CA19.9 was isolated by PCR-based differential screening in a search for developmentally regulated brain genes. The cDNA was excised from an RNA display gel and cloned as described [2]. A database search showed that CA19.9 is 100% identical to a portion of the mouse *Csrp* gene (GenBank accession number D88793) [N. Hashimoto, unpublished]. CA19.9 was radiolabeled as described [3] and used as a molecular probe to hybridize a DNA panel from the parental strains used to generate the BSS backcross.
Linkage mapping: an *Xba*I RFLP (SPRET/Ei-specific band: 0.5 kb; C57BL/6JEi-specific band: 2.3 kb) was identified. Southern blots of *Xba*I-digested DNA from the two parental strains and 94 N_2 progeny were hybridized with radiolabeled CA19.9. The resulting strain distribution pattern was typed into the MapManager 2.6 program [4], resulting in the unequivocal assignment of *Csrp* to proximal mouse Chromosome (Chr) 3 (Fig. 1).
Previously identified homologs: The human CSRP gene has been mapped to Chr 1q24-q32 [5]. The location of *Csrp* in the BSS map reveals a new region of synteny between mouse Chr 3 and human 1q, distinct from the one previously described at a more telomeric location [6,7] (see Fig. 1b). Likely orthologs of *Csrp* have also been found in the rat [8], chicken [9], and quail genomes [10].
Discussion: Our genetic mapping work allows *Csrp* to be assigned to proximal mouse Chr 3. In the BSS linkage map, *Csrp* cosegregates with *D3Mit60* and *Il7*. The latter shows a more centromeric localization (proximal to *Agtr1b*) in the BSS map than in the consensus MGD map. The chromosomal position of *Csrp* on mouse Chr 3 suggests that synteny between MMU3 and human 1q is split in two distinct segments.

Csrp encodes a protein containing two zinc-finger domains of the LIM type [11]. The protein was first isolated in chick through an in vitro biochemical screening as a 23-kDa interactor of zyxin [12].

The chicken Cysteine-Rich Protein (cCRP) is present at high levels in terminally differentiated muscle cells [9]. In chick embryo fibroblasts, cCRP is found at detectable levels in stretch fibers near adhesion plaques, as well as in the nucleus [9]. The C-terminal LIM domain of cCRP displays structural identity to the zinc-finger domain of GATA-1 [13], suggesting that the protein might bind DNA.

We have demonstrated that the *Csrp* gene is tightly regulated

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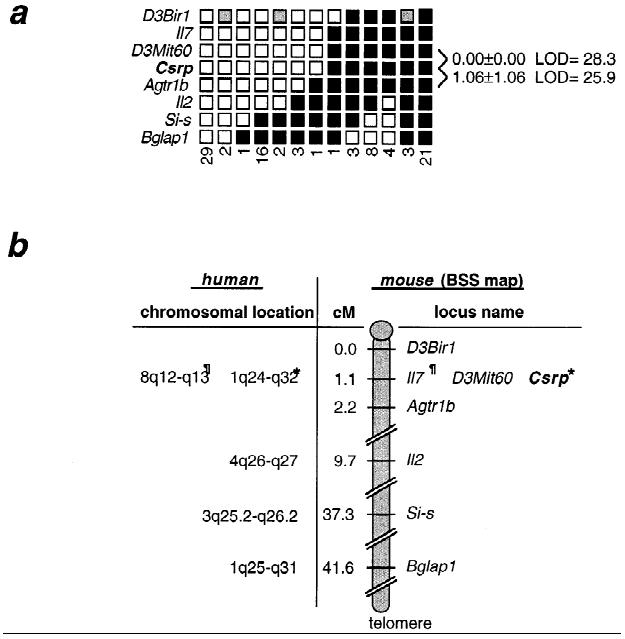

Fig. 1. a: Haplotype and linkage analysis of *Csrp* and flanking Chr 3 loci in the 94 individual N_2 progeny of the BSS backcross. Filled squares: BS genotype; empty squares: SS genotype; stippled squares: untyped. Below each column are the numbers of N_2 progeny members sharing each given haplotype. Recombination frequencies ± standard errors and LOD scores are on the right. **b:** Sketch of loci/marker loci independently mapped to the BSS Chr 3 map, and genetic distances (cM) between each locus and the most centromeric one in the map. Also indicated are some of the human chromosomal regions syntenic to various segments of mouse Chr 3. Online references and information on loci mapped through the BSS panel can be obtained from http://www.jax.org/resources/documents/cmdata/.

during mouse brain development (C. Alli, unpublished). Studies of its potential function in central nervous system differentiation are currently in progress.

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Localization of the ileal sodium-bile salt cotransporter gene (*Slc10a2*) to mouse Chromosome 8

