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Identification of expressed bovine class I MHC genes at two loci and demonstration of physical linkage

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Abstract. A cDNA library prepared from lymphocytes of a cow (E98), homozygous at major histocompatibility complex (MHC) loci (BoLA phenotype w10, KN104), was screened with a bovine MHC class I probe. Of the cDNA clones isolated, two, (2.1 and 5.1) were selected and showed divergence at both 5′ and 3′ termini. E98 DNA was digested with rare-cutter enzymes (*Sfi* I, *Mlu* I, *Not* I, and *Cla* I) and fragments were size-separated by field inversion gel electrophoresis (FIGE). Hybridization with an entire class I cDNA probe revealed multiple fragments generated by each enzyme. When the 3′untranslated regions (UT) of 2.1 and 5.1 were used as probes, only one fragment was revealed in each digested sample, showing locus specificity of these probes in cattle. Further, DNA of transfected mouse fibroblasts L4 (expressing KN104) and L10 (expressing w10) hybridized to the 3′UT regions of clones 2.1 and 5.1, respectively. Northern blot analysis of the mRNA of the L4 and L10 transfected cells provided further evidence that the cDNA clones 2.1 and 5.1 code for the BoLA-KN104 and BoLA-w10 class I molecules respectively, and thus these represent the products of two different genes. A long range physical mapping of the *BoLA-w10* and *KN104* genes was performed using FIGE analysis of DNA of an homozygous and an heterozygous animal. This analysis revealed that the *BoLA-w10* and *KN104* genes are separated by not more than 210 kilobases (kb) and that they are components of a multigene family spanning 1550 kb. As the *w10* gene is at the *BoLA-A* locus we assign the *KN104* gene to a *B* locus.

Introduction

Class I major histocompatibility complex (MHC) antigens are highly polymorphic molecules involved in the presentation of foreign antigens to effector cells of the immune system (Klein 1975; Zinkernagel and Doherty 1974). In mouse (H-2) and man (HLA), class I antigens are coded by several genes which occupy several hundred kilobases (kb) of DNA (Steinmetz et al. 1986; Carroll et al. 1988). Although the number of classical class I loci is limited to six for the *H-2* complex (*H-2K1*, *K*, *D1*, *D2*, *D3*, and *L*) of the *Balb*/c mouse and four for the *HLA* complex (*HLA*, *A*, *B*, *C*, and *E*), 20 to 30 additional nonclassical sequences are found in the class I region. They correspond to genes expressed in a tissue-specific manner (*Qa* and *TL* in mice) or pseudogenes. Most of them are clustered telomeric to the *H-2D* or *HLA-A* genes (Steinmetz et al. 1986; Strivastava et al. 1987).

In cattle, the *BoLA* complex is currently defined as a single allelic series coding for 32 specificities, *w1*, *w2*, . . . *w32*, (Bull et al. 1988). In addition to these internationally-defined allospecificities, many laboratories have raised alloantisera which do not cluster with the international panel but which define local specificities. In the case of zebu (*Bos indicus*), the East African cattle, several locally-defined specificities have been characterized and are termed KN (Kenya Nairobi). Some of these are typical of African breeds (Kemp et al. 1988). In our laboratory, a panel of monoclonal antibodies directed against BoLA

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class I molecules has been developed. In addition to clustering with reagents defining allospecificities, some of these antibodies have proved valuable tools in defining class I antigens at the molecular level. Evidence was obtained that one haplotype could generate at least two distinct class I proteins (Bensaid et al. 1988; Kemp et al. 1990). Evidence that these molecules could be encoded by two different genes came from transfections of bovine DNA into mouse fibroblasts. The DNA donor animal, E98, was homozygous at the *MHC* by consanguinity and of BoLA type w10, KN104 (Kemp et al. 1988, 1990). Two clones of transfected murine L cells, L4 and L10, were obtained and bound monoclonal antibodies with reactivity in population and family studies correlating with the allospecificities w10 and KN104, respectively (Toye et al. 1990).

In order to gain further insights into the genomic organization of the *BoLA* class I region, and to demonstrate that the w10 and KN104 allospecificities are controlled by genes at two loci, class I cDNA clones were isolated from a library derived from peripheral blood lymphocytes of animal E98. After sequence analysis, the 3′ UT regions of two different clones were used as probes on genomic DNA of L4, L10, and bovine cells digested with rare cutter enzymes and on mRNA of L4 and L10. DNA analysis was performed using pulsed-field techniques in order to determine the degree of linkage between the two genes and to attempt a long range map of the bovine class I *MHC* region.

Materials and methods

Cattle, cells, and MHC phenotyping. Serological characterization of class I BoLA phenotypes was performed with an automated microlymphocytotoxicity assay as described by Kemp and co-workers (1988). The two animals studied were of the Boran (*Bos indicus*) breed. B641 is an *MHC* heterozygous of BoLA-type w10, KN104/KN18 (Bensaid et al. 1988). E98 is homozygous for the w10, KN104 haplotype. The animal is the product of inbreeding. Homozygosity is supported by class II phenotyping, mixed lymphocyte reactivity, and biochemical analysis, in addition to class I typing. Peripheral blood mononuclear cells (PBMC) from B641 were immortalized with the intracellular protozoan parasite *Theileria parva* by the method of Stagg and co-workers (1981). The mouse fibroblast lines L4 and L10, were obtained by transfecting total bovine genomic DNA from E98 into LMTK$^-$ mouse cells (Toye et al. 1990).

