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A genetic map of bovine Chromosome 7 with an interspecific hybrid backcross panel

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Abstract. A genetic linkage map of bovine Chromosome (Chr) 7 was generated with a *Bos taurus* × *Bos gaurus* interspecific hybrid backcross panel. This study included six previously mapped microsatellites and five unmapped expressed genes that were identified by PCR-based restriction fragment length variants (RFLVs). The gene order (from centromere to telomere) and the map distances (in centimorgans) are as follows: cen–*BM2607*–11.2–*LDLR*–3.6–*AMH,CSF2*–11.2–*BP41*–19–*BM6117*–19–*SPARC*–14.4–*FGFA*–15.5–*BM1853*–11.2–*RASA*–18.8–*ILSTS006*. Previous comparative synteny mapping demonstrated that bovine Chr 7 shares homologous regions with both HSA5q and HSA19p. A break or fusion between *AMH* and *CSF2* in an ancestral chromosome is suggested to account for the current arrangement of these homologous segments in the human and bovine genomes. In this study, we demonstrate that a short proximal portion of BTA7 is homologous with HSA19p, while a larger distal portion of BTA7 is homologous with human Chr 5q. The orientation of these conserved human segments on BTA7 is also demonstrated. Our data show that the linear order of genes has not been conserved within the homologous region of HSA5 and BTA7, and one chromosomal translocation or inversion is proposed to account for this difference.

Introduction

A comprehensive map of the bovine genome is becoming an increasingly valuable tool for the study of mammalian chromosomal evolution as well as for mapping economically important traits and marker-assisted selection. Bovine gene mapping has been facilitated by comparative gene mapping, that is, mapping cattle homologs of genes that have been mapped in humans and mice in order to identify regions of syntenic conservation. Unfortunately, comparative gene mapping involving cattle has had to rely almost entirely on somatic cell genetics and synteny mapping that does not address the conservation of gene order. Answers to questions of whether the gene order within conserved synteny is conserved or disrupted and how homology segments are rearranged among different species requires the establishment of an ordered map rich in expressed coding genes (type I markers). Abundant polymorphism and relative ease of developing and genotyping have made microsatellites the markers of choice for linkage mapping, and consequently two microsatellite-based genetic linkage maps of the bovine genome have been published (Barendse et al. 1994; Bishop et al. 1994). These maps provide a valuable foundation for mapping of economically important traits. They are not powerful tools for comparative mapping, however. Only the Barendse and associates (1994) map includes sufficient numbers of type I markers for comparative mapping with other mammals, and it is lacking type I markers on most chromosomes. It does contain enough, however, to demonstrate that conservation of gene order between humans and cattle is not necessarily conserved within the extensive segments of conserved synteny.

Lack of genetic variation within the species is a major obstacle for incorporating type I loci into the bovine linkage map. A *Bos taurus* × *Bos gaurus* interspecific hybrid backcross panel has been developed in our laboratory to map loci that are monomorphic in *Bos taurus* (Riggs et al. submitted). The interspecific hybrid approach has been used successfully by mouse geneticist since the late 1970s (Bonhomme et al. 1979). Although male mammals are usually sterile in such wide crosses (Haldane's rule), the females are maximally heterozygous and extremely valuable for backcrossing and linkage analysis (Copeland et al. 1993; Avner et al. 1988). *Bos taurus* × *Bos gaurus* produces a true interspecific hybrid with fertile females and sterile males. Whereas *Bos taurus* × *Bos indicus* crosses are common in the animal breeding world, they are probably inappropriately classified as different species and certainly do not demonstrate the extent of genetic variation found between *taurus* (or *indicus*) and *gaurus*.

Previous comparative mapping has shown that genes mapped to BTA7 are distributed over two human chromosomes, HSA5q and HSA19p (O'Brien et al. 1993). Eleven loci, including five unmapped type I loci *AMH, LDLR, FGFA, SPARC, CSF2* and six previously mapped microsatellite loci *BM2607, BP41, BM6117, BM1853, ILSTS006, RASA* were used to generate a linkage map of bovine Chr 7 from our interspecific hybrid backcross. RASA is also a type I locus, although it contains a microsatellite in the noncoding region that facilitated its incorporation into previous linkage maps (Eggen et al. 1992). The five unmapped type I loci were chosen to represent the two homologous human chromosomes with the expectation that the orientation of the two human chromosome segments relative to BTA7 could be determined.