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Species: Mouse
Locus name: Ileal sodium-dependent bile salt transporter (solute carrier family 10, member 2)
Locus symbol: *Slc10a2*
Map position: *Slc10a2* is found to map to Chromosome (Chr) 8 with a LOD score of 25.6; centromere–*D8Mit155*–1.1 ± 1.1 cM–*Slc10a2*–1.1 ± 1.1 cM–*D8Bir3*–1.1 ± 1.1 cM–*Sox1*–10.8 ± 3.2 cM–*D8Mit4* (Fig. 1A and B).
Method of mapping: Linkage analysis was performed with the BSS Backcross DNA Panel (The Jackson Laboratory, Bar Harbor, Maine) [1]. Linkage was established with the program MapManager Classic (distributed via World Wide Web, URL: http://mcbio.med.buffalo.edu/mapmgr.html) [2].
Database deposit information: Mouse Genome Database Accession No. MGD-JNUM-42394.
Molecular reagents: Oligonucleotide primers based on the 3′ untranslated region of *Mus musculus* (strain ICR) cDNA (GenBank Accession No. AB002693), which contains a CA repeat [$(CA)_{22}$], were used to amplify genomic DNA purified from inbred strains.
Allele detection: Genotyping was performed by PCR amplification of Simple Sequence Length Polymorphisms (SSLP) as previously described [3]. The primer pair with the sequences 5′-GAC AAT GAA ATG GAC TCC AGG-3′ (forward primer) and 5′-TAC GAT CGT ACG CTC ATG TAC-3′ (reverse primer) identified a polymorphism between inbred strains C57BL/6JEi and SPRET/Ei.
Previously identified homologs: The assignment of mouse *Slc10a2* to Chr 8 (Fig. 1B) is consistent with previous mapping of human SLC10A2 to the homologous region of Chr 13q33 with human/rodent cell hybrids and fluorescence in situ hybridization analysis [4].
Discussion: Bile salts are synthesized in the liver from cholesterol and secreted into the biliary tree and then the small intestine, where they serve dispersive and solubilization roles in absorption of dietary fat, cholesterol, and fat-soluble vitamins [5]. These important detergent-like molecules are efficiently reabsorbed from the intestine by active and passive transport mechanisms and returned to the liver (enterohepatic circulation) [5]. Although less polar bile salts are reabsorbed in part by passive diffusion mechanisms throughout the small intestine, taurine and glycine conjugates, being fully ionized, are almost totally dependent upon an active transport system located in the distal ileum [6]. The gene encoding this bile salt transporter (also abbreviated IBAT for ileal bile acid transporter) was first cloned from Syrian hamster using an expression cloning strategy [7]. The cloning of rat, rabbit, and human homologs followed, and the transporter was shown to be expressed in ileum, cecum, rectum, large cholangiocytes, and kidney [8–11]. The cotransporter was proven to possess a counter-ion stoichiom-

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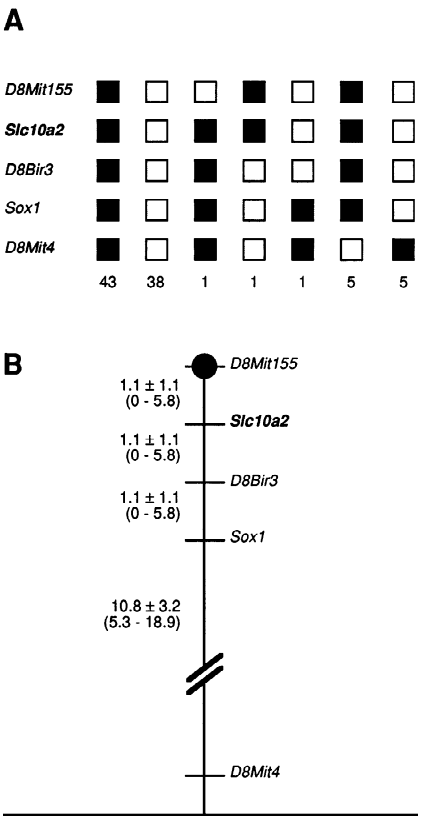

Fig. 1. Position of *Slc10a2* with respect to markers on mouse Chr 8. **(A)** Haplotype diagram of BSS backcross progeny. Filled boxes represent mice carrying C57BL/6JEi alleles. The number of mice that carry each haplotype is indicated at the bottom of each column. **(B)** Map distances are given in cM ± SE with 95% confidence intervals.

etry of two sodium ions per bile salt anion, resulting in sodium-dependent as well as electrogenic transport [12].

Point mutations of the ileal sodium-bile salt cotransporter, e.g., P290S [9], L243P, and T262M [13], can produce primary bile acid malabsorption in humans, a disease expressed clinically by chronic diarrhea, steatorrhoea, failure to thrive, as well as reduced plasma levels of cholesterol and fat-soluble vitamins, all characteristic of interrupted enterohepatic circulation of bile salts [5]. Bile salt spillage into the cecum and the proximal colon can lead also to enterohepatic cycling of unconjugated bilirubin and enterorenal delivery of oxalic acid. The subsequent elevation of bilirubin levels in bile (hyperbilirubinbilia) [14] may increase the risk for ''black'' pigment gallstones, composed principally of polymerized calcium salts of monoanionic unconjugated bilirubin [15], and high oxalic acid concentrations in urine (hyperoxaluria) may lead to calcium oxalate renal calculi [16].

We have employed Quantitative Trait Loci (QTL) mapping [17] to identify lithogenic genes that effect cholesterol gallstone formation in inbred mice [18]. *Lith* genes dysregulate hepatic lipid metabolism in inbred mice, inducing cholesterol supersaturation with biliary hypersecretion of cholesterol and bile salts [19]. Cloning of the genes that underlie QTL for cholelithiasis is challenging, but is facilitated by complete cataloguing of all genes relevant to the trait [17]. Genetic mapping of *Slc10a2* should help, therefore, in searching for QTL candidate genes involved in pathophysiology of the enterohepatic circulation as well as gallstone formation in inbred mouse models.

