Available online at www.sciencedirect.com

SCIENCE DIRECT

Gene 339 (2004) 71–78

GENE
AN INTERNATIONAL JOURNAL ON GENES AND GENOMES

www.elsevier.com/locate/gene

Polymorphism in the bovine *BOLA-DRB3* upstream regulatory regions detected through PCR-SSCP and DNA sequencing☆

M.V. Ripoli*[,1], P. Peral-García, F.N. Dulout, 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

Received 9 December 2003; received in revised form 2 April 2004; accepted 1 June 2004
Available online 19 July 2004
Received by T. Gojobori

Abstract

In the present work, we describe through polymerase chain reaction-single strand conformation polymorphism (PCR-SSCP) and DNA sequencing the polymorphism within the *URR-BoLA-DRB3* in 15 cattle breeds. In total, seven PCR-SSCP defined alleles were detected. The alignment of studied sequences showed six polymorphic sites (four transitions, one transversion and one deletion) in the interconsensus regions of the *BoLA-DRB3* upstream regulatory region (URR), while the consensus boxes were invariant. Five out of six detected polymorphic sites were of one nucleotide substitution in the interconsensus regions. It is expected that these mutations do not affect significantly the level of expression. In contrast, the deletion observed in the sequence AY364455 between CCAAT and TATA boxes could have some effect on affinity interactions between the promoter region and the transcription factors. The *URR-BoLA-DRB3* DNA analyzed sequences showed moderate level of nucleotide diversity, high level of identity among them and were grouped in the same clade in the phylogenetic tree. In addition, the phylogenetic tree, the similarity analysis and the sequence structure confirmed that the fragment analyzed in this study corresponds to the *URR-BoLA-DRB3*. The functional role of the observed polymorphic sites among the regulatory motifs in bovine needs to be analyzed and confirmed by means of gene expression assays.
© 2004 Elsevier B.V. All rights reserved.

Keywords: Bovine; Class II promoter; *BoLA-DRB3*; Polymorphism

1. Introduction

The upstream regulatory region (URR) of major histocompatibility complex (MHC) class II genes is highly conserved in humans and murines. This region of approximately 200 bp is located upstream the initiation of transcription and is composed of highly conserved sequence motifs that include from 5′ to 3′, the W, X, Y, CCAAT and TATA like boxes. These motifs are highly conserved among all MHC-D region genes studied up to date (for review, see Benoist and Mathis, 1990; Glimcher and Kara, 1992).

The expression of the MHC class II D regions genes is tissue specific and is controlled by a carefully coordinated action between the cis-acting elements mentioned above and a group of trans-acting factors. This system either activates or represses transcription through the interaction with RNA polymerases, and it is necessary for optimal constitutive and cytokine-induced gene expression. Briefly, the Y and the X1 cis-acting elements are bound by the NF-Y/CBF and the RFX trans-acting factors, respectively. In addition, the RFX complex, which is composed of RFXANK/RFXB, RFX5 and RFXAP, also binds the S/W cis-acting element, while the CREB protein binds to the X2 box. These trans-acting factors not only bind the DNA, but also interact among them

Abbreviations: MHC, major histocompatibility complex; *BoLA-DRB3*, bovine MHC class II D regions gene; SSCP, single strand conformation polymorphism; URR, upstream regulatory region; *CIITA*, class II transactivator; *HLA-DRB*, human MHC class II D regions gene; *DQB*, MHC class II D region gene; *I-Eb* and *I-Ea*, murine MHC class II D regions genes.

☆ The nucleotides sequences data reported in this paper have been submitted to the GenBank nucleotide sequence database and have been assigned the accession numbers AY040327, AF510446, AY364454, AY364455 and AY570362.

* Corresponding author. Tel./fax: +54-221-4211799.
E-mail address: mvripoli@fcv.unlp.edu.ar (M.V. Ripoli).
[1] Fellow from the Comisión Nacional de Investigaciones Científicas y Técnicas (CONICET).

