

Physical localization and order of genes in the class I region of the bovine MHC

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Summary

Fluorescence *in situ* hybridization (FISH) analyses were used to order 16 bacterial artificial chromosomes (BAC) clones containing loci from the bovine lymphocyte antigen (BoLA) class I and III regions of bovine chromosome 23 (BTA23). Fourteen of these BACs were assigned to chromosomal band locations of mitotic and pachytene chromosomes by single- and dual-colour FISH. Dual-colour FISH confirmed that class II *DYA* is proximal to and separated from BoLA class I genes by approximately three chromosome bands. The FISH results showed that tumour necrosis factor α (TNFA), heat shock protein 70 (*HSP70.1*) and 21 steroid dehydrogenase (*CYP21*) are closely linked in the region of BTA23 band 22 along with BoLA class I genes, and that male enhanced antigen (*MEA*) mapped between *DYA* and the *CYP21/TNFA/HSP70.1* gene region. All BAC clones containing BoLA class I genes mapped distal to *CYP21/TNFA/HSP70.1* and centromeric to prolactin (*PRL*). Myelin oligodendrocyte glycoprotein (*MOG*) was shown to be imbedded within the BoLA class I gene cluster. The cytogenetic data confirmed that the disrupted distribution of BoLA genes is most likely the result of a single large chromosomal inversion. Similar FISH results were obtained when BoLA *DYA* and class I BAC clones were mapped to discrete chromosomal locations on the BTA homologue in white-tailed deer, suggesting that this chromosomal inversion predates divergence of the advanced ruminant families from a common ancestor.

Keywords bovine, MHC, BoLA, gene mapping.

Introduction

The general organizational features of the bovine lymphocyte antigen (BoLA) complex on BTA23 has been determined by analysis of somatic cell hybrids, radiation hybrids, linkage, and fluorescent *in situ* hybridization (FISH) (Leveziel & Hines 1984; Andersson *et al.* 1988; Skow *et al.* 1988, 1996; Band *et al.* 1998, 2000). However, a high-resolution map showing the arrangement of genes in the *BoLA* class I region

has yet to be constructed based on contiguous large insert clones. This study used single- and dual-coloured FISH to describe the distribution of recombinant bacterial artificial chromosomes (BACs) containing known *BoLA* genes and to construct a high resolution molecular map of the *BoLA* class I region. In the process of mapping genes of the class I region by FISH, we confirmed the locations and order of several additional genes on BTA23. For comparative purposes, FISH analysis was also performed using the BoLA BACs hybridized to chromosomes of white-tail deer.

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Accepted for publication 17 May 2001

Materials and methods

Recombinant clones containing BoLA genes were identified by polymerase chain reaction (PCR) (Table 1) in a bovine BAC library constructed in the vector pBeLO BACII

Table 1 Polymerase chain reaction primers and conditions used to screen the bovine BAC library for class I sequences and seven other BTA23 loci used in this study.

Locus symbol	BAC clone	Size (kbp)	Primers	Primer sequence	Annealing temperature	Fragment size (bp)
BoLA DYA	27	126	DY α U DY α L1	5' CTCGAATTCGGCACTTACGGCACAAATG 3' – 28 mer 5' CAGGGCGCTCTGAACTTCAA 3' – 21 mer	58	174
MEA	546	68	Bovmeal-For Bovmeal-Rev	5' GGGATGGAGAAGATGTAGTTGCTG 3' – 24 mer 5' GCTCGGATCCAACCTCCTCTGAAG 3' – 24 mer	56	700
CYP21	124	94	21OH #1	5' GGTTACAGTCCATGAGTTTGCAAAG 3' – 25 mer	57	204
	138	131	21OH #2	5' GCTCGGATCCAACCTCCTCTGAAG 3' – 25 mer		
HSP 70-1	121	94	Hsp70-1 up Hsp70-1 down	5' GCGCGCTTTGGGGCTCAGGGC 3' – 21 mer 5' TAGCTTGAGTTTGGCATGAAT 3' – 21 mer	57	305
TNF	283	161	TNF For TNF Rev	5' GAAGAGGTGAGTTTCTGGCCGGC 3' – 23 mer 5' CCACCTGGGGACTGCTGGGGAGA 3' – 23 mer	61	590
BoLA I	76	149	BUCON	5' GCTACGTGGACGACACGCA 3' – 19 mer	57	700
	110	80	BLCON	5' CCTTCCGTTCTCCAGGTATCT 3–22 mer		
	126	70				
	159	75				
	171	204				
	187	115				
	235	112				
	296	105				
	336	60				
	361	40				
MOG	137	140	Mog U Mog L	5' ATCTCCAGGAAAGAACGCT 3' – 19 mer 5' CACATTCGGATCCTGAGGG 3' – 20 mer	52	188
PRL	287	65	PRL-F PRL-R	5' CTGTGTGGTCAGGACTCCCTGTC 3' – 23 mer 5' GAAACCCATTAGACCAAGCATGC 3' – 24 mer	58	268