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Erythroid Krüppel-like transcription factor (*Eklf*) maps to a region of mouse Chromosome 8 syntenic with human Chromosome 19

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Species: Mouse
Locus name: Erythroid Krüppel-like factor
Locus symbol: *Eklf*
Map position: *Eklf* is located on mouse Chromosome (Chr) 8: centromere–*Jund*–1.9 ± 1.1 (cM ± SE)–*Mlr*–4.8 ± 1.8–[*Eklf*, *Junb*]–9.3 ± 2.7–*Mt1*. No recombinants were detected between *Eklf* and *Junb* in 165 animals typed in common, suggesting that the

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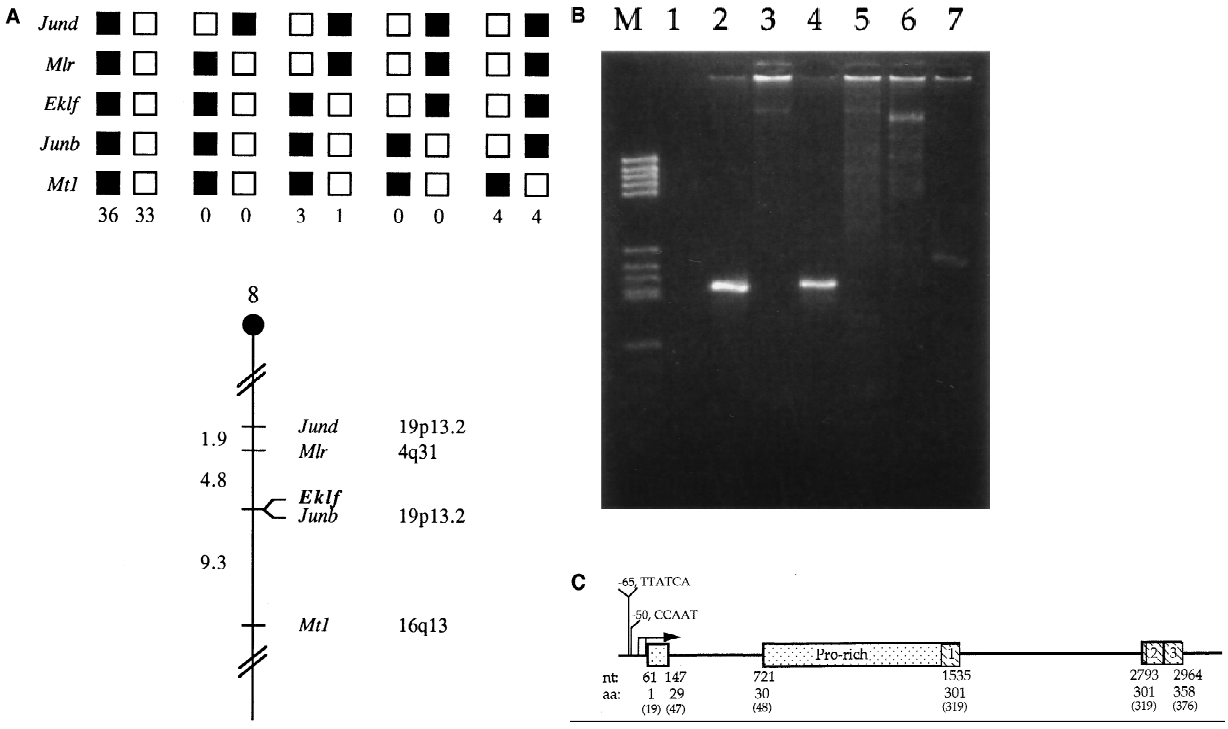

Fig. 1. (A) *Eklf* maps to the central region of mouse Chr 8. *Eklf* was placed on Chr 8 by interspecific backcross analysis. The segregation of *Eklf* and flanking genes in 81 backcross animals that were typed for all loci is shown at the **top.** For individual pairs of loci, more than 81 animals were typed. The shaded boxes represent the presence of a C57BL/6J allele, and white boxes represent the presence of an *M. spretus* allele. The number of offspring inheriting each type of chromosome is listed at the bottom of each column. A partial Chr 8 linkage map showing the location of *Eklf* in relation to linked genes is shown at the **bottom.** Recombination distances in centiMorgans between loci are shown to the left of the chromosome, and the positions of loci in human chromosomes, where known, are shown to the right. References for the human map positions of loci can be obtained from the Genome Data Base (a computerized database maintained by the Welch Medical Library, Johns Hopkins University, Baltimore, Md.) **(B)** PCR analysis of chromosomal DNA. Human EKLF primers within intron 2 [9] were used in PCR reactions (1 min at 95°; 1 min at 54°; 1 min at 72° in Qiagen Q Buffer) with: (1) water; (2) 1 μg human chromosomal DNA; (3) 1 μg mouse/human Chr 1 hybrid DNA (Coriell GM13139); (4) 1 μg hamster/human Chr 19 hybrid DNA (Coriell GM10012) (5) 1 μg mouse DNA; (6) 1 μg hamster DNA. Lane 7 (a control for lane 3) is derived from a reaction with murine EKLF intron primers with mouse/human Chr 1 DNA. Lane M contains DNA molecular weight marker V (Boehringer). **(C)** Diagram of the murine EKLF transcription unit (Genbank accession number AF019074). Boxed areas are exons, and lines between them are introns. Stippled boxes indicate the proline-rich region of EKLF, diagonally hatched boxes denote each of the three EKLF zinc fingers. The location of the transcription initiation sites are indicated by the arrow, and subsequent nucleotide (nt) numbering is based on defining the distal initiation site (see [2]) as +1. Locations of translation initiation, intron/exon boundaries, and CAAT and GATA1 binding sites are shown. Inclusive amino acid (aa) numbers are indicated (aa 1-358), as are their correspondence to the numbering in [2] (aa 19-376 in parentheses).

two loci are within 1.8 cM of each other (upper 95% confidence limit).

Method of mapping: Interspecific backcross progeny were generated by mating (C57BL/6J × *M. spretus*) F_1 females and C57BL/6J males as described [1]. This interspecific backcross mapping panel has been typed for over 2400 loci that are well distributed among all the autosomes as well as the X Chr. A total of 205 N_2 mice were used to map the *Eklf* locus.

Database deposit information: The MGD accession number is MGD-CREX-160. The Genbank accession number is AF019074.