0378-1119/$ - see front matter © 2004 Elsevier B.V. All rights reserved.
doi:10.1016/j.gene.2004.06.002

and with the class II transactivator (CIITA), forming a large transcriptosome complex (for review, see Ting and Trowsdale, 2002).

A number of loci- and allele-specific sequence polymorphisms have been described in the URR of *DQB* and *DRB* genes in human and mouse. Some of these polymorphisms were found in the regulatory motifs (i.e. X1, Y and CCAAT boxes), while others were observed among consensus boxes (Andersen et al., 1991; Pascale et al., 1993; Singal et al., 1993a; Reichstetter et al, 1994; Singal and Qiu, 1994; Cowell et al., 1998; Mitchinson and Roes, 2002). Polymorphisms found in conserved consensus sequences could affect DNA–protein interactions (Singal et al., 1993b; Emery et al., 1993). Consequently, these mutations may confer allelic differences in expression, inducibility and/or tissue specificity of class II molecules, affecting the transcriptional levels and the immune response. An example of this is the analysis of the activity of normal and mutated promoters of *DR52* associated with *DRB1* and *DRB3* genes as having a dominant role of the X box in controlling relative level of expression of these genes (Emery et al., 1993). In their paper, Singal and Qiu (1996) confirmed early data on the effect of nucleotide variation in the X1 box on the level of expression of *HLA-DRB1* genes. These authors also demonstrated that polymorphism in the Y box affecting the inverted CCAAT sequence also plays a dominant role in the transcriptional activity of *DRB1* promoters.

In a previous work, we characterized the sequence organization of *URR-BoLA-DRB3* gene (Ripoli et al., 2002) as a region of approximately 200 bp composed of highly conserved sequence motifs, including from 5′ to 3′ the W, X, Y, CCAAT and TATA like boxes. The aim of the present work is to describe through polymerase chain reaction-single strand conformation polymorphism (PCR-SSCP) and DNA sequencing the polymorphism within the *URR-BoLA-DRB3* in 15 cattle breeds. In total, seven PCR-SSCP defined alleles were detected in the analyzed animals. This analysis would allow us to identify mutations within and among regulatory boxes that, potentially, could affect both the level and the tissue specificity of *BoLA-DRB3* gene expression.

2. Materials and methods

2.1. Blood samples and DNA isolation

To explore the *BoLA-DRB3* promoter variation, blood samples were collected from 162 unrelated adult animals belonging to 15 domestic cattle breeds. These breeds included six South American Creole cattle that are bred under extensive management and low level of selection (Saavedreño Creole, Argentino Creole, Yacumeño Creole, Chusco Creole, Bolivian Chaqueño Creole, Bolivian Chaqueño Creole of the Valley), six highly selected European breeds (Aberdeen Angus, Jersey, Holstein, Hereford, Charolais, Normande), two humped breeds (Nelore, Brahman), and one synthetic breed (Brangus). Additional information about these breeds could be found in http://www.fao.org. Moreover, 11 animals (1 sire, 5 dams and 5 offsprings) belonging to a half-sib family were included to analyze the Mendelian inheritance of the gene.

Genomic DNA from blood lymphocytes and recombinant plasmid DNA isolation were performed by the DNAzol™ method and S.N.A.P. Plasmid Purification System (Invitrogene, Carlsbad, CA, USA), respectively.