Materials and methods

Interspecific hybrid backcross family (IHB). The F_1 interspecific cattle × gaur hybrid female, produced by mating a gaur bull to a Holstein cow, was backcrossed to an Augus bull. Twenty-eight backcross fetuses from this mating were recovered from recipient cows at approximately 30 days after fertilization. DNAs were extracted from fibroblast cultures of the recovered fetuses as well as from primary fetal tissue and placenta (Riggs et al. submitted).

Primer design. With the computer program "Mac Vector 4.1.4", primers for type I loci were designed from published bovine sequences with the exception of CSF2, which was from a porcine sequence and synthesized in the Department of Veterinary Pathobiology, Texas A&M University. The primers were designed to amplify noncoding regions of the loci to facilitate the detection of DNA variation. Table 1 lists the primers and restriction enzymes that were used to detect interspecific variation and PCR condi-

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Table 1. Primer sequences, PCR conditions, and restriction enzymes that detect RFLVs of five type I loci on bovine Chr 7.

Locus	Primer sequences	Annealing temperature (°C)	Product size (bp)	Restriction enzyme	References
AMH	5′-acttgaccaccttcgcagtgtg-3′ 5′-tggaacctcagcaagggtgttg-3′	60.7	760	*Msp*I	Cate et al. 1986
LDLR	5′-tcactcaaaggcagcaagaacac-3′ 5′-catcttcacgcacaaagtaaggg-3′	58	718	*Cfo*I	Hobbs et al. 1985
FGFA	5′-aaacgggcggagagagacctac-3′ 5′-aacagcttgcaggctgaggg-3′	60.4	315	*Taq*I	Philippe et al. 1992
SPARC	5′-atgtagaatccgcctgcctacc-3′ 5′-cttccaagcctttcctcctctc-3′	57.7	716	*Hae*III	Young et al. 1989
CSF2	5′-tggcagcatgtggatgccatc-3′ 5′-tgatagactgggtttcacaggaag-3′	58.7	~2500	*Taq*I	Inumaru and Takamatsu, 1995

tions used for typing the five type I loci. Primers for the type II loci are published elsewhere and not included in this table.

PCR-based RFLVs. The PCR was performed in 10-μl reactions containing 1.5 mM magnesium chloride, 0.2 μM dATP, dGTP, dCTP, dTTP, 0.5 u of Taq DNA polymerase (Boehringer Mannheim) for 1 cycle at 94°C for 3 min followed by 40 cycles at 94°C for 30 s, 55–60°C for 30 s, 72°C for 50 s-2 min, and one cycle of extension at 72°C for 10 min. Since the product size for CSF2 was larger than 2 kb, expand™ high fidelity Taq DNA polymerase was used in the PCR reaction according to the manufacturer's instructions. PCR products were digested with 2–4 u of several restriction enzymes and incubated at appropriate temperature for 2 or more hours. The samples were then subjected to electrophoresis in 3% agarose gel. We use the term restriction fragment length variant (RFLV) to distinguish these interspecific variants from restriction fragment length polymorphism (RFLP) within a species.

Microsatellite typing. The microsatellites were selected from existing linkage maps to insure complete coverage of Chr 7 with our map and to facilitate integration of the IHB map with the rapidly expanding linkage maps of microsatellite loci. The PCR conditions for microsatellites were the same as those for PCR-RFLVs except that either forward or backward primers were labeled with 1.0 μCi of γ-^{33}P (2000 Ci/mmol). The PCR products were mixed with an equal volume of loading dye, denatured by heating to 95°C for 3 min, and chilled on ice for 2 min. The DNA fragments were separated on 6% denaturing Long Ranger gel (FMC Rockland, Me., USA), and run at 85 watts for 2–3 h. The gels were vacuum dried, and the variants were visualized by overnight exposure.