Preparation of RNA. Total RNA was prepared following the acid phenol/guanidinium thiocyanate method of Chomczynski (1987). Messenger RNAs from E98 PBMC were purified with an oligo(dt)-cellulose column (Collaborative Research Inc., Bedford, MA).

Cloning and characterization of class I cDNA molecules. Messenger RNAs from E98 PBMC were copied into cDNA using the BRL synthesis kit (BRL Laboratories, Gaitheisburg, MD). Second stranded cDNA synthesis, methylation of internal *Eco* RI sites, ligation into λ gt11 arms, and in vitro packaging were performed using the Amersham cDNA cloning kit (Amersham, UK) The resulting library was screened using the bovine class I cDNA clone cBoLA 1 (kindly provided by Dr. Pamella Brown, Institute for Animal Physiology and Genetics Reasearch, Edinburgh). Positive clones were grown and the inserted cDNAs were transferred into the plasmid pUC19 and the bacteriophage M13. The cDNA were sequenced using sequenase (US Biochemicals, Cleveland, OH) and the dideoxy method of Sanger (1977). Two clones, 5.1 and 2.1, were selected for further studies. Both of the 3′ untranslated (UT) regions of the class I cDNA were characterized by a *Pst* I 230 base pairs (bp) fragment upstream of the polyadenylation signal.

Preparation and labeling of the probes. The entire class I cDNAs were isolated by digesting the recombinant plasmids with *Eco* RI while the 3′UT fragments were obtained upon double digestion with *Eco* RI and *Pst* I. The appropriate fragments were purified after electrophoresis in low melting point agarose (BRL laboratories, Gaithesburg, Ohio). Labeling with ^{32}P-dCTP was performed using the nick translation method of Rigby (1977) or the random priming method of Feinberg and Vogelstein (1983). Between $1\times10^6-2\times10^6$ cpm of labeled probes were used, after heat-denaturation, per milliliter of hybridization solution.

Northern blotting. Twenty micrograms of total RNA were loaded in each lane of a formaldehyde 1% agarose gel (BRL). Samples were electrophoresed at 120 V for 2 h (Maniatis et al. 1982). An RNA ladder (BRL) was used to provide molecular weight standards. After electrophoresis, the gel was soaked twice for 10 min in distilled water and then incubated in 50m*M* NaOH 10m*M* NaCl for 20 min and neutralized in 0.1*M* Tris pH 7.4. RNA was transferred onto a nylon filter (Hybond, Amersham, UK) by capillarity. After dyring, the filter was UV-irradiated for 4 min. Prehybridization was performed for 4 h at 42°C in 0.1% SDS, 0.1% sodium pyrophosphate, 4×SSC, 10×Denhardt's solution, 50% deionized formamide (Merck, Darmstadt, FRG) containing 250 μg/ml of sonicated salmon sperm DNA (Pharmacia, Uppsala, Sweden). Hybridizations were performed at 42°C for 16 h in 0.1% SDS, 6×SSC, 5×Denhardt's solution, 0.1% sodium pyrophosphate, 10% dextran sulphate, and 50% deionized formamide containing 100 μg of salmon sperm DNA, to which radiolabeled probe was added. The filter was washed twice for 5 min at room temperature in 2×SSC, 0.25% SDS and twice for 45 min in 0.1×SSC, 0.25% SDS at 68°C. After exposure to Fuji X-Ray films, the filter was stripped in boilling water with 0.1% SDS for 15 min.

FIGE analysis of genomic DNA. Peripheral blood mononuclear cells (PBM) of donor cattle and fibroblasts were washed in PBS and suspended at a final concentration of 10^7 cells/ml in melted 0.75% LMP agarose (BRL) in phosphate buffered saline (PBS) and dispensed in precooled block formers. Blocks were treated with 0.5 mg/ml of proteinase K (BRL) in 10mM Tris, 250mM EDTA pH 8, 0.5% SDS at 50°C for 48 h. Blocks were then washed extensively in 10m*M* Tris/1m*M* EDTA pH 8. Pieces of 5×6 mm were cut from the agarose blocks, equilibrated with the appropriate restriction enzyme buffer, and DNA was digested with 30U of *Sfi* I, 20 units (U) of *Not* I, 40 U of *Mlu* I or 50 U of *Cla* I (New England Biolabs; Beverly; MA, USA) for 16 h. When double digestions were performed, after the first digestion, agarose slices were rinsed for 1 h in the second restriction enzyme buffer and DNA was digested with the second enzyme for 16 h. Agarose slices were then equilibrated in 0.5×TBE and loaded into slots in 1% agarose gels. The FIGE unit was made in our laboratory according to protocols described by Carle and co-workers (1986). Periodic inversions were driven by a BBC computer following a linear gradient ramp. Electrophoresis was performed for 28 h at 12°C at 280 V. Chromosomes of *Trypanosoma brucei brucei* were prepared as described by Shah and co-workers (1987) and used as molecular mass standards. At the end of the run, size separated DNA was stained, depurinated and transferred onto nylon membranes (Amersham, UK) according to standard procedures (Maniatis et al. 1982). UV irradiated filters were prehybridized, hybridized and washed as described for the northern blots.