Molecular reagents: We excluded the EKLF zinc finger region from our hybridizations to avoid potential cross-reaction with other family members by using a probe that spans nucleotides 271–841 of the EKLF cDNA sequence [2].

Allele detection: The presence or absence of the 5.4-kb *M. spretus*-specific *Bam*H1 fragment was followed in backcross mice (Fig. 1A). A description of the probes and RFLPs for loci linked to *Eklf* has been reported previously [3,4].

Discussion: EKLF is a zinc finger-containing, erythroid cell-specific transcription factor that binds to and transactivates from the CACCC element present in the adult human and murine β-globin promoter [2]. Point mutations at its DNA target site, including those known to give rise to β-thalassemia, disrupt EKLF binding and thus its ability to activate transcription [5]. Molecular [6] and genetic [7,8] studies indicate that EKLF is critical for the transcriptional switch that leads to high-level adult β-globin expression.

The map location of *Eklf* is in a region of homology with human Chr 4 and 19 (Fig. 1A); in particular, *Junb* maps to 19p13. Given the tight linkage between *Junb* and *Eklf* in mouse, we addressed whether human EKLF resides on Chr 19 as well. We utilized rodent/human hybrid DNA samples (Coriell) that contained either human chromosome 1 or 19 for PCR analysis with human EKLF [9] gene-specific primers within intron 2. Figure 1B demonstrates that a product of the correct size (190 bp) as the positive control is observed only in the Chr 19-containing sample. As a result, we conclude that EKLF resides in syntenic regions of mouse Chr 8 and human Chr 19. Consistent with these expectations, and while these experiments were in progress, human EKLF was shown to be located at 19p13 [10].

To complete the molecular comparison between the human and murine orthologs of EKLF, we determined the nucleotide sequence of 6350 bp surrounding the murine transcription unit, schematized in Fig. 1C. Beyond the conserved coding sequence and the locations of translation initiation, intron, and termination sites when compared with the human transcription unit [9], the murine and human units contain conserved 5′- and 3′-untranslated sequences, and conserved proximal promoter sequences [11].

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Mapping of three members of the mouse protein disulfide isomerase family

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Species: Mouse
Locus names: Glucose-regulated protein 58 (*Grp58*), endoplasmic reticulum protein 72 (*Erp72*), protein disulfide isomerase (*P4hb*).
Locus symbols: *Grp58, Erp72,* and *P4hb.*
Map positions: *Grp58* is localized on mouse Chromosome (Chr) 2: centromere–*D2Mit35*–(9.5 ± 3.7 cM)–*D2Mit14*–(12.7 ± 4.2 cM)–*Grp58*/*D2Mit30*–(5.7 ± 3.2 cM)–*D2Nds3*–(1.8 ± 1.8 cM)–*D2Mit19*–(4.5 ± 2.6 cM)–*D2Ucla1*/*D2Ucla2. Erp72* is localized on mouse Chr 6: centromere–*D6mit1*/*Pon*–(3.5 ± 2.4 cM)–*D6Mit48*–(12.3 ± 4.1)–*Erp72*–(3.0 ± 2.1 cM)–*D6Nds4*–(6.1 ± 2.9)–*Ghrhr*/*lit*/*D6Ucla2*–(5.1 ± 2.9)–*D6Mit17. P4hb* is located on mouse Chr 11: centromere–*D11Mit59*–(1.6 ± 1.6 cM)–*Crhr*–(3.2 ± 2.2 cM)–*Pkca*–(1.6 ± 1.6)–*D11Ucla2*–(9.5 ± 3.7)–*P4hb*–(8.9 ± 3.8 cM)–*D11Mit11* (Fig. 1).
Method of mapping: Southern blot analysis was performed on a panel of 67 DNA samples from a [(C57BL/6J × *Mus spretus*)F1 × C57BL/6J] backcross typed for more than 400 markers [1]. Linkage was detected with Map Manager QTb8 v2.6.5 [2].
Database deposit information: The accession number for *Grp58* and *Erp72* is MGD-JNUM-40697; *P4hb* has the accession numbers MGD-MRK-13114, MGD-MRK-15082.
Molecular reagents: cDNA probes were prepared by RT-PCR with mRNA from mouse liver with primers based on published sequence. PCR primers were as follows: *Grp58,* (upstream) 5′-TGGACTGCACTGCCAACACA-3′ and (downstream) 5′-TTCCAGGTTCTTACAGTGGC-3′ [3]; *Erp72,* (upstream) 5′-GCCGTTGACTATGATGGCTCCAGG-3′ and (downstream) 5′-