Linkage analysis. Linkage analysis was based entirely on segregation of alleles in the highly heterozygous hybrid female. Since the phase of all allelic combinations is known, recombination was calculated directly as the number of recombinants over total offspring. A computer program "Map Manager" version 2.6 was used to identify the haplotype distribution among backcross offspring and to determine gene order by minimizing multiple crossing-over.

Results

The F_1 hybrid female was heterozygous at all 11 loci tested in this study. Figure 1 shows the allelic transmission of five RFLVs in part of the interspecific family. Data for the six microsatellites are not shown. The pairwise LOD scores among the eleven loci tested are shown in Table 2. Fig. 2 shows the haplotypes of the 11 loci among 28 offspring. In establishing order of loci on the map, loci were placed to minimize double and triple crossovers (one of each). *AMH* and *CSF2* did not recombine in the 28 meioses observed. Kosambi's mapping function was used to convert the recombination frequency into map distance in centimorgans (CM). The gene order (from centromere to telomere) and the recombination distance were as follows: cen–*BM2607*–11.2–*LDLR*–3.6–*AMH,CSF2*–11.2–*BP41*–19–*BM6117*–19–*SPARC*–14.4–*FGFA*–15.5–*BM1853*–11.2–*RASA*–18.8–*ILSTS006*. The total length of the BTA7 map established in this study is 124 cM, with average marker interval of 11.2 cM.

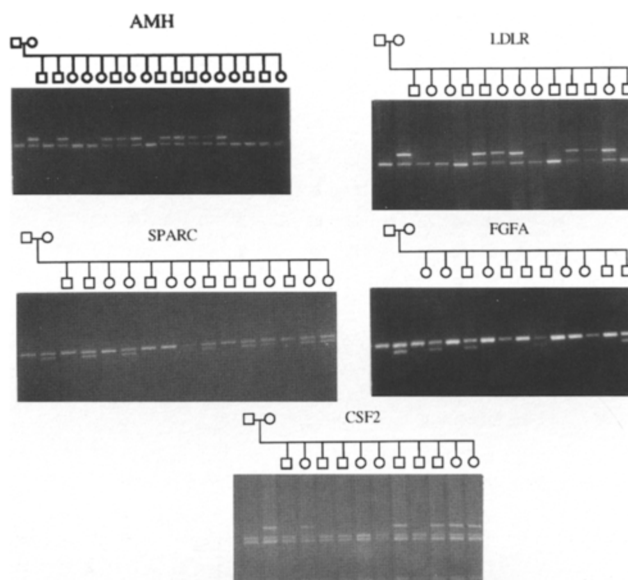

Fig. 1. Agarose gel electrophoresis showing the allele segregation of PCR-based RFLVs at five loci in part of the *Bos taurus* × *Bos gaurus* interspecific hybrid backcross panel.

Discussion

The loci *AMH, LDLR, FGFA, SPARC,* and *CSF2* were previously assigned to bovine Chr 7 (syntenic group 22) by somatic cell genetics (Zhang and Womack 1992; Rogers et al. 1991; Dietz et al. 1992). The microsatellites *BM2607, BP41, RASA, BM1853, ILSTS006* were genetically mapped to bovine Chr 7 in one of the two comprehensive linkage maps published to date (Barendse et al. 1994; Bishop et al. 1994). In the present study, the 11 loci were incorporated into a common linkage map of bovine Chr 7 with the *Bos taurus* × *Bos gaurus* interspecific hybrid backcross family.

The *Bos taurus* × *Bos gaurus* interspecific hybrid backcross is the first interspecific backcross panel established in cattle specifically for linkage analysis. Although it presently consists of only 28 backcross offspring, it effectively ordered all loci included in this study with the exception of *AMH–CSF2*. The genetic map constructed by studying this family is necessarily a female-specific map that measures recombination in oogenesis. The genetic map of BTA7 constructed from the IHB has a length of 124 cM, only slightly shorter than the Bishop and colleagues map (Fig. 3), which is approximately 128 cM. Comparison of these two maps revealed a consistent linear order of loci, but the distances between adjacent loci varied to some extent, possibly owing to the small sample size

Table 2. LOD score of adjacent loci on bovine Chr 7.

