

Nucleotide sequence of the upstream regulatory region of BoLA-DRB†

M. V. Ripoli, S. Díaz,* P. Peral-García & G. Giovambattista

Summary

The sequence of the proximal upstream regulatory region (URR) of the bovine DRB genes was amplified using oligonucleotide primers designed from the consensus among DRB sequences from different species. The obtained DNA sequence was 234 bp long and composed of highly conserved sequence motifs, showing the same organization as the HLA-DRB, H2-IAb, H2-IEb and ELA-DRB genes.

In this paper we report the sequence of the proximal upstream regulatory region (URR) of the bovine DRB genes. These are the first sequence data on the upstream regulatory regions of MHC class II genes in cattle.

The reverse oligonucleotide primer (5'-GAGAAATA-CAGACACACCATGC-3') was designed from the consensus among DRB sequences from different species: BoLA-DRB (accession numbers U77067-68, U78548 and D45357), SLA-DRB (M55165-6), HLA-DRB (S72812 and L07838-40), H2-IEb and H2-IAb (X86151-6). As a forward primer we used the oligonucleotide proposed by Turco *et al.* (1990) (5'-TGTTTCAGAAAAGGACCTTC-3'), which was designed from the consensus among HLA-DRB sequences corresponding to the promoter region of the DRB genes. A polymerase chain reaction (PCR) was carried out in a total volume of 25 µl comprising 2.5 mM MgCl₂, 20 mM Tris-HCl (pH = 8.4), 50 mM KCl, 100 µM of each dNTP, 0.5 µM of each primer, 1.0 unit Taq polymerase (Gibco BRL, Life-Technologies, Grand Island, NY) and 50–100 ng DNA template. The genomic DNA was extracted from whole blood from one animal of the Saavedreño Creole breed.

The amplification profile consisted of 1 min at 94 °C, followed by 30 cycles of 45 s at 94 °C, 45 s at 55 °C and

45 s at 72 °C, with a final extension of 3 min at 72 °C. The amplification products were cloned into a dT-tailed pGEM-T easy vector (Promega, Madison, WI), and three clones of each PCR product were sequenced on an Applied Biosystems 377 automated sequencer (Bio-Resource Center, Cornell University, Ithaca, NY), using a T7 universal primer.

All the sequenced clones exhibited 100% sequence similarity to each other. The nucleotide sequence of the proximal upstream control region of the BoLA-DRB genes (GenBank accession number AY040327) was 234 bp long and composed of highly conserved sequence motifs that included, from the 5' to the 3' direction, W, X, Y, CCAAT and TATA-like boxes (Fig. 1), showing the same organization of the conserved regulatory elements as the HLA-DRB, H2-IAb, H2-IEb and ELA-DRB genes (e.g. Louis *et al.*, 1993, 1994; Singal *et al.*, 1993; Singal & Qiu, 1994). Furthermore, the BoLA-DRB URR nucleotide sequence had higher identity with HLA-DRB sequences than with HLA-DQB sequences (data not shown). This suggests that the sequence AY040327 corresponds to a BoLA-DRB promoter. However, we are still unable to assign this sequence to a specific BoLA-DRB gene, as at least three BoLA-DRB genes have been reported to exist (Andersson *et al.*, 1986; Muggli-Cockett & Stone, 1988). BoLA-DRB3 is the most expressed DRB gene in cattle, while DRB1 is expressed at a low level and DRB2 is a pseudogene (Burke *et al.*, 1991).

Acknowledgements

This research was supported by grants PICT 99 N°0805216 of the Secretaría de Ciencia y Técnica de la Nación, Comisión de Investigaciones Científicas de la provincia de Buenos Aires (CICBA) and Japan International Cooperation Agency (JICA).

References

- Andersson, L., Bohme, J., Peterson, P.A. & Rask, L. (1986) Genomic hybridization of bovine class II major histocompatibility genes. 2. Polymorphism of DR genes and linkage disequilibrium in the DQ-DR region. *Animal Genetics*, **17**, 295.
- Burke, M.G., Stone, R.T. & Muggli-Cockett, N.E. (1991) Nucleotide sequence and northern analysis of a bovine major histocompatibility class II DR beta-like cDNA. *Animal Genetics*, **22**, 343.
- Louis, P., Eliaou, J.-F., Kerlan-Candon, S., Pinet, V., Vincent, R. & Clot, J. (1993) Polymorphism in the regulatory regions of

Centro de Investigaciones en Genética Básica y Aplicada (CIGEBA), Facultad de Ciencias Veterinarias, Universidad Nacional de La Plata, La Plata, Argentina.

† The nucleotide sequence data reported in this paper have been submitted to the GenBank nucleotide sequence database and have been assigned the accession number AY040327.

* Fellow of the Comisión Nacional de Investigaciones Científicas y Técnicas (CONICET).