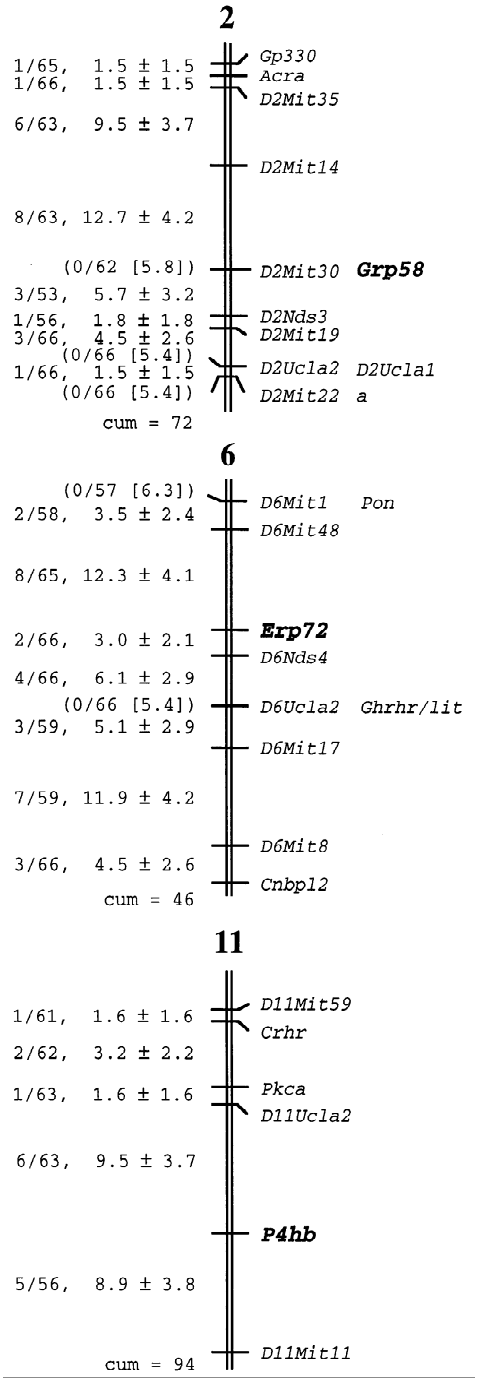

Fig. 1. Mapping of *Grp58, Erp72,* and *P4hb* to mouse Chrs 2, 6, and 11 respectively in an interspecific backcross [(C57BL/6J × *Mus spretus*)F_1 × C57BL/6J]. The chromosome is drawn to scale with the centromere at the top and the distance of the most distal marker from the centromere indicated at the bottom (cum., in centiMorgans). The ratios of the number of recombinants to the total number of informative mice and the recombination frequencies ± standard errors (in centiMorgans), for each pair of loci, are indicated to the left of the chromosome. For pairs of loci that cosegregate, the upper 95% confidence interval is shown in parentheses. *Ucla* markers were reported in Warden et al. [1].

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GGAGAGCGCTTGCTGAGCTCC-3′ [4]; *P4hb*, (upstream) 5′-GTCTTCATGTCCAGCTACTTG-3′ and (downstream) 5′-GGCTGGTCGGTAGTCCTGG-3′ [4].

Allele detection: An RFLV for *Grp58* was identified with *TaqI*, where fragments of 3.0 kb in C57BL/6J and 3.6 kb in *M. spretus* were used for scoring. An RFLV for *Erp72* was identified with *BglII*, which generated fragments of 5.2 and 6.8 kb for C57BL/6J and 5.2 and 5.8 kb for *M. spretus.* An RFLV for *P4hb* was identified with *PvuII*, which produced a fragment of 3.5 kb for C57BL/6J and 3.8 kb for *M. spretus.*

Previously identified homologs: The mouse PDI gene (*P4hb*) was mapped previously to the distal region of Chr 11 with a [(C57BL/6J × *Mus spretus*)F_1 × C57BL/6J] interspecific cross [8]. The human PDI gene has been mapped by somatic cell hybrids to Chr 17q25 [5]. The human Grp58 is located on Chr 15q15 [6]. To date, no homolog for mouse *Erp72* has been mapped in humans. No known mutations are present in the regions where *P4hb, Erp72,* and *Grp58* are located.

Abbreviations: For discussion purposes, we designate the *Grp58,* gene product as Grp58 (glucose-regulated protein 58), although it has also been called PI-PLC, ERp57, ERp60, ERp61, and PDI-Q2. The *Erp72* gene product is called ERp72 (endoplasmic reticulum protein 72). We designate the *P4hb* gene product as PDI (protein disulfide isomerase), although it has also been called ERp59.

Discussion: The PDI family represents a group of thioredoxin-related proteins that reside in the lumen of the ER [7]. Here we report the chromosomal position for three members of this family, *Grp58, Erp72,* and *P4hb* (Fig. 1). *P4hb,* mapped previously in an interspecific cross identical to the one used here, placed this locus about 12 cM distal to *Pkca* on Chr 11 [8]. Figure 1 shows that this map position is very similar to ours, placing *P4hb* about 11 cM from *Pkca.*

The PDI family is characterized by the presence of thioredoxin-like domains containing the motif CGHC; this motif contains the redox-active cysteine residues involved in the disulfide isomerase activity of PDI [7,9]. The PDI family members are soluble proteins that are retained within the ER by the C-terminal retention signals -KDEL, -KEEL, and -QEDL for PDI, ERp72, and Grp58 respectively [10]. PDI is the best characterized protein of this gene family and is well known for its function as a protein-folding enzyme that catalyzes disulfide bond isomerization [11]. PDI has the capacity to bind a wide variety of peptides at a domain separate from its CGHC active site, and this domain may function to target PDI to newly synthesized polypeptides as they are translocated into the ER [11]. In addition, PDI is part of complex enzyme systems operating in the ER: PDI is a subunit of the enzyme prolyl-4-hydroxylase, catalyzing the formation of hydroxyprolyl residues in nascent collagen-like polypeptides; PDI is also a subunit of the microsomal triacylglycerol transfer protein, functioning in the formation of nascent, triglyceride-rich lipoproteins [7].

While ERp72 and Grp58 also contain thioredoxin-like domains, they do not display PDI activity *in vitro* [12]. Nevertheless, it has been shown that ERp72, but not Grp58, can replace the growth-essential PDI function in yeast [12]. In addition, ERp72 is involved in ER-specific protein degradation [13], and Grp58 has recently been implicated as a glycoprotein-specific molecular chaperone [14].

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Linkage mapping of α3, α5, and β4 neuronal nicotinic acetylcholine receptors to rat Chromosome 8

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Species: Rat (*Rattus norvegicus*)

Locus name: α3,α5 and β4 neuronal nicotinic acetylcholine receptor gene cluster

Locus symbol: *D8Bord1* defining *Chrna3, Chrna5, Chrnb4*

Map position: *D8Bord1* is linked to *D8Mgh4* (13, 29.9), *D8Mit3* (14, 27.1), *Apeh* (32,7), *D8Mgh2* (36, 6.3), *D8Mit6* (52, 2.1) (cM distance and pairwise Lod score).