(Shizuya *et al.* 1992) from DNA of an Angus bull (Cai *et al.* 1995). The DNA was isolated by a standard alkaline lysis protocol (Birnboim & Doly 1979) as modified by Qiagen. Terminal sequences of each BAC clone were end-rescued by inverse PCR performed on *EcoRI* digested and self-ligated BAC DNAs as described by Cai *et al.* (1995) except that rescue of right end sequences was modified using the primers:

REPCR2 (5'GCGGATAACAATTTTCACAGG3')

RIPCR1 (5'CAC CGT CTT TCA TTG CCA TAC3')

at an annealing temperature of 50 °C. Ends of each insert were sequenced by dideoxy methods (Sanger *et al.* 1977) and unique PCR primers were developed for each end-sequence of each insert to order overlapping BACs prior to FISH analysis. Chromosomal FISH followed standard protocols (Pinkel *et al.* 1986) as previously described (Gallagher *et al.* 1998). DNA from BAC clones was used in single- and dual-colour FISH to mitotic and pachytene chromosomes. The BAC clones were first assigned to chromosome bands by single-colour FISH, then dual-colour FISH was used to establish locus order when possible. After detection of fluorescence from the DNA probes, FISH preparations were

mounted in antifade solution containing the fluorescent dye Hoechst 33258. The FITC (green), Cy3 (red) and FITC/CY3 (yellow) probe-signals relative to the Hoechst 33258 counterstain were photographed on colour print film using an epifluorescence microscope equipped for simultaneous visualization of probe and counterstain fluorescence. Bovine chromosomes were identified according to domestic cattle standard nomenclature (Reading Conference 1980; ISCNDA 1990) as modified by Popescu *et al.* (1996).

Results and discussion

We isolated 16 recombinant BACs containing DNA sequences from *BoLA* or other genes on BTA23 (Table 1). End-specific PCR permitted the identification of overlapping clones and alignment of 13 BACs into three contiguous segments (Fig. 2). Using mitotic chromosome preparations, we mapped 14 BAC clones by single-colour FISH to precise band locations on BTA23. The BAC27 contains *DYA* sequences and mapped proximally at bands 13–14 (Fig. 1a) consistent with previous results (Skow *et al.* 1996). This BAC was used as a landmark probe in subsequent dual FISH analysis. Individual BACs containing Myelin