Received 11 November 2001; revised 20 February 2002; accepted 5 May 2002

Correspondence: G. Giovambattista, Centro de Investigaciones en Genética Básica y Aplicada (CIGEBA), Facultad de Ciencias Veterinarias, Universidad Nacional de La Plata, C.C. 296, C.P. B1900AVW, La Plata, Argentina. E-mail: ggiovam@fcv.medvet.unlp.edu.ar

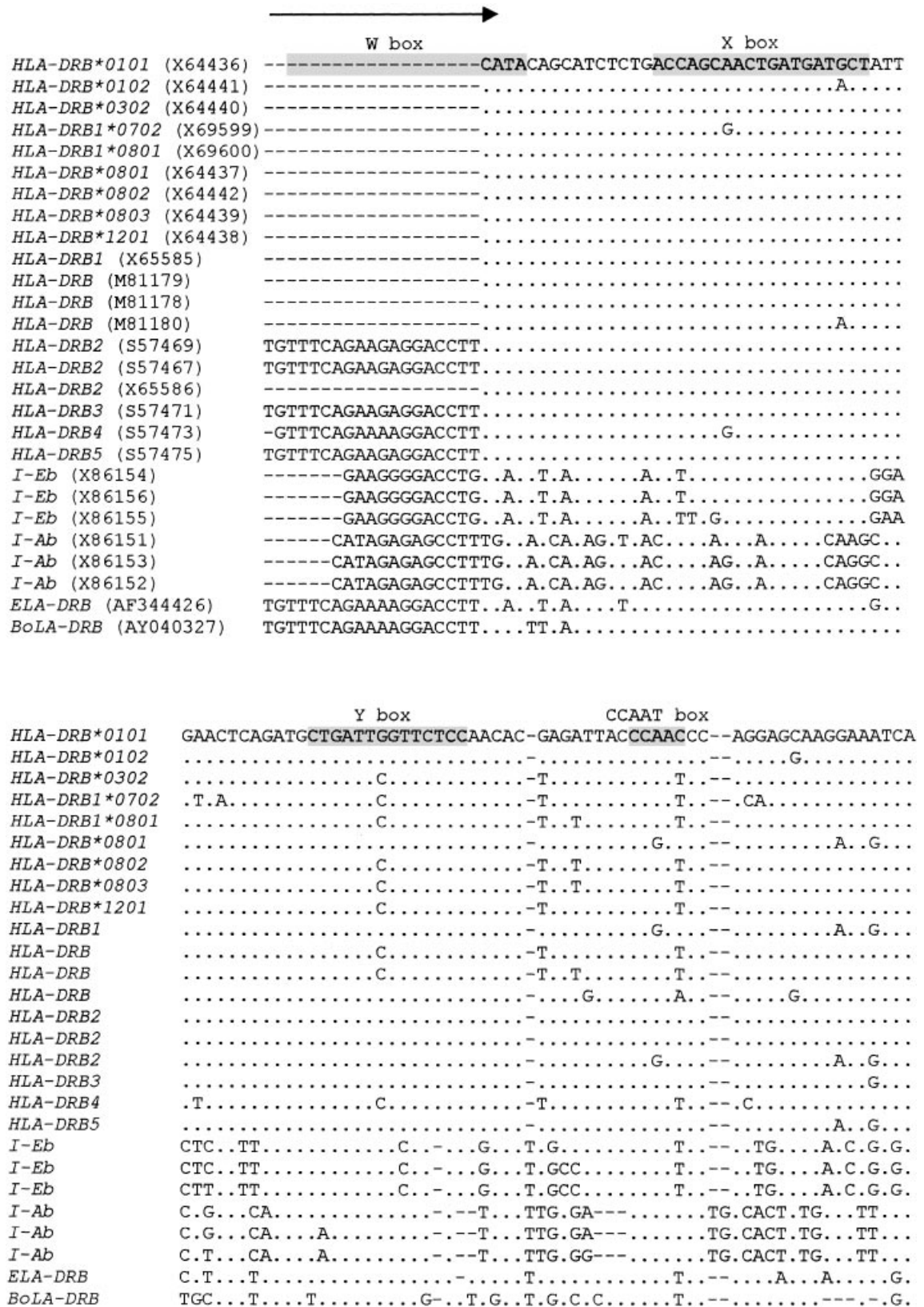


Figure 1. Alignment of the nucleotide sequence of the upstream regulatory region (URR) of BoLA-DRB with those of HLA-DRB, IAb, IEb and ELA-DRB. The A of ATG is designated as +1. Dots indicate nucleotide identity to the HLA-DRB1*0101 sequence (X64436), and dashes (-) represent gaps introduced to achieve the best alignment. Boxed sequences represent the W, X, Y, CCAAT and TATA regions. Arrows indicate the location of the primers.