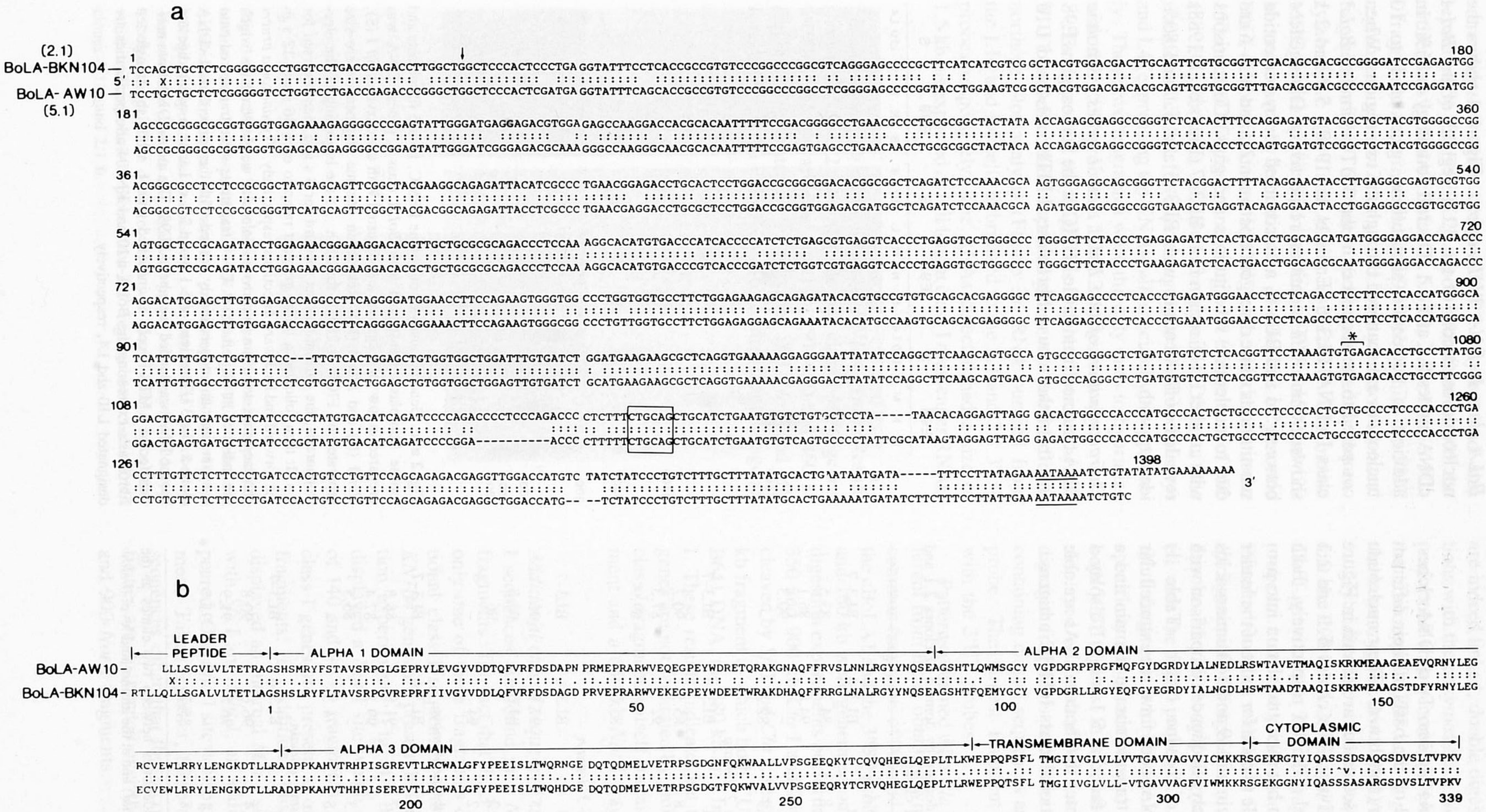


Fig. 1. a Nucleotide sequence comparison of the cDNA clones 2.1 and 5.1. The twelve first nucleotides of clone 2.1 are not shown. The *arrow* indicates the first nucleotide coding for the mature protein. The *star* indicates the stop sodon. the polyadenylation sites are *underlined* and the *Pst* I sites are *boxed*. (−) indicates deletions of nucleotides in order to maximize identities. Sequence comparisons were performed using the HIBIO-DNASIS software from Hitachi. **b** Amino-acid sequence comparison of the translated cDNA clones 2.1 and 5.1. Sequence comparisons were performed using the HIBIO-PROSIS software from Hitachi. (:) indicates a full match while (.) indicates a permissive substitution. (−) indicates a deletion of amino-acids in order to maximize identities.

Results

BoLA class I cDNA 5′coding regions. Two cDNA clones, 5.1 and 2.1 were selected on the basis of their different restriction enzyme pattern (not shown). Their nucleotide and predicted amino-acid sequences are shown in Figure 1a and Figure 1b respectively. The clones 5.1 and 2.1 have lengths of 1378 and 1396 bp respectively. Both cDNAs are not full length but their translation into proteins show that they are able to code for most of the leader peptide and a mature protein of 339 and 340 amino-acids for 2.1 and 5.1, respectively. Sequence comparison with other class I antigens of bovine origin (Table 1) demonstrate that these cDNAs code for three extracellular domains ($\alpha 2$ and $\alpha 3$), a transmembrane domain and a cytoplasmic domain. The fact that 5.1 and 2.1 display a polyadenylation site indicates that both mRNAs are able to be translated into functional class I MHC antigens.

Table 1. Sequence comparison domain by domain of clones 2.1 and 5.1 and two other BoLA class I clones.