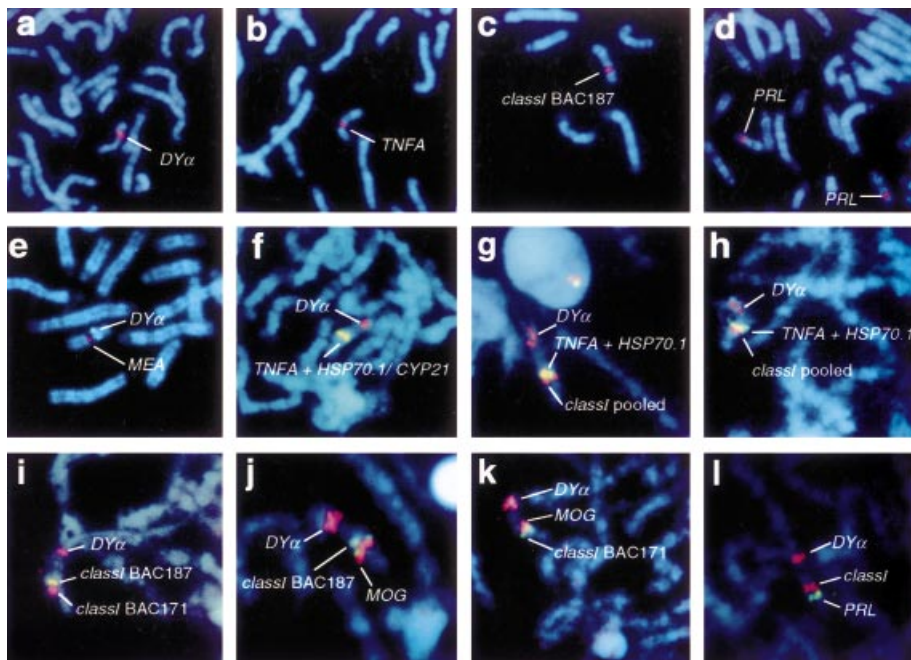


Figure 1 Single- and dual-colour FISH results for BTA23 BACs to mitotic chromosomes (a) *DYA* BAC27-Cy3, (b) *TNF* BAC283-Cy3, (c) class I BAC187-Cy3, (d) *PRL* BAC287-Cy3 and pachytene chromosomes, (e) *MEA* BAC546-Cy3, *DYA* BAC27-FITC, (f) *DYA* BAC27-Cy3, *TNF* BAC283 + *HSP70-1* BAC121-FITC, *CYP21* BAC138-Cy3, (g) *DYA* BAC27-Cy3, *TNF* BAC283 + *HSP70-1* BAC121-FITC, class I BAC126 + BAC171 + BAC187-Cy3, (h) *DYA* BAC27-Cy3, *TNF* BAC283 + *HSP70-1* BAC121-FITC, class I BAC76 + BAC110 + BAC159 + BAC235-Cy3, (i) *DYA* BAC27-Cy3, class I BAC187-FITC, class I BAC171-Cy3, (j) *DYA* BAC27-Cy3, class I BAC187-FITC, *MOG* BAC137-Cy3, (k) *DYA* BAC27-Cy3, *MOG* BAC137, class I BAC187-FITC and (l) *DYA* BAC27-Cy3, class I BAC159-Cy3, *PRL* BAC287-FITC. The DNA of BAC clones joined by '+' symbol were pooled, labelled and detected as a single probe. Red and green probe signals resulted from streptavidin-Cy3 and antidigoxigenin-FITC fluorescence detection of biotin and digoxigenin labelled probes, respectively. Yellow probe signal was caused by overlap in FITC and Cy3 fluorescence.

oligodendrocyte glycoprotein (*MOG*), *CYP21*, *HSP70.1*, and *TNFA* (Fig. 1b) and all BACs containing one or more *BoLA* class I sequences (Fig. 1c) mapped to band 22. The BAC287 containing *PRL* (Fig. 1d) mapped to band 23, telomeric to the *BoLA* class I genes. The BAC546 containing male enhanced antigen (*MEA*) (Fig. 1e) mapped to band 15, between *DYA* and the *BoLA* class I genes.

The ordering of *BoLA* BAC clones on pachytene chromosomes by dual-colour FISH using the *DYA* clone to anchor the centromeric end of BTA23 showed that *CYP21*, *HSP70.1* and *TNFA* co-localize on pachytene chromosomes (Fig. 1f), indicating that these genes are tightly linked. The region containing *TNFA* and *HSP70.1* mapped centromeric to *BoLA* class I BAC clones but overlap in probe signal seen as yellow fluorescence (Fig. 1g and h) suggests that *TNF* and *HSP70* are physically nearer the class I region than is *CYP21*. Co-localization of *CYP21*, *HSP70.1* and *TNFA* on pachytene chromosomes led us to conclude that all three genes are centromeric to the *BoLA* class I genes used in this study. Analysis of the overlapping BAC clones containing class I sequences demonstrated that the class I

clones identified in this study are distributed over a region of BTA23 large enough to be resolved by FISH analysis. The BAC187 mapped proximal to BAC171 and BAC126 (Fig. 1i), positioning the end of the class I region marked by BAC187 as centromeric to BAC126 and BAC171, which appear to mark the distal boundary of the class I region. The *MOG* mapped distal to BAC187 (Fig. 1j) and proximal to BAC171 (Fig. 1k); therefore *MOG* is imbedded among *BoLA* class I genes as has also been observed in humans and mice (Pham-Dinh *et al.* 1995). It is clearly evident from dual-colour FISH to pachytene chromosomes that *PRL* is distal to the *BoLA* class I genes (Fig. 1l), consistent with our mapping of all *BoLA* class I clones to band 23 by single-colour FISH. The order of loci determined by chromosomal FISH and by overlap analysis of BACs (Fig. 2) in this study is in agreement with the order of loci on the radiation hybrid (RH) map of BTA23 (Band *et al.* 1998). These results also indicate that the arrangement of genes in *BoLA* is very similar or identical to the arrangement of genes in the homologous regions of *HLA* (Shiina *et al.* 1999; The MHC Sequencing Consortium 1999).