2.2. PCR amplification and SSCP analysis of the URR

Animals were preselected before sequencing by PCR-SSCP analysis to include a range of different SSCP patterns. The reverse oligonucleotide primer (5′-GAGCCTCCAGAGAAATACAGGC-3′) was designed from the consensus among different *BoLA-DRB3* sequences (Accession Numbers D37952, D37953, Y18202, Y18201, Y18203, D37954, AF037315, U77067 and D45357). As a forward primer, we used the oligonucleotide proposed by Turco et al. (1990) (5′-TGTTTCAGAAAGGACCTTC-3′), which was designed from the consensus among *HLA-DRB* sequences corresponding to the promoter region of the *DRB* genes. The polymerase chain reaction (PCR) was carried out in a total volume of 25μl comprising 2.5 mM $MgCl_2$, 20 mM Tris–HCl (pH 8.4), 50 mM KCl, 100 μM each dNTP, 0.5 μM each primer, 1.0 unit Taq polymerase (Invitrogene) and 50–100 ng DNA template. The amplification profile consisted of 1 min at 94 °C, followed by 30 cycles of 45 s at 94 °C, 45 s at 58 °C and 45 s at 72 °C, with a final extension of 3 min at 72 °C. Ten microliters of each PCR product were added to 16 μl of denaturing loading dye (95% v/v formamide, 20 mM EDTA, 0.05% w/v Bromophenol Blue, 0.05% w/v xylene cyanol) and diluted with 10 μl of H_2O. The samples were then heated at 96 °C for 10 min, cooled on ice for at least 5 min and loaded onto a 10% polyacrylamide gel (38:1, acrylamide/bisacrylamide). Electrophoresis was carried out at 4 °C, 200 V in 0.5 × TBE buffer for 16 h. The gels were subsequently fixed in 5% ethanol, stained with 0.2% $AgNO_3$ and revealed with 2% $CaCO_3$.

2.3. Cloning and DNA sequencing

The *URR-BoLA-DRB* amplification products were cloned into pCR®2.1-TOPO® (TOPO TA Cloning®, Invitrogen Life Technologies). *Escherichia coli* competent cells (strain *TOP10*) were transformed with the recombinant plasmid and selected by kanamicyn–white/blue screening. Inserts for positive clones were confirmed by PCR and SSCP. PCR products corresponding to different SSCP patterns were chosen for DNA sequencing. Three clones of each selected PCR product were sequenced with an Applied Biosystems 377 automated sequencer, using ABI PRISM ready reaction

dye-terminator and T7 universal primer (BioResource Center, Cornell University, Ithaca, NY, USA).

2.4. BoLA-URR nomenclature

In the present work, we used two different nomenclatures to designate the *BoLA-URR* alleles. At first, the promoter variants were named with capital letters according to the SSCP variants. After each allele was cloned and sequenced, they were named according with their GenBank accession number.

2.5. Sequence analysis and statistical methods

Identity between pairs of DNA sequences were estimated by means of BLAST2 version blastn 2.0.8 (The Baylor College of Medicine Search Launcher; Altschul et al., 1997). Multiple alignments of the nucleotide sequences of the *URR-BoLA-DRB3* were carried out using the CLUSTAL-W version 1.7 (Baylor College of Medicine, Human Genome Sequencing Center, Houston, TX, USA; Thompson et al., 1994). Neighbor-joining trees for the human, mouse and bovine DRB upstream regulatory region sequences were constructed based on the Jukes–Cantor and Kimura two-parameter models, using Mega 2.1 (Pennsylvania State University, PA, USA; Kumar et al., 1994). Bootstrap values based on 1000 replicates were calculated to test the tree topology. Nucleotide diversity among sequences was estimated using the Kimura's two-parameter method included in the software ARLEQUIN 2.0 (Schneider et al., 2000).

2.6. Variability at population level

Gene frequencies within each breed were determined by direct counting. The genetic variability was evaluated through the observed number of alleles (n_a) and gene diversity (h_e according to Nei, 1987). The F_{ST} index was used as an estimator of genetic subdivision and genetic differentiation among studied cattle breeds. These parameters were calculated using the software ARLEQUIN 2.0 (Schneider et al., 2000).