Method of mapping: Linkage analysis of *D8Bord1* and the other Chromosome (Chr) 8 markers was carried out in a 192 F_2 progeny from SHR/N × LEW/N cross (parental strains were purchased from Iffa credo, France). The Mapmaker v3.0 was used to estimate the map location and distances between loci.

Molecular reagents: The primers used were derived from the sequence of the genomic region between β4 and α3 genes [1] (Genbank S74125) containing sequence repeats (GTAT)n.
forward primer: 5′CCAGATGTCATCACCCTGTG 3′
reverse primer: 5′GAACCGTGGAAGTTGATGCT 3′

Method of allele detection: There was a 4-bp difference (probably one repeat) between *D8Bord1* PCR products of LEW and SHR rats. Alleles were detected on 3% agarose gel or on 6% polyacrylamide gel as previously described [2].

Previously identified homologs: The α3, α5, and β4 gene cluster has been mapped to human Chr 15q24, (CHRNA3, CHRNA5 and CHRNB4, respectively) [3] and to mouse Chr 9 (*Acra3, Acra5,* and *Acrb4* respectively) [4,5].

Discussion: Recently, we detected a major quantitative trait locus

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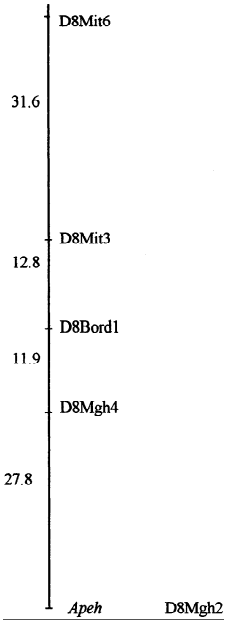

Fig. 1. Genetic linkage map of rat Chr 8. Numbers on the left side are cM distances (Kosambi function).

for behavioral hyperactivity of WKHA/WKY rats on rat Chr 8 near the *Apeh* (also called *Acph*) marker [2]. The α3 neuronal nicotinic acetylcholine receptor gene had been mapped close to the *Apeh* marker on mouse Chr 9 [4] and thus represented a good positional candidate for the hyperactivity QTL since these receptors are present on striatal nerve terminals where they modulate the release of dopamine [6]. We have found a rat microsatellite marker (*D8Bord1*) for this α*3* gene which in the rat forms a cluster with α*5* and β*4* genes [7] and we have mapped this cluster >32cM from *Apeh* (Fig. 1). There was no polymorphism between the WKY and WKHA rat strains for *D8Bord1;* therefore, we could not test genetic linkage between this locus and behavioral hyperactivity, but this study suggests that it is outside the hyperactivity QTL. This study confirms the high degree of homology between rat Chr 8 and mouse Chr 9. The α*3*, α*5* and β*4* gene cluster is the eighth gene localization shared between rat Chr 8 and human Chr 15q21-24.

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The cat immunoglobulin lambda light chain gene maps to Chromosome D3p12-p11

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Species: Cat (*Felis catus*)
Locus name: Immunoglobulin light chain Cλ
Locus symbol: IGL@
Map position: cat Chr D3 at band p12-p11
Method of mapping: FISH. A λ phage clone, which contains 15-kb inserts hybridizing with the cat *IgL* Cλ cDNA fragment (420 bp) [1], was isolated from a commercially purchased feline genomic library (Lambda FIX II library, Stratagene). The probe was labeled with biotin-16-dUTP by nick translation and mixed with a 10-fold excess of sheared cat genomic DNA to avoid hybridization to the repetitive sequences. The signal was detected by FITC-avidin (Boehringer) overlay as described [2]. Prometaphase chromosomes were counterstained with propidium iodide and DAPI for identification of individual chromosomes. A proposed nomenclature for cat karyotype [3] was adapted to localize the gene.
Data base deposit information: European Patent Publication Number of the cat *IgL C*λ gene is 0417486A.
Molecular reagents used for mapping: FIGL1, a 15-kb genomic DNA probe containing a 420-bp cat *IgL* Cλ cDNA sequence, was used in FISH analysis.
Previously identified homologs: Human and mouse homologs, *IGL @* and *Igl-l*, have been mapped on Chr 22q11.1-q11.2 and Chr 16B1-B5, respectively [5].
Discussion: The hybridization signals were clearly located on the proximal region of the short arm of cat Chr D3, but the doublet signals on both homologs of Chr D3 were observed in few metaphases (Fig. 1). Of over 30 metaphases surveyed, none of the other chromosomes showed significant hybridization. We therefore conclude that the cat *IGL @* maps to Chr D3p12-p11, with the most likely position at p12.2. In addition, the experiments using a cat × rodent somatic cell hybrid panel have indicated *IGL @* maps to cat Chr D3 [1].