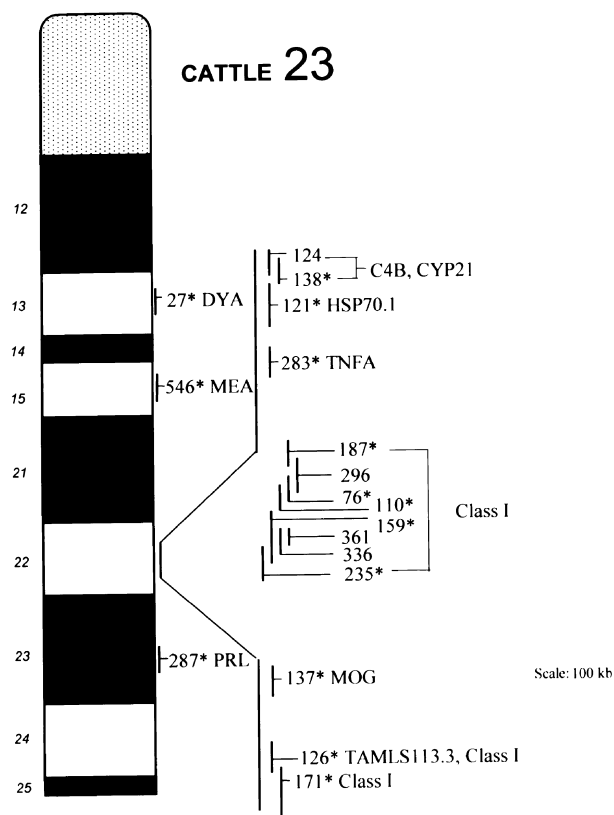


Figure 2 Arrangement of BACs on BTA23 as indicated by FISH analysis and end-overlap identification of contiguous BAC clones for the *BoLA* class I and III regions. The BAC clones marked with '*' were used for chromosome localization by FISH analysis.

Thus, chromosomal position may be useful as an additional parameter for investigating function and evolution of these genes.

Mapping performed by linkage analysis (Andersson *et al.* 1988; Stone & Muggli-Cockett 1993), radiation hybrid analysis (Band *et al.* 1998, 2000) and chromosomal FISH (Skow *et al.* 1996; Hess *et al.* 1999; these data) indicate that *BoLA* is subdivided into two discrete regions on BTA23. Comparative analysis of the gene order in *BoLA* with the MHC of mouse and man, which occur as tightly linked gene clusters, revealed that the disruption of *BoLA* likely was caused by a single large chromosomal inversion (Band *et al.* 1998). Conservation of chromosome banding patterns for BTA23 and its homologues among the advanced pecoran families Bovidae, Cervidae, Giraffidae and Antilocapridae (Gallagher *et al.* 1994) predicts that the chromosomal inversion that resulted in subdivision of *BoLA* predates the divergence of these families from a common ancestor. As demonstrated in cattle, FISH of *BoLA* DYA and class I BAC clones confirmed that these genes map to discrete chromosomal locations on the homologue

of BTA23 in white-tailed deer (data not shown). This supports the evidence that chromosome band homologues identified among and within the artiodactyl families of ruminants are indicative of gene order conservation, and that the organization of the cattle MHC is an ancestral condition for the advanced pecorans.

Acknowledgements

This research was supported by USDA-NRICGP grant number 97-35 205-5074 to L. C. S. and the Cattleman's Beef Association and by USDA NRICGP grants number 9 437 205-12 224 and number 95-37 205-2273 to J. F. T. and S. K. D. We thank Sue Ann Berend for her technical help in collecting the meiotic material, and Dr Jim Womack and Dr Dave Stelly for providing access to their cytogenetic facilities.

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