3. Results and discussion

3.1. Identification of the URR-BoLA-DRB3 polymorphism by SSCP analysis

In this study, we described through PCR-SSCP and DNA sequencing the allelic-specific polymorphisms within the *URR-BoLA-DRB3* in 15 cattle breeds. In total, seven PCR-SSCP defined alleles were detected for the analyzed animals. The different band patterns observed were denominated from "A" to "G", as shown in Fig. 1a. To confirm the identity of the SSCP variants in putative heterocygous animals, two different analysis were carried out. First, the PCR products from homocygotes and heterocygotes cattle were cloned, and the SSCP patterns were observed in the cloned DNA compared with those previously detected in genomic DNA. Second, the Mendelian inheritance of these SSCP variants was verified by family segregation. In all cases, the alleles observed in the offspring were present in either the dam or the sire (Fig. 1b).

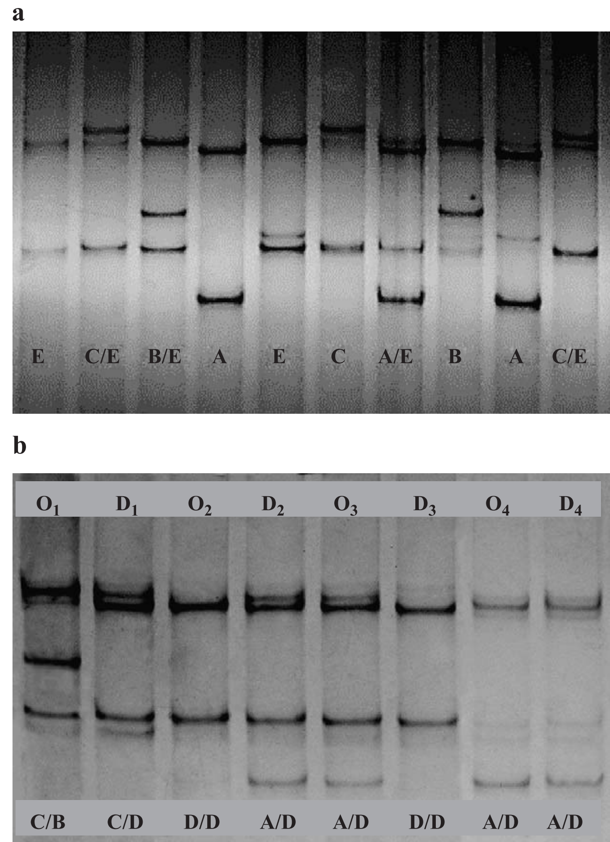

Fig 1. (a) Some PCR-SSCP defined alleles detected for the analyzed animals. Inferred patterns are indicated at the bottom. (b) The Mendelian inheritance of SSCP variants was verified by family segregation. The alleles observed in the offspring were present in either the dam or the sire. Inferred patterns are indicated at the bottom. O and D indicated offspring and dam, respectively.

The gene frequencies estimated for each breed were calculated (data not shown). The n_a within each studied breed varied between 2 and 5. The C and E variants were detected in all breeds, the A, B, D and F alleles were observed in 10, 7, 10 and 2 breeds, respectively, while the G variant was privative of Holstein breed. The h_e ranged from 0.268 in the Jersey breed to 0.816 in the Brahman breed (Table 1). Significant differences across the studied bovine breeds were detected with F_{ST} ($F_{ST}=0.1188$, P value = 0.000). This significant genetic differentiation among breeds is probably a consequence of both allele combination within each breed and their gene frequency profiles (Table 1). The result is in agreement with previous studies that showed significant levels of genetic subdivision based on serological, protein and DNA sequences of BoLA-DRB3

Table 1
Genetic variability of BoLA-DRB3-URR within the studied populations through the PCR-SSCP method