In humans, chromosome translocations involving the Ig and TCR genes are frequently found in B- and T-cell malignancies, and

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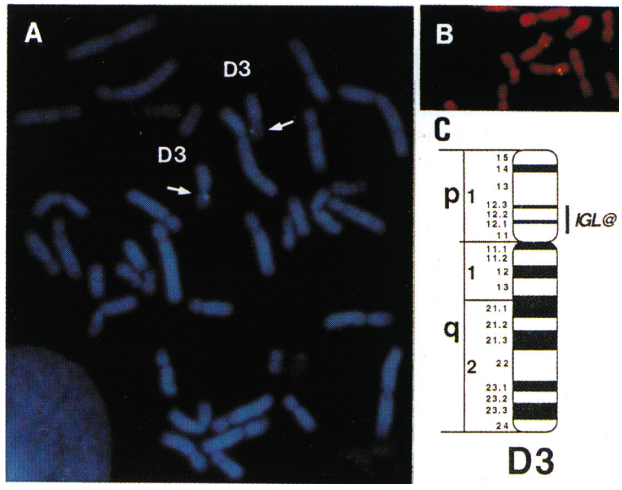

Fig. 1. FISH analysis of the cat *IGL* Cλ gene. **(A)** Arrows indicate specific hybridization of the cat *IgL* Cλ gene to Chr D3 in a normal cat prometaphase spread. **(B)** A partial metaphase showing symmetrical twin spots on both homologs of cat Chr D3. **(C)** Ideogram of the cat Chr D3. Bar indicates the *IGL @* locus.

this may play an important role in lymphoid tumorigenesis [6,7]. The *IGL @* locus is known to be involved in the t(8;22) chromosome translocation in a variant form of Burkitt's lymphoma in which the c-*MYC* gene is juxtaposed to the upstream region of Cλ gene segment of the *IGL @*, thereby activating the oncogene [8]. Furthermore, the locus is involved in a case of the t(3;22) chromosome translocation in B-cell non-Hodgkins lymphoma in which the *BCL6* gene is translocated to the Jλ2 gene segment, causing promotor substitution [9]. Thus, it is of interest to investigate whether any particular chromosome translocations involve Ig light chain genes in cat lymphomas and leukemias because the cat is being used as a model for human diseases.

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SINEVA polymorphism and mapping of the bovine pregnancy-associated glycoprotein 1 gene

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Species: Bovine
Locus Name: Pregnancy-associated glycoprotein-1
Locus symbol: *PAG1B*
Map position: *PAG1B* maps on BTA29 (Texas nomenclature, [1]), linked to the anonymous markers *ABS14* [2,3] (Z: 7.03, θ: 0.24), *DIK94* [3,4] (Z: 12.56, θ: 0.21), *RM40* [3] (Z: 3.18, θ: 0.17), and *ILSTS081* [3] (Z: 4.11, θ: 0.30) and to *IGF2* (Z: 3.17, θ: 0.19). The probable marker order is cen–*DIK94-RM40-PAG1B-ILSTS081*–tel.
Method of mapping: A total of 174 animals representing seven informative families of the International Bovine Reference Panel (IBRP) were genotyped for linkage analysis. Genotypes were merged with the cattle Genotypic Database, and all possible pairwise comparisons were performed with CRI-MAP (v2.4 SunOs).

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Somatic cell hybrid analysis was performed with a well-characterized panel of 38 hamster–bovine hybrid clones [5].
Database deposit information: GenBank accession number: L27833 [6].
PCR primer sequences: Forward: 5′ - TTTCTCTCTGTCCCCA-CTGC-3′ (SINE region). Reverse: 5′-AGCCTTTCCTCCA-CTTCACC-3′.
Method of detection: The GenBank and EMBL database were searched for sequences homologous to several *Bos taurus* SINEs (Short Interspersed Nuclear Elements). The 5′ flanking sequence of the bovine pregnancy-associated glycoprotein 1 gene showed 85% identity in a 167-bp overlap to the SINE sequence Bov-B (accession no. X64125; [7]). An A/T rich tract was found at the 3′ end of the overlapping region, showing the $(T)_7GGT(A)_{21}$ repeat motif. PCR primers were designed on the basis of the flanking sequence. The amplified fragment showed the expected size (249 bp), and PCR products varied in length in denaturing polyacrylamide gel electrophoresis. Mendelian inheritance of six alleles was observed in the IBRP families.

Furthermore, analysis of the bovine-hamster hybrid cell panel with these *PAG1B* primers confirmed the assignment of this gene to bovine Chr 29 with a significant correlation coefficient of 0.9 (according to Chevalet and Corpet, [8]).
Previously identified homolog: To our knowledge, this work is the first report of the assignment of *PAG1B* or any other pregnancy-specific protein gene.
Discussion: Bovine pregnancy-associated glycoprotein 1 (also called pregnancy-specific protein B) is a member of the aspartic proteinase family secreted by the chorionic epithelium of the placenta and suggested to be enzymatically inactive [9]. Bovine *PAG1B* can be detected in maternal blood soon after implantation until term. Because of their relative abundance and localized expression, it is possible that these glycoproteins have a function during the pregnancy, but that function remains unclear.

The *PAG1B* polymorphism described in this paper could be considered as a SINEVA (SINE variable poly(A) [10,11]). Even though the bovine genome seems to present a minor amount of SINEVA polymorphism compared with other human and domestic species [11] and di- and trinucleotide repeats appear the most frequent SINE-associated polymorphism, with a database search, we have found several of these polymorphisms (unpublished data). Because of their presence near or inside gene sequences and their high degree of polymorphism, these SINEVA markers can be used for linkage mapping of conserved regions. The polymorphism reported in this work has allowed us to map the *PAG1B* gene, showing that the SINEVA analysis is a useful mapping tool in the bovine genome.

Our data show linkage between *PAG1B* and *IGF2* on BTA29, and it corresponds to part of HSA11 [12–14], where *IGF2* is located (Genome Data Base). Since this is the first time that a *PAG1B* gene has been mapped in any species, we confirmed the assignment using two different mapping strategies, linkage and somatic cell hybrid analysis. We expect that this gene would appear on human Chr 11.

