

# Localization of bovine lymphocyte antigen (BoLA) *DYA* and class I loci to different regions of Chromosome 23

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The class II loci of the major histocompatibility complex (MHC) of humans (HLA) and mice (H-2) are embedded among other loci of the MHC, which spans approximately 4 mb of DNA and has a recombination length of about 2 cM (Campbell and Trowsdale 1993; Himmelbauer et al. 1993).

In contrast, recombination analysis of class II genes in the bovine lymphocyte antigen complex (BoLA) revealed two distinct clusters of class II loci about 17 cM apart (Anderson et al. 1988). One cluster designated IIa contains the bovine homologs of the human DQ and DR gene families and is tightly linked to other BoLA genes, including *HSP70*, *CYP21*, and several class I loci (Bishop et al. 1994). The second cluster, IIb, contains the *DYA*, *DYB*, *DOB* loci and a novel class II gene designated *DIB* (Stone and Muggli-Cockett 1990). The disruption of the BoLA class II loci into two clusters suggests that the BoLA complex has undergone significant rearrangements not yet observed in the MHCs of humans or mice, but that apparently have occurred in the MHCs of chickens (Miller et al. 1994) and swine (Smith et al. 1995). Alternatively, the bovine class IIa and IIb gene clusters could be in physical proximity but undergo frequent recombination owing to one or more recombination hotspots. Recombination hotspots have been reported in the HLA and H-2 regions (Shiroishi et al. 1990; Steinmetz et al. 1986; Yoshino et al. 1994), and anomalous recombination frequencies in this region among individual bulls have been reported (Park et al. 1995).

To test whether the BoLA IIb cluster mapped to a different chromosomal location than the other BoLA loci, we performed fluorescence in situ hybridization (FISH) analysis of bovine Chromosome (Chr) 23, using large insert clones containing *DYA* or class I sequences as fluorescent probes. In this report, we demonstrate that DNA probes containing bovine *DYA* sequences map to a different region of Chr 23 than does DNA containing class I sequences, consistent with the reported recombination distance of 17 cM between these genes.

Recombinant clones of bacterial artificial chromosomes (BACs) containing *DYA* or class I sequences were isolated by PCR screening of a bovine BAC library (Cai et al. 1995) with primer pairs that have been previously described (Van Eijk et al. 1992; Garber et al. 1994). Authenticity of each clone was confirmed by sequencing of the respective PCR products and comparing with published sequences.

BAC clones 6 and 76 were identified by PCR as containing *DYA* and class I sequences, respectively, and DNAs from these clones were prepared for FISH. The sequence of the PCR product from BAC 6 differed at only a single position, <sup>112</sup>A→<sup>112</sup>G, from the published sequence of *DYA* exon 2 (Van der Poel et al. 1990), as amplified by Van Eijk and colleagues (1993). The presence of

<sup>112</sup>G creates a recognition sequence for *HhaI* and therefore an additional polymorphism marking exon 2 of *DYA* (Skow 1996).

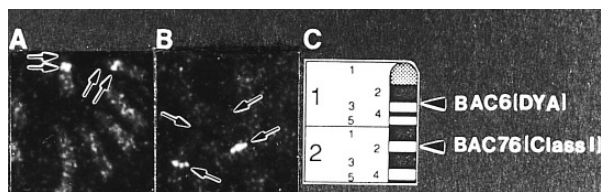
Sequence analysis (not presented) of the PCR products of BAC 76 identified at least three BoLA class I genes present in this clone including sequences that are >98% identical with published sequences for BSN, BS1b, and BSX (Garber et al. 1994).