Breed	*N*	n_a	h_e	Observed PCR-SSCP variants
Aberdeen Angus	11	5	0.749	A, B, C, D y E
Hereford	11	2	0.312	C y E
Holstein	10	5	0.784	A, C, E, F y G
Jersey	10	2	0.268	C y E
Charolais	10	3	0.668	C, E y F
Normande	4	2	ND	C y E
Creole Argentine	26	4	0.732	A, C, D y E
Creole Saavedreño	12	5	0.580	A, B, C, D y E
Creole Chaqueño	12	5	0.764	A, B, C, D y E
Creole of Valley	10	4	0.784	A, C, D y E
Creole Chusco	7	5	ND	A, B, C, D y E
Creole Yacumeño	10	5	0.668	A, B, C, D y E
Nelore	11	4	0.775	A, C, D y E
Brahman	10	5	0.816	A, B, C, D y E
Brangus	8	4	0.692	B, C, D y E

between breeds (i.e., Maillard et al., 1989; Mikko and Andersson, 1995; Giovambattista et al., 2001; Takeshima et al., 2003; Ripoli et al., in press).

3.2. Analysis of URR-BoLA-DRB3 polymorphism by DNA sequencing

In a previous work, we characterized the sequence organization of the *URR-BoLA-DRB3* gene (Ripoli et al., 2002), founding that this region is about 222 bp from the +1 position to the W box and is composed of highly conserved sequence motifs that include from 5′ to 3′ direction, the W, X, Y, CCAAT and TATA like boxes. In the present study, we selected the most frequent SSCP variants for cloning and DNA sequencing. All sequenced clones corresponding to the same SSCP-defined variant exhibited 100% sequence similarity to each other.

DNA sequences of the *BoLA-URR* variants defined by PCR-SSCP showed that the A pattern match the sequence AF510446 previously published by Ripoli et al. (2002), while the B, C, D and E SSCP variants corresponded with new DNA sequences AY364454, AY364455, AY570362 and AY040327, respectively. Furthermore, neither of the sequences present here corresponded with the sequence AJ488500 reported by Russell (2002).

The AF510446, AY040327, AY364454, AY364455, AY570362 *URR-BoLA-DRB3* variants reported in this study and the AJ488500 sequence reported by Russell (2002) were aligned (Fig. 2). The alignment showed six polymorphic sites (four transitions, one transversion and one deletion) in the interconsensus regions: one polymorphic site between the X and the Y boxes (position −161), two between the Y and the CCAAT boxes (positions −138 and −135), one deletion in the sequence AY364455 between the CCAAT and TATA boxes (position −104) and two downstream of the TATA box (positions −27 and −21). However, an examination of these sequences did not evidence polymorphism within consensus boxes, showing the same conserved motifs at the X, Y CCAAT and TATA boxes. The polymorphic sites resulted in a nucleotide diversity of 0.0109 ± 0.008, as estimated by the Kimura two-parameter method.

These data contrasted with the results reported in mouse and human. In both cases, polymorphic positions were detected in both the regions of transcription factor binding and the interconsensus regions (e.g. Cowell et al., 1998). Furthermore, variability in bovine and human is distributed evenly across the promoter region, while in mouse, polymorphic sites are clustered in two groups, one in the 3′ half of the promoter and one dense group centered over the X box (Cowell et al., 1998). Also, the bovine URR-BoLA-DRB3 seems to be less polymorphic than the orthologous regions in mouse and human (Kimura two parameters$_{mouse}$: 0.0211 ± 0.014; Kimura two parameters$_{human}$: 0.0846 ± 0.042).

The absence of polymorphism within regulatory boxes and the moderate level of variability in the URR-BoLA-DRB3 could be the consequence of one or more of the following points. First, the nucleotide diversity estimated in this study corresponds to a unique functional gene, while the variability calculated in humans included interloci and allelic polymorphisms. Second, the relatively low variation compared with the human and mouse could be reflecting a lack of genetic diversity in domesticated cattle resulting from a small founding population. And third, these results could indicate the existence of positive selection, which would be eliminating mutations within the conserved motif that could affect the transcription factors binding site and, as a result, the tightly regulatory process. Regarding this last possibility, Cowell et al. (1998) suggested two alternative hypotheses to explain the maintenance and removal of mutations in the regulatory region. The first one proposed that the level of variation in the promoter box regions may simply be tolerated rather than actively selected, while the second hypothesis postulated that it may be that diversity in the boxes was actually selected in the past but has reached its maximum tolerable level, whereas nonbox regions have continued to accumulate changes. The last alternative seems to be the case of the URR-BoLA-DRB3. However, further studies are necessary to estimate the total amount of nucleotide diversity present in the proximal regulatory region of this gene in domestic cattle.