	0.11	0.12	0.13	0.14	0.15	0.16	0.17	0.18	0.19	0.20
BM2607–LDLR	4.288	4.278	4.259	4.230	4.193	4.148	4.097	4.040	3.977	3.909
LDLR–AMH	6.104	6.009	5.910	5.806	5.699	5.589	5.474	5.357	5.237	5.113
AMH–CSF2	7.012	6.874	6.735	6.595	6.453	6.309	6.163	6.016	5.866	5.715
CSF2–BP41	4.288	4.278	4.259	4.230	4.193	4.148	4.097	4.040	3.977	3.909
BP41–BM6117	2.472	2.548	2.607	2.653	2.686	2.708	2.720	2.723	2.718	2.705
BM6117–SPARC	2.472	2.548	2.607	2.653	2.686	2.708	2.720	2.723	2.728	2.705
SPARC–FGFA	3.380	3.413	3.433	3.441	3.439	3.428	3.409	3.381	3.347	3.307
FGFA–BM1853	3.380	3.413	3.433	3.441	3.439	3.428	3.409	3.381	3.347	3.307
BM1853–RASA	4.288	4.278	4.259	4.230	4.193	4.148	4.097	4.040	3.977	3.909
RASA–ILSTS006	2.472	2.548	2.607	2.653	2.686	2.708	2.720	2.723	2.718	2.705

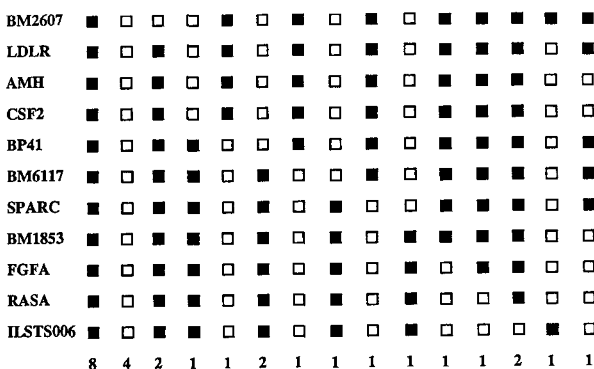

Fig. 2. Haplotype distribution of 28 interspecific backcross offspring for 11 loci on BTA7. Solid squares represent cattle alleles, open squares gaur alleles. The numbers at the bottom of each column indicate the total number of offspring inheriting each haplotype.

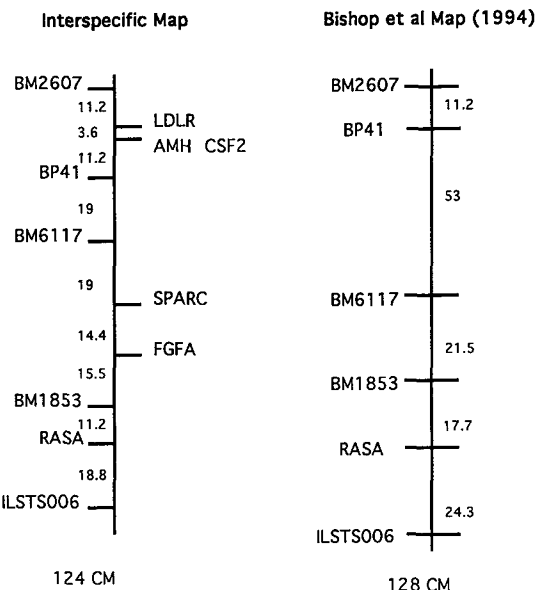

Fig. 3. Comparison of linkage maps of bovine Chr 7 from interspecific hybrid backcross panel with Bishop and coworkers (1994) linkage map of microsatellites.