		Alpha 1 domain		
	2.1	5.1	BL3-6	BL3-7
2.1	–	85.8	84.7	83.5
5.1	78.9	–	94.8	86.1
BL3-6	75.6	91.1	–	90.3
BL3-7	72.2	76.7	82.2	–
		Alpha 2 domain		
	2.1	5.1	BL3-6	BL3-7
2.1	–	84.8	85.5	90.2
5.1	78.3	–	92	87.3
BL3-6	72.2	88	–	89.9
BL3-7	83.7	79.3	82.6	–
		Alpha 3 domain		
	2.1	5.1	BL3-6	BL3-7
2.1	–	93.5	97.8	96
5.1	89.1	–	94.6	92.8
BL3-6	96.7	90.2	–	96
BL3-7	94.6	90.2	94	–
		Transmembrane domain		
	2.1	5.1	BL3-6	BL3-7
2.1	–	92.7	91.8	88.8
5.1	89.2	–	99.1	87.4
BL3-6	86.5	94.3	–	86.5
BL3-7	83.3	77.8	74.3	–
		Cytoplasmic domain		
	2.1	5.1	BL3-6	BL3-7
2.1	–	95.4	96.6	90.8
5.1	85.7	–	96.5	91.9
BL3-6	92.9	93.1	–	91.7
BL3-7	78.6	82.8	85.7	–

Figures express percentage of identities between two clones at the nucleotide level (*above the diagonal*) and at the amino-acid level (*below the diagonal*).

BoLA class I cDNA 3′UT regions. Figure 1 shows the nucleotide sequences of the 3′UT regions of the *BoLA* cDNA clones 5.1 and 2.1. Overall homology is 86%. In addition to nucleotide subtitutions, gaps of 1 to 10 nucleotides are scattered through the two regions. When compared to the sequences of the 3′UT regions of *BoLA* class I cDNA BL3-6 (Ennis et al. 1988), 5.1 and 2.1 showed 95 and 87% identities, respectively. Divergences between 5.1 and BL3-6, are accounted for by nucleotide substitutions while divergence between *2.1* and BL3-6 are due to nucleotide substitutions and gaps. Comparisons with another 3′UT region from BL3-7 (Ennis et al. 1988) revealed 86% identities with BL3-6 and 5.1 and 80% identity with 2.1.

Characterisation of two BoLA class I genes. Genomic DNA obtained from cattle PBMC of the homozygous E98 and from the mouse fibroblasts LMTK$^-$, L4 and L10