3.3. Phylogenetic analysis of promoter region from bovine, human and murine DRB genes

The neighbor-joining tree showed two major clusters that include DRB and DQB promoter sequences, respectively (Fig. 3). The same tree topology was found using the Jukes–Cantor and the Kimura two-parameter distances. The first cluster comprises three groups corresponding to the *HLA-DRB*, *I-Eb* and *BoLA-DRB3* promoter sequences, respectively, supported by bootstrap values higher than 67%. The

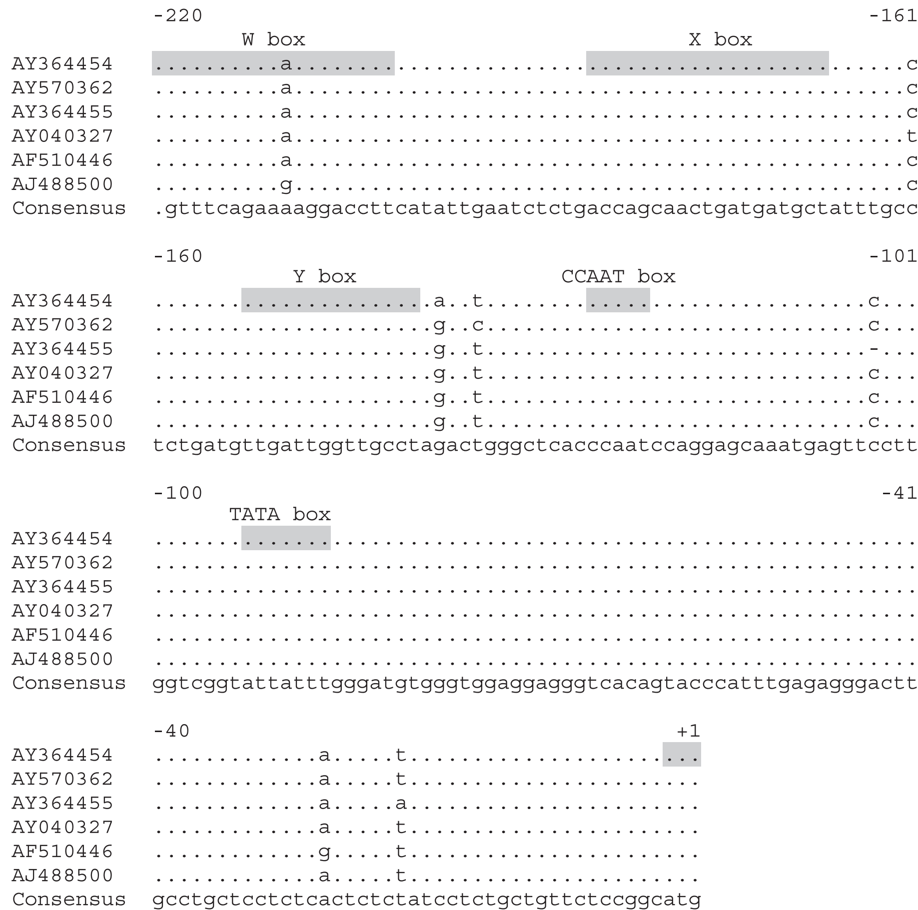

Fig 2. Alignment of the nucleotide sequences of upstream regulatory regions (URR) of *BoLA-DRB* AY364454, AY040327, AY364455, AF510446, AJ488500 and AY570362 *URR-BoLA-DRB3* variant). The A of ATG is designated as +1. Dots indicate nucleotide identity to the consensus sequence, and dashes (–) represent gaps introduced to achieve the best alignment.

bootstrap values were 100% for the *I-Eb* and *Bola-DRB3* promoter sequences clusters, while the bootstrap value for the *HLA-DRB* group was 67%. The second cluster included two groups that correspond to *HLA-DQB* and I-Ab URR sequences (100% bootstrap value in each cluster).