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Human class III POU genes, POU3F1 and POU3F3, map to Chromosomes 1p34.1 and 3p14.2

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Species: Human
Locus name: class III POU transcription factor
Locus symbol: POU3F1 and POU3F3
Map positions: 1p34.1 (POU3F1) and 3p14.2 (POU3F3)
Method of mapping: Fluorescence in situ hybridization (FISH) [1]
Molecular reagents: A whole phage DNA containing the human genomic sequence was used as a probe for POU3F1 assignment, and a 2.2-kbp human DNA fragment containing the carboxyl-terminal coding and the 3′ flanking regions was used for POU3F3 assignment to reduce nonspecific signals.
Discussion: The vertebrate class III POU transcription factors consist of four members, POU3F1 (Scip/Oct-6/Tst-1), POU3F2 (Brain-2/N-Oct3), POU3F3 (Brain-1), and POU3F4 (Brain-4). The chromosomal location of the four murine class III POU genes has been determined by interspecific backcross analysis [2,3]. On the basis of mouse–human chromosomal homologies, human POU3F1 and POU3F3 are expected to map to 1p and 2q, respectively. The location of POU3F1 was consistent with this prediction, mapping to Chromosome (Chr) 1p34.1. Contrary to the prediction, however, POU3F3 was mapped to Chr 3p14.2. Chr 3p14.2 is a translocational breakpoint associated with renal cell carcinoma [4,5]. The fragile histidine triad gene, alteration of which causes lung, head, and neck cancers, has been identified at 3p14.2 [6,7]. Neither genes nor diseases have so far been identified at 1p34.1, but most mice homozygous for the mutant POU3F1 allele die soon after birth [8,9].

The human POU3F2 and POU3F4 genes have already been mapped to Chr 6q16 and Xq21.1, respectively [10,11]. Therefore, all four of the human class III POU genes map to chromosomal locations that are different from one other. A phylogenetic tree of the class III POU genes constructed with the POU domain sequences shows that the POU3F1, POU3F2, POU3F3, and POU3F4 genes have emerged in a common ancestor of vertebrates [12]. Recent studies on the genome structure of vertebrates have suggested that genome duplication has occurred at least twice in the early stage of vertebrate evolution; four homologous complexes such as Hox and MHC are interspersed in the mammalian gene [13–15]. The present data showing interspersed chromosomal locations of the four human class III POU genes are consistent with this idea.

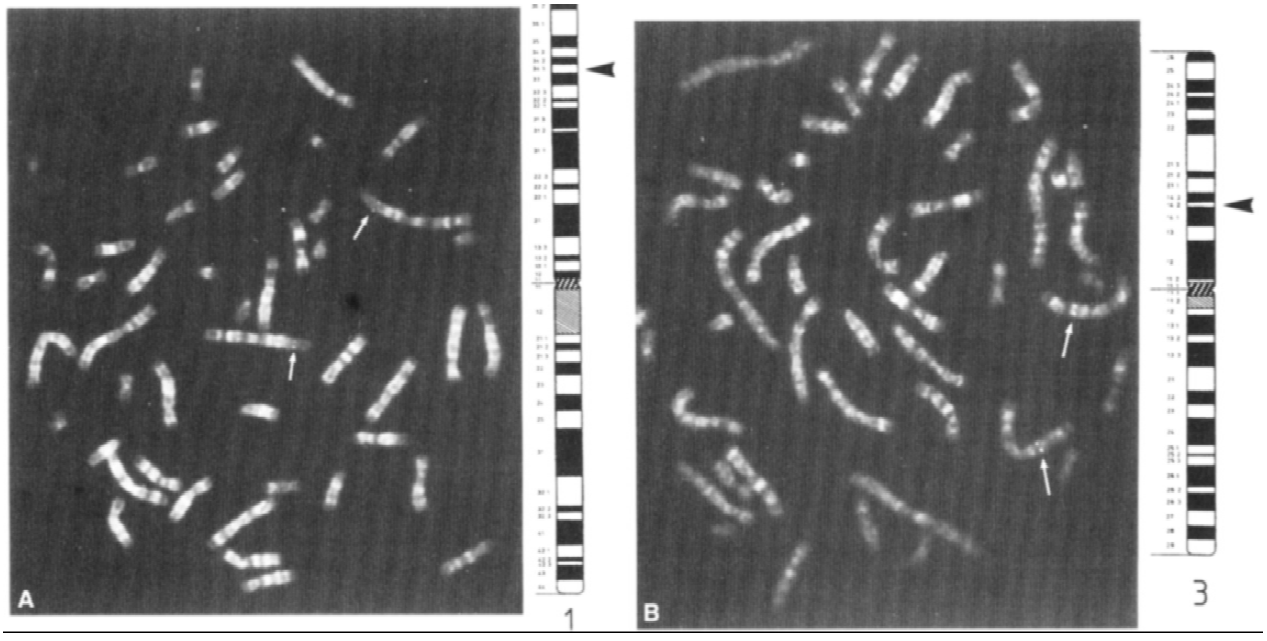

Fig. 1. In situ hybridization of the human POU3F1 (a) and POU3F3 (b) genes to 1p34.1 and 3p14.2, respectively. Hybridization signals were detected with HNPP on Q-banded chromosomes [1].

The mammalian genome is made up of a mosaic of very long (more than 200 kilobases) DNA segments called isochores with different GC contents [16]. In each isochore the GC content is fairly homogeneous [17], and GC-rich genes are located mainly on special subsets of R-positive (G-negative) chromosomal bands in mammals, whereas AT-rich genes are located on G-positive bands [18]. The human POU3F1, POU3F2, and POU3F3 genes have higher GC contents, whereas the human POU3F4 gene has a lower GC contents [12]. Therefore, it is expected that the human POU3F1, POU3F2, and POU3F3 genes are located on G-negative bands, and the POU3F4 gene on a G-positive band. The chromosomal assignment of the POU3F1, POU3F3, and POU3F4 genes is consistent with this expectation. As 6q16 is divided into three subregions, G-positive 16.1, G-negative 16.2, and G-positive 16.3, fine mapping remains to be done for POU3F2.

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