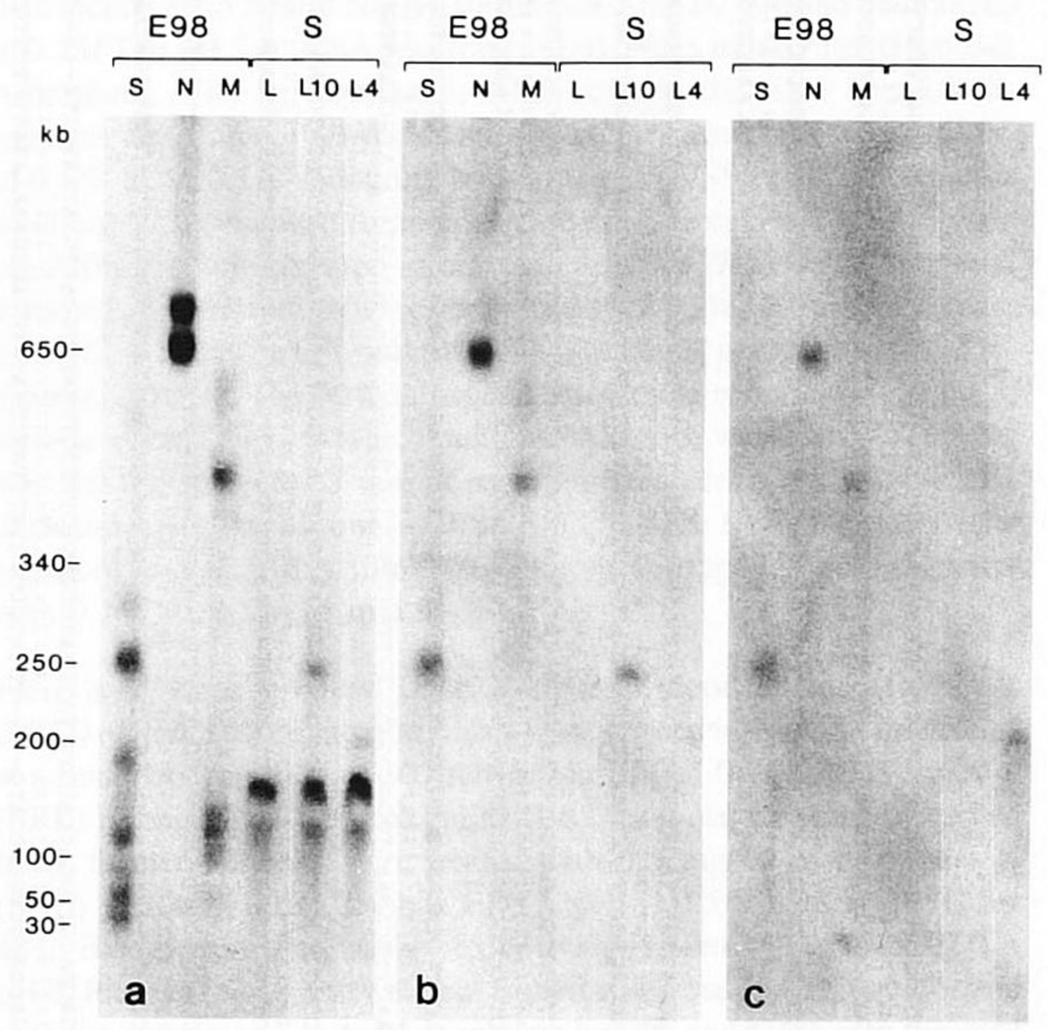


Fig. 2 a–c. Genomic analysis of bovine PBMC, mouse fibroblasts and mouse fibroblasts transfected with *BoLA* class I genes. DNA was prepared in low melt agarose and digested with the enzymes *Sfi* I (S), *Not* I (N) and *Mlu* I (M). Restriction enzyme fragments were size separated by FIGE at 280 V for 28 h. At the beginning of the electrophoresis, the current was set forward for 3 s and then reversed for 1 s. It then followed a linear gradient ramp to each 36 s and 12 s in the forward and backward directions respectively. *Trypanosoma brucei* chromosomes were run to provide molecular weight standards which are indicated at the left in kb. DNA fragments were transferred onto a nylon filter and consecutively probed with the entire class I cDNA clone **a**, the 3′UT regions of 5.1 **b** and 2.1 **c**. Lanes in which digested bovine DNA was loaded are labelled E98. The mouse DNA from non-transfected LMTK$^-$ cells is indicated with L while the transfected fibroblasts expressing the BoLA-w10 and KN104 allospecificities are designated L10 and L4, respectively.

were digested with "rare cutter" enzymes and size separated by FIGE (Fig. 2). Upon hybridization with the entire class I cDNA cBoLA 1, *6 Sfi* I, *2 Not* I and *3 Mlu* I fragments were revealed in E98 DNA while the 3′UT regions of clones 2.1 and 5.1 both detected identical fragments of 250, 650 and 450 kb generated by the enzymes *Sfi* I, *Not* I and *Mlu* I, respectively. The mouse MHC is cleaved by *Sfi* I into fragments of 150 and 120 kb as assessed by hybridization of cBoLA I on genomic DNA of non-transfected LMTK$^-$ cells. In addition to the 150 and 120 *Sfi* I fragments, single *Sfi* I fragments of 250 and 200 kb were revealed on genomic DNA of the transfected cells L10 (expressing the w10 allospecificity) and L4 (expressing the KN104 allospecificity) respectively. These two fragments were the only ones to hybridize with the 3′UT probes 5.1 and 2.1, respectively.

Evidence that the fragments of DNA detected by the 3′ UT probes contain expresed genes was provided by a northern blot analysis (Figure 3). RNA purified from the L10 and L4 cells hybridized to the 5.1 and 2.1 3′UT probes respectively. The RNAs detected had a size of 1.5 kb, as expected of full lenght class I messenger RNAs.

Physical mapping of the w10 and KN104 genes. Results shown in Figure 2 show that the two class I *MHC* genes, *w10* and *KN104* are positioned in 250, 650 and 450 kb fragments generated by the enzymes *Sfi* I, *Not* I and *Mlu* I, respectively, in E98 DNA. To confirm that these genes are indeed linked, double digestion in pairwise combinations with the enzymes *Sfi* I, *Not* I, *Mlu* I and *Cla* I were performed with genomic DNA obtained from PBMC of the homozygous E98 and *T. parva*-transformed cells of the heterozygous B641 (BoLA-w10, KN104/KN18).

Hybridization with the cBoLA I class I cDNA revealed that the E98 DNA 250 kb *Sfi* I fragment was cleaved by *Cla* I generating a 210 kb fragment, to which the 3′UT probes 5.1 and 2.1 preferentially hybridized (Figure 4). Patterns revealed with B641 DNA were more complex. *Sfi* I generated 8 fragments revealed by the cBoLA class I probe. The 250 kb *Sfi* I fragment preferentially hybridized with the 3′UT probes 5.1 and 2.1 while the 380 kb *Sfi* I fragment, which correlates with the KN18 allospecificity (result not shown) had a weak reativity with both 3′UT regions. In addition, the 380 kb *Sfi* I fragment displayed an internal *Not* I site. The resulting double-digested fragments had sizes of 340 and 40 kb which containing class I sequences as revealed by the cBoLA1 probe. The largest fragment hybridized preferentially with the 3′UT probes.

Patterns obtained with *Cla* I and B641 DNA were different from those obtained with E98 DNA although two common fragments of 900 and 350 kb were observed with the cBoLA1 and the 3′UT probes. *Cla* I fragments of 250 and 160 kb were generated only in E98 DNA. In double digestion experiments performed with *Cla* I and *Sfi* I, the 350 and 900 kb *Cla* I and 250 kb *Sfi* I fragments were cleaved by *Sfi* I and *Cla* I, respectively, to generate a 210 kb fragment to which both 3′UT probes hybridized. With B641 DNA, the 380 kb *Sfi* I fragment was not cut by *Cla* I. These results indicate that the *BoLA-w10* and *KN104* genes are not separated by more than 210 kb and that this class I fragment is itself contained in a 450 kb *Mlu* I fragment and a 650 kb *Not* I fragment in E98.