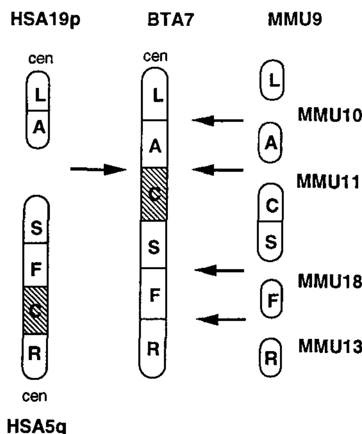

Fig. 4. A schematic diagram of the conservation and rearrangement of loci in cattle, humans, and mice. Putative break point and the location of insertion are indicated. L, A, C, F and R represent the genes *LDLR, AMH, CSF2, FGFA,* and *RASA*.

in the IHB. The incorporation of microsatellites into the IHB linkage analysis serves to integrate this map with high-density reference maps constructed from within species crosses (Barendse et al. 1994; Bishop et al. 1994).

Homologs of the type I loci (including *RASA*) were either physically or genetically mapped to human and mouse chromosomes. Establishing the order of these loci in cattle allowed further examination of linkage relationships across human, cattle, and mouse. The genes used in this study were distributed over two segments of human chromosomes, HSA5q and HSA19p (O'Brien et al. 1993). *LDLR, AMH* are located on HSA19p, and *FGFA, SPARC, CSF2, RASA* are on HSA5q. The gene order of cen–*LDLR–AMH– CSF2–SPARC–FGFA–RASA* in cattle suggests that a break could have occurred between *AMH* and *CSF2* in an ancestral chromosome during the divergence of the human and cattle genomes. Further comparison of the linear order of these genes across human and cattle demonstrates that a small proximal portion of cattle Chr 7 is homologous with human Chr 19p with centromeres in a common orientation, while a larger distal portion of cattle Chr 7 is homologous with human Chr 5q with the centromeres in a reverse orientation (Fig. 4). On HSA19, *LDLR* and *AMH* were mapped to different subbanding regions in the order cen–*LDLR–AMH* (Ropers et al. 1992). Thus, the order of *LDLR, AMH* and their relative orientation to a centromere is conserved between HSA19p and BTA7. *SPARC* and *FGFA* were assigned to HSA5q31–33 by in situ hybridization, whereas both *CSF2* and *RASA* were assigned to separate bands, HSA5q23 and HSA5q13, respectively. Radiation hybrid and in situ hybridization mapping of HSA5 suggests the most likely order of genes as cen–*RASA–CSF2–FGFA–SPARC* (Thangavelu et al. 1992; Warrington et al. 1992; Warrington and Bengtsson 1994; Saltman et al. 1993). The gene order is apparently not conserved within the homologous region of HSA5q and BTA7. A nonreciprocal translocation or a simple inversion is proposed to account for the disruption of gene order (Fig. 4). Since *CSF2* was the only gene involved in the rearrangement of order in this study, the inversion hypothesis needs to be confirmed by mapping more loci around *CSF2* in cattle Chr 7. The inversion suggested for the different gene order between human and cattle need not exist between mouse and cattle, since *CSF2* and *SPARC* are conserved on mouse Chr 11. The other four genes, *LDLR, AMH, FGFA,* and *RASA,* are each located on different chromosomes, MMU9, MMU10, MMU18 and MMU13,

respectively (Justice et al. 1992; Seldin et al. 1991; Johnson and Davisson 1992; King et al. 1991), requiring multiple breaks to explain the organization of cattle and mice genomes relative to a common ancestor. These data support the previous demonstration that chromosomal conservation between cattle and human is more extensive than that of either of them with mice (Womack 1988, 1993).

In summary, a genetic linkage map of BTA7 was generated with an interspecific hybrid backcross family. This map is composed of five type I loci and six type II loci. Comparison of gene organization in cattle and human requires a breakpoint between *AMH* and *CSF2*, and a nonreciprocal translocation or a chromosomal inversion is proposed to explain the disruption of gene order. These results show the importance of including type I loci into comparative linkage maps in order to explore the evolutionary events underlying mammalian genome organization and to make full use of the wealth of information generated by the human and mouse genome project.

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