Several authors demonstrated that the URR sequence organization of DRB differs from the paralogous DQB promoter (Benoist and Mathis, 1990; Glimcher and Kara, 1992). This divergence was clearly evidenced in the phylogenetic tree, which exhibited two different clusters for the promoters. As mentioned above, within each major cluster, all URR sequences belonging to the same species were grouped together. This fact suggests that the observed polymorphisms could have occurred after the divergence of the three included orders. In the case of cattle, the moderate level of nucleotide diversity, the high percentage of identity and the short length of the branch within its cluster could evidence that the polymorphism reported in this study occurred more recently.

The pair-wise comparisons among all URR-BoLA-DRB3 DNA sequences showed an identity ranging from 98.3% to 99.2%. These values of similarity decreased when bovine URR-DRB3 sequences were compared with human and mouse DRB promoters (varying from 58.6% to 76.3% and 67.9% to 70.8%, respectively). As expected, the lowest values of identity were obtained when URR-BoLA-DRB3 sequences were compared with human and mouse DQB regulatory region (19% to 25% and 25.4% to 26.4%, respectively). The identity results were in concordance with the topology of the phylogenetic tree. In addition, the phylogenetic tree, the similarity analyses and the sequence structure confirmed that the fragment analyzed in this study corresponds to URR-BoLA-DRB3.

3.4. Putative implications of URR-BoLA-DRB3 polymorphism

The promoter variation may likely affect inducibility or tissue specificity and may also play a role in susceptibility to infectious diseases, being both independent of coding region variation. Five out of six detected polymorphic sites were one nucleotide substitution in the interconsensus regions. It would be expected that these mutations do not affect the level of expression. In contrast, the deletion