Additional class I sequences. As shown in Figure 2 class I sequences are found in 2 *Not* I, 4 *Mul* I and 6 *Sfi* I fragments. The fact that the 3′UT probes hybridized with only one of these fragments clearly indicates that additional class I sequences other than the *BoLA-w10* and *KN104* genes are present in the genome. In double digestion experiments (Fig. 4), the 180 kb *Sfi* I fragment displayed a *Not* I site which resulted in the appearance of 140 and 40 kb fragments. This demonstrates that the class I genes are present at one extremity of both *Not* I fragments. In addition, this 180 kb *Sfi* I fragment displayed an internal *Mlu* I site. Upon double digestion with *Sfi* I and *Mlu* I, an additional 40 kb fragment appeared. One *Mlu* I site is also present in a 75 kb *Sfi* I fragment. Taken together, these results indicate that class I sequences other that those coding for known allospecificities are distributed over the entire length of both 650 and 900 *Not* I fragments.

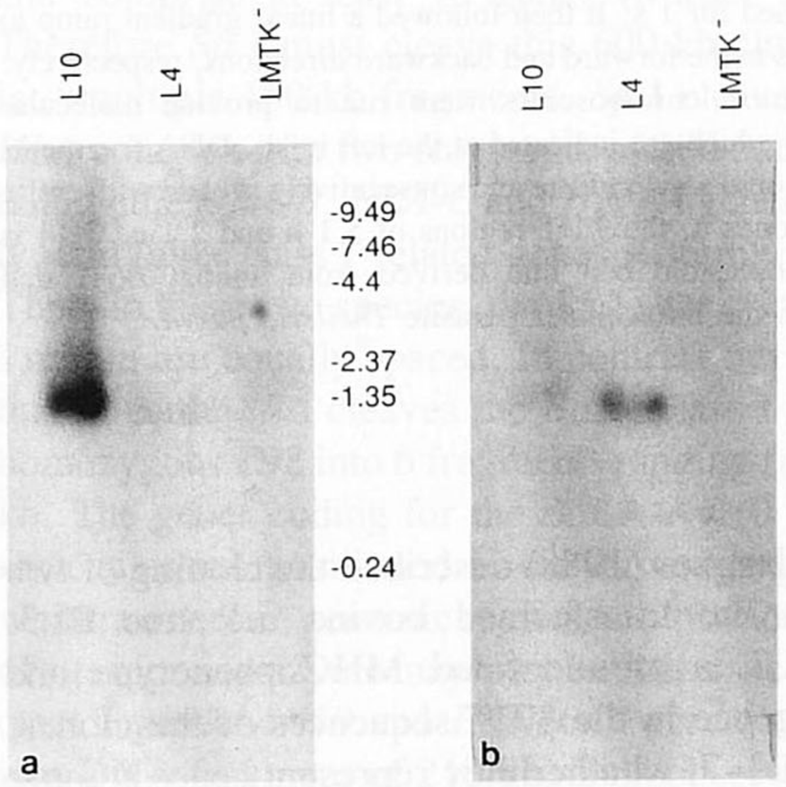
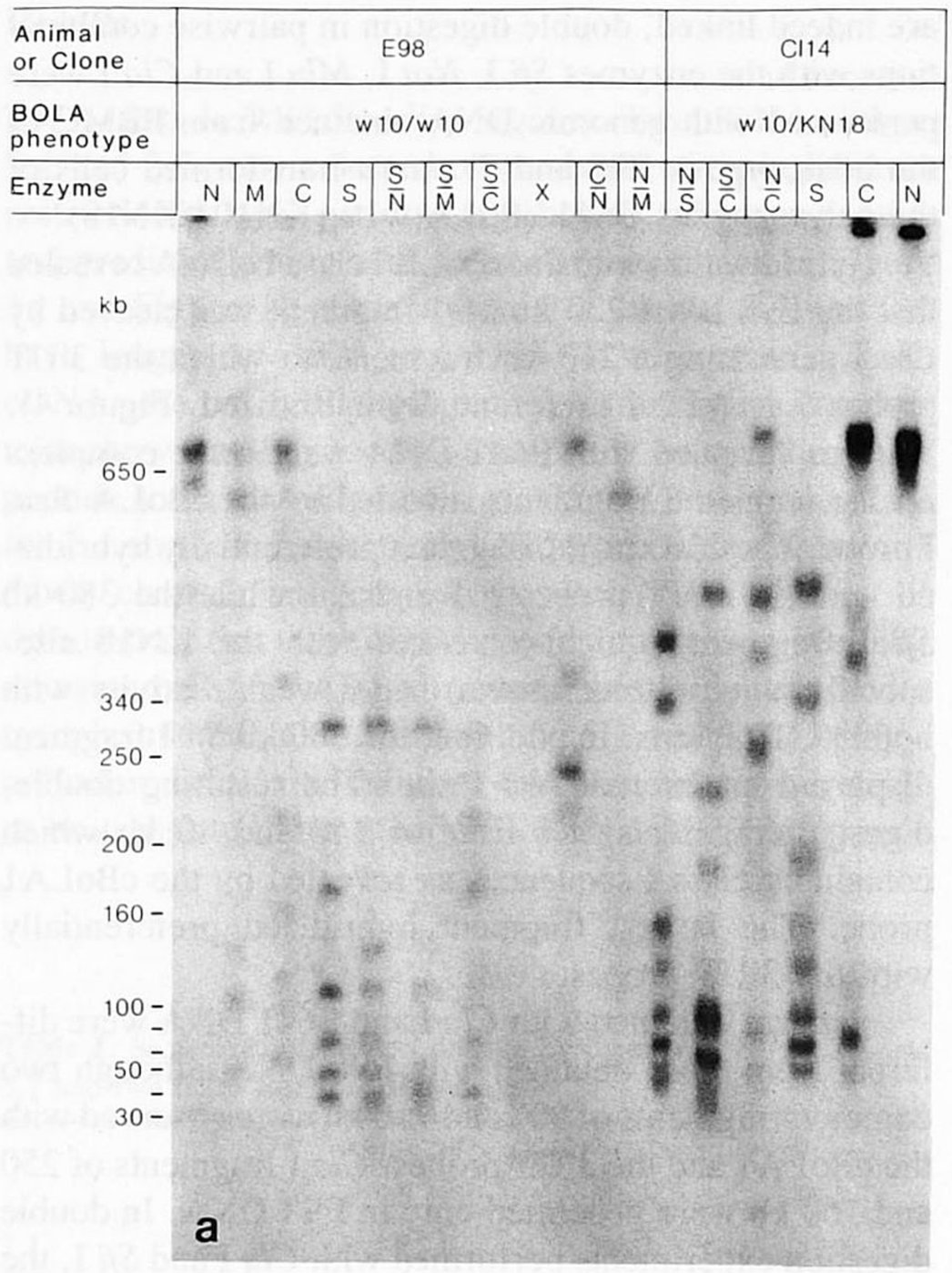