Probe labeling, purification, hybridization, and detection were performed as previously described (Gallagher et al. 1993). One microgram of DNA (vector + insert) of each genomic clone was biotinylated and separately hybridized to preparations of cattle chromosomes harvested from fibroblast cultures (Gallagher and Womack 1992). Standard air-dried chromosomal preparations were aged for 2 days at 37°C prior to FISH. The probes were hybridized at 10 ng/μl and 37°C overnight. Cattle genomic DNA enriched for the highly repeated fraction (low Cot) were included in the hybridization mix to suppress repeat sequences present in the probes. Post-hybridization washes were in 50% formamide, 2 × SSC, and then 2 × SSC (40°C), followed by avidin-fluorescein chemical detection. QFH-banding and propidium iodide (PI) counterstaining of the cattle chromosomes were previously described (Gallagher and Womack 1992), and photographs of probe signal and PI counterstaining were taken on Kodak Gold Ultra 400 film. In turn, QFH-bands were visualized and chromosomes identified at the microscope. By shifting between visualization of probe signal and QFH-bands, each probe was mapped to a chromosomal band region. The QFH-band pattern of chromosomes showing probe hybridization was compared with chromosomes of a QFH-band karyotype arranged according to the Reading Conference (1980) GTG-band standard (Gallagher and Womack 1992). Idiograms of ISCND (1989) were used when assigning loci to chromosome bands.

DNA from both genomic clones demonstrated primary sites of hybridization detected as intense fluorescent signal on both chromatids (Fig. 1A, 1B). For each probe, we selected ten metaphase spreads for scoring based on the criterion that both chromatids of at least one chromosome were labeled. Six of ten spreads scored for BAC 6 (*DYA*) showed intense probe signal on both chromatids of each Chr 23 and seven of ten spreads probed with BAC76 (class I) DNA had both chromatids labeled on each Chr 23. QFH banding performed subsequent to FISH was sufficient for chromosome identification and localization of probe signal.

DNA from BAC6 (*DYA*) hybridized to BTA 23 at the boundary of bands 12–13. In contrast, BAC 76 (class I) DNA localized to BTA 23 band 22, the same band region to which BoLA and HSP70-2 had previously been localized (Fries et al. 1986; Gallagher et al. 1993; Iannuzzi et al. 1993). The chromosomal positions of BAC6 and 76 DNA are summarized on ISCND standard idiogram of Chr 23 (Figure 1C).

The positioning of BAC6 (*DYA*) and BAC76 (class I) DNA to bands 12–13 and 22 respectively reveals that the physical location



**Fig. 1.** Chromosomal localizations of bovine BAC clones 6 (DYA) and 76 (class I) to different regions of Chr 23. The partial metaphases showed hybridization of BAC 6 (A) and BAC 76 (B) to BTA 23. Arrows at the termini of chromosomes indicate the position of the BTA 23 centromere, whereas those positioned interstitially indicate sites of intense probe signal. It is evident that the DYA-containing BAC 6 maps nearer the centromere than does the BoLA class I containing BAC 76. Probe signal for both BAC clones is placed relative to the BTA 23 idiogram (C), with the DYA probe mapping to the boundary of bands 12–13, and the class I probe mapping to band 22.

of these sequences is consistent with observed recombination values. Bovine Chr 23 has an estimated recombination length of 66 cM (Bishop et al. 1994), and the physical positions of BAC6 and 76 are separated by approximately  $\frac{1}{3}$  of the length of Chr 23. If one assumes colinearity between the physical and recombination maps of BTA23, then the expected recombination distance between these loci would be approximately 22 cM, similar to the reported recombination frequency of 17cM (Andersson et al. 1988). Although recombination values in this region may vary more than twofold among different animals of the same sex (Park et al. 1995), the data presented here do not support the presence of a recombinational hotspot as contributing to this variation. Furthermore, this organizational feature of the bovine MHC is likely to occur in other artiodactyls, because similar FISH results have been observed in water buffalo (*Bubalus bubalis*) (Gallagher, unpublished), and homologs of genes in BoLA IIb have been identified in Cervidae, Giraffidae, Ovidae, as well as other Bovid species (Mann et al. 1993; Stone and Muggli-Cockett 1993; Wright et al. 1994).

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