TATA box

```

HLA-DRB*0101 GTAA-CTTCCT-CCCTATA-A-CTTGAATGTGGGTGGAG-GGGTTCA-TAGTTCTC---CCTG
HLA-DRB*0102 .....-T.....G...G.....T---...
HLA-DRB*0302 .....A.....-.....T---...
HLA-DRB1*0702 .....T.....-T.....A..G.....T---...
HLA-DRB1*0801 .....A.....-.....T---...
HLA-DRB*0801 .....C.....-T.....T---...
HLA-DRB*0802 .....A.....-.....T---...
HLA-DRB*0803 .....A.....-.....T---...
HLA-DRB*1201 .....A.....-.....T---...
HLA-DRB1 .....C.....-T.....T---...
HLA-DRB .....A.....-.....T---...
HLA-DRB .....A.....-.....T---...
HLA-DRB .....C.....-T.....G..A.....T---...
HLA-DRB2 .....AA..C.....-T.....T---...
HLA-DRB2 .....-.....T---...
HLA-DRB2 .....-.....T---...
HLA-DRB3 .....C.....C.....-T.....T---...
HLA-DRB4 .....T.....-T.....A..G.....T---...
HLA-DRB5 .....C.....C.....-T.....T---...
I-Eb A.GTC..GT...T..T..T..A.C.A..A..AA...-A.AAT..A...-----A.A.
I-Eb A.GCC..GT...T..T..T..A.C.A..A..AA...-A.AAT..A...-----A.A.
I-Eb A.GTC..GT...T..T..T..A.C.A..A..AA...-A.AAT..A...-----A.A.
I-Ab .ATCA...-AGG...C.G.A..T.CT.--TC..A..G...CA..GC..G.G.GAGT...
I-Ab .ATCA...-CAGG...C.G.A..T.CT.--TC..A..G...CA..GC..G.G.GAGT...
I-Ab .ATCA...-AGG...C.G.A..T.CT.--TC..A..G...CA..GC..G.G.GAGT...
ELA-DRB ..TCG..AG...-GG...T...G.....GAA.G...-...C...--A...
BoLA-DRB ..TC...GG...GG...T...-T...G.....GA..G...-C...A.C.A--TT...
    
```



```

HLA-DRB*0101 AGTGAGACTTGCCTGCTTCTCTGGCCCTGGTCCCTGTCCTGTTCTCCAGCATGGTGTGTCTG
HLA-DRB*0102 .....CT.....
HLA-DRB*0302 .....G.....
HLA-DRB1*0702 .....CA.....C.....
HLA-DRB1*0801 .....G.....
HLA-DRB*0801 ..A.....C..C.....T.....
HLA-DRB*0802 .....G.....
HLA-DRB*0803 .....G.....
HLA-DRB*1201 .....G.....
HLA-DRB1 ..A.....C..C.....T.....
HLA-DRB .....G.....
HLA-DRB .....G.....
HLA-DRB .....C.....
HLA-DRB2 .....-----
HLA-DRB2 .....-----
HLA-DRB2 ..A.....C..C.....T.....
HLA-DRB3 ..A.....C..C.....-----
HLA-DRB4 .....CA.....C.....-----
HLA-DRB5 ..A.....C..C.....-----
I-Eb TC...AG.....T.CC.....A.T...T--G.C...C.C..G.....-----
I-Eb TC...AG.....T.CC.....A.T...T--G.C...C.C..G.....-----
I-Eb TC...AG.....T.CC.....A.T...T--G.C...C.C..G.....-----
I-Ab ---.T...G..A..-A...-TG..T.A.AGA..-----
I-Ab ---.T...GC.A..-A...-TG..T.A.AGA..-----
I-Ab ---.T...GC.A..-A...-TG..T.A.AGA..-----
ELA-DRB .....C.CT..CA.....T--..C.G.....-----
BoLA-DRB ..A.G.....C...C..T.TCTA...C.G.....G.....
    
```

Figure 1. Continued.

- HLA-DRB genes correlating with haplotype evolution. *Immunogenetics*, **38**, 21.
- Louis, P., Pinet, V., Cavadore, P., Kerlan-Candon, S., Clot, J. & Eliaou, J.-F. (1994) Differential expression of HLA-DRB genes according to the polymorphism of their regulatory region. *Comptes Rendus de L'Académie des Sciences, Paris*, **137**, 161.
- Muggli-Cockett, N.E. & Stone, R.T. (1988) Identification of genetic variation in the bovine major histocompatibility complex DR-beta like genes using sequenced bovine genomic probes. *Animal Genetics*, **19**, 213.
- Singal, D.P. & Qiu, X. (1994) Polymorphism in the upstream regulatory regions and level of expression of HLA-DRB genes. *Molecular Immunology*, **31**, 1117.
- Singal, D.P., Qiu, X., D'Souza, M. & Sood, S.K. (1993) Polymorphism in the upstream regulatory regions of HLA-DRB genes. *Immunogenetics*, **37**, 143.
- Turco, E., Manfras, B.J., Ge, L., Rudert, W.A. & Trucco, M. (1990) The X boxes from promoters of HLA class II B genes at different loci do not compete for nuclear protein-specific binding. *Immunogenetics*, **32**, 117.