Fig. 3 a, b. Northern blot analysis of mouse fibroblasts expressing bovine class I allospecificities. RNA from LMTK$^-$ cells and transfected fibroblasts, L10 and L4, expressing the BoLA-w10 and KN104 allospecificities respectively was purified as described in *Materials and methods* and loaded (20 μg per lane) on a formaldehyde 1% agarose gel. Size separated RNA was blotted onto a nylon filter which was consecutively probed with 3′UT regions of the class I cDNA clones 5.1 **a** and 2.1 **b**.



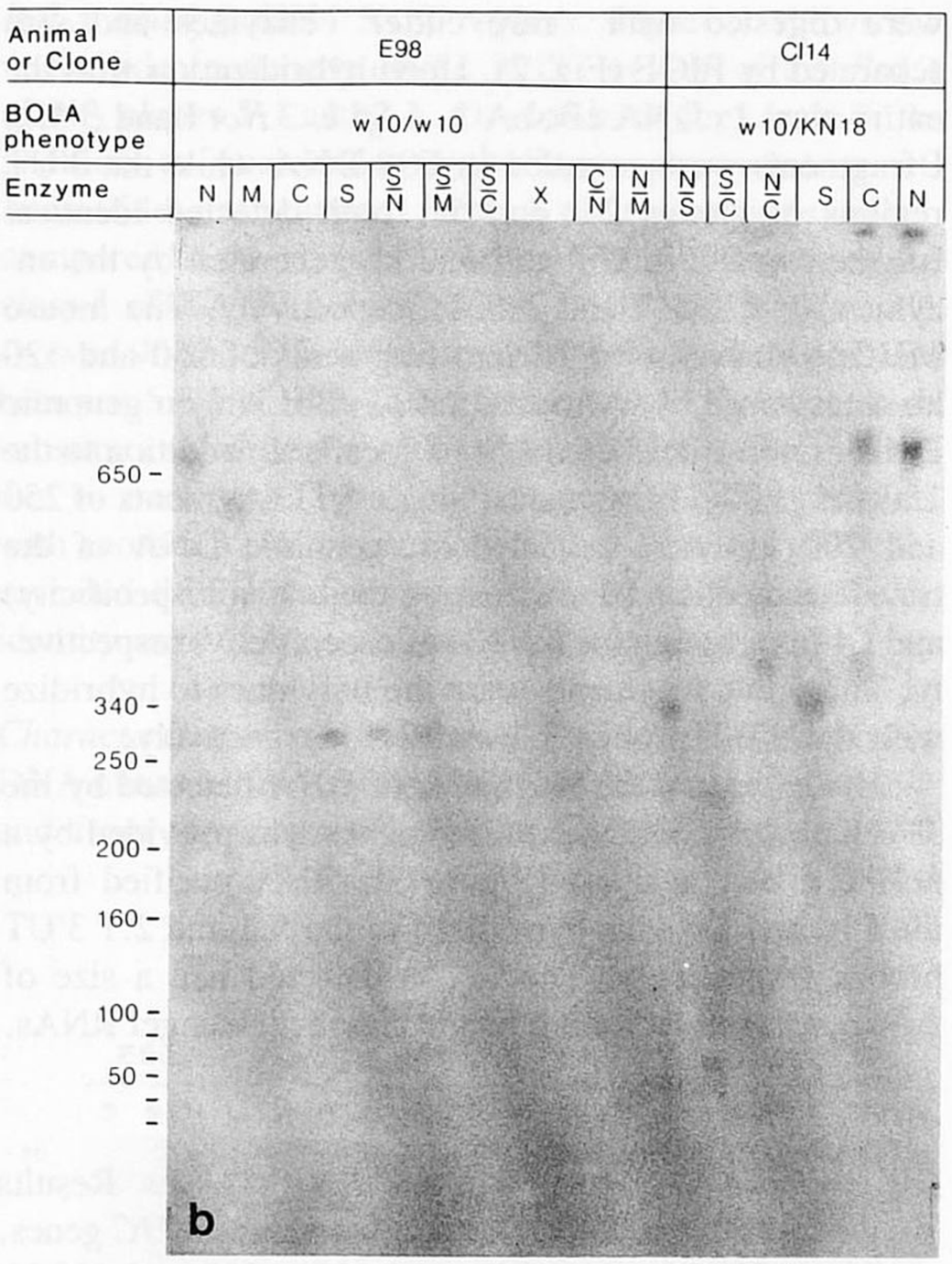


Fig. 4 a–c. Genomic analysis of the class I region of two animals, E98 and B641 homozygous and heterozygous at their MHC respectively. DNA was prepared in low-melt agarose and digested in single and double combinations with the restriction enzymes *Sfi* I (S), *Not* I (N), *Mlu* I (M) and *Cla* I (C). Samples were run for 28 h at 280 V by FIGE. At the beginning of the electrophoresis the current was set forward for 3 s and then reversed for 1 s. It then followed a linear gradient ramp to reach 24 s and 8 s in the forward and backward directions, respectively. *Trypanosoma brucei* chromosomes were run to provide molecular weight standards which are indicated at the left in kb. DNA fragments were transferred onto a nylon filter and consecutively probed with entire cDNA class I clones **a**, the 3′UT regions of 5.1 **b** and 2.1 **c**. Cl14 is a cloned lymphoblastoid cell line derived from animal B641 and transformed with the intracellular parasite *Theileria parva*.

Discussion

Ennis and colleagues (1988) described the cloning of two cDNAs from the transformed bovine cell line BL3. However, BL3 is of undefined MHC phenotype and despite divergence in the 3′UT sequences of the clones, BL3–6 and BL3–7, whether they represent genes at more than one locus, is a matter of speculation. We have cloned two distinct cDNAs coding for class I BoLA molecules from an MHC homozygous animal. The two clones 5.1 and 2.1, were in part selected for study on the basis of divergence at their 3′ termini. As shown in other species, 3′UT regions of alleles at a single locus may display 96% identities whereas identities of alleles at different loci can

vary from 74 to 95% (Ennis et al. 1988). On this basis, 5.1, 2.1 and BL3–7 would represent the products of three different loci. This is supported by the fact that BL3–7 has, at the end of the 3′UT region, a tract of 62 nucleotides with a second polyadenylation signal not found in many of the other three cDNA clones. However, it is questionable whether such assumptions based on homologies are applicable in cattle. In this respect, it is important to note that the 3′UT regions of 5.1 and 2.1 do not hybridize to genomic DNA of all cattle (Bensaid et al. manuscript in preparation).

The fact that the 3′UT regions of the clones 5.1 and 2.1 hybridized exclusively to genomic DNA of the L10 and L4 cells, respectively, demonstrates conclusively that the transfected fibroblasts contain different *BoLA* class I genes and that the 5.1 cDNA is the product of the *w10* gene (*A* locus) of animal E98, whereas the 2.1 cDNA is the product of the *KN104* gene, which we will assign to a *B* locus. As far as we are aware, this constitutes the first formal demonstration of a *BoLA-B* locus.

Utilising the cDNA clones and FIGE analysis, we have been able to obtain information on the genomic organization of the *BoLA* region. In the present study, we concentrated on the patterns obtained with the enzyme *Sfi* I in the cattle and mouse class I *MHC* regions, since data with this enzyme have already been reported for the *HLA* complex (Chimini et al. 1987; Carroll et al. 1987). In our studies, the class I *H-2* region was cleaved into two fragments of 150 and 120 kb. The 150 kb fragment appears to contain most of the class I genes and although the *H-2K* region is relatively short with two tightly linked genes, the *H-2D* and *Qa* regions span more than 600 kb and comprise at least 13 genes (Muller et al. 1987). Therefore *Sfi* I must cleave this 600 kb cluster of genes into multiple 150 kb fragments. *Sfi* I cleaves that class I *HLA* complex into two fragments of 200 and 250 kb containing the *HLA-B*, *HLA-C* and *HLA-A* genes, respectively, and other class I-related genes (Chimini et al. 1987). Thus, in these two species, the *Sfi* I sites lying in the class I region are equally spaced. In contrast our results show that in cattle *Sfi* I cleaves the *BoLA* class I region of the homozygous E98 into 6 fragments ranging from 45 to 250 kb. The genes coding for the BoLA-Aw10 and BKN104 molecules are positioned in the largest fragment. Thus, whithin a certain genomic region, the location and number of sites for an enzyme may vary between species. The two class I genes *Aw10* and *BKN104*, were found clustered in a DNA fragment of 210 kb. This observation support the idea that the *BoLA-A* and *B* genes are organized in a similar manner to the *HLA-B* and *C* genes. However, conclusive demonstration of this must await detection of linkage between the complement genes (*C4* and *C2*) and the class I *BoLA-A* and *B* genes.

Class I sequences other than those which can be assigned to the *BoLA-A* and *B* genes have been found and located on different *Sfi* I, *Mlu* I and *Not* I DNA fragments. This confirms that the *BoLA* class I region is a multigene family spanning several hundreds of kilobases. Of interest is a fragment of 180 kb generated by *Sfi* I and including both *Not* I and *Mlu* I sites. This characteristic makes it the linking fragment between the 650 and 900 kb *Not* I fragments which should contain the entire class I MHC. One of these 'extra' genes might be expressed and represent a *BoLA-C* gene equivalent of *HLA-A*. In this respect, it is of interest to note that a previous biochemical analysis of activated T cells has indicated the presence of class I-related molecules which display common biochemical features with mouse Qa antigens (Bensaid et al. 1989).

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