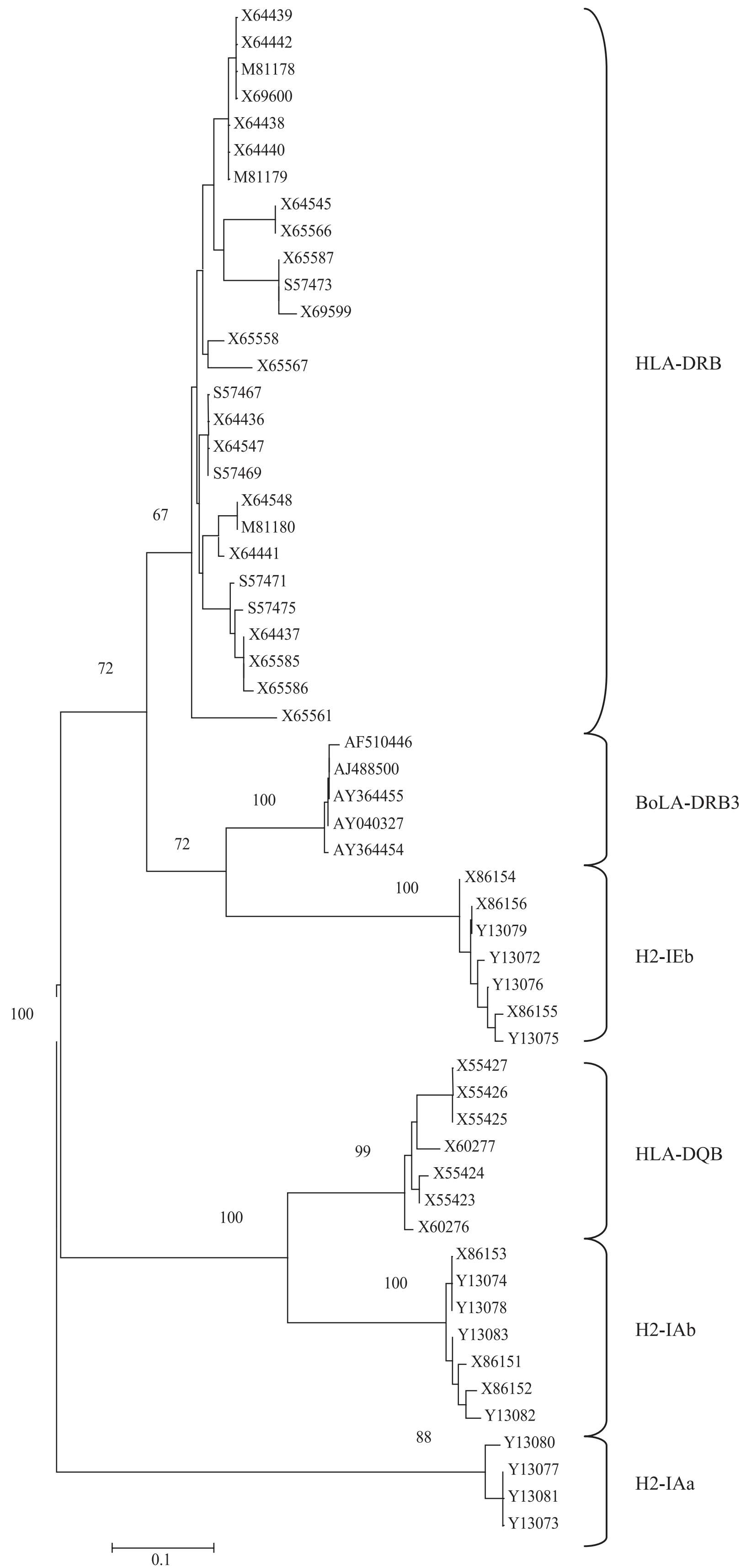

Fig 3. Phylogenetic tree of URR sequences from human and bovine *DRB* and *DQB* genes, and murine IEb and IAb orthologous genes, and murine IAa genes.

observed in the sequence AY364455 between the CCAAT and TATA boxes could have some effect on affinity interactions between the promoter region and the transcription factors. This assumption is supported by evidence reported in procariotes and eucariotes. In procariotes, the distance separating the − 35 and − 10 sites is between 16 and 18 bp in 90% of the promoters. This distance is critical in holding the two sites at the appropriate separation for the geometry of RNA polymerase (Lewin, 2000). In human, Hake et al. (2003) observed that the difference among all class II promoters and the DOB promoter is the distance between X2 and Y boxes. While all other class II gene promoters have 14 or 15 nucleotides between these boxes, the corresponding length for the DOB promoter is only 13 nucleotides (Servenius et al., 1987). This difference may partially explain why DOB is only weakly expressed in B cells compared to other class II genes. Furthermore, this one-nucleotide difference might explain the high affinity interactions between the DRA promoter and the transcription factors RFX and NF-Y. Recently, Heldt et al. (2003) demonstrated in human the functional consequences of variation close to the TATA box. Since the DNA–protein binding and functional analyses were not done in the present study, the functional role of detected polymorphisms in the promoter region in the regulation of the expression of *BoLA-DRB3* gene is unknown.

In humans, the high level of linkage disequilibrium between HLA-DQ promoters and their exons has made impossible to assess the significance of promoter variation on disease. For this reason, it is important to know about linkage disequilibrium between *BoLA-DRB3* promoter sequences and the corresponding exon 2 sequences. This research is actually being carried out by our group.

In summary, the PCR-SSCP and DNA sequencing technique used in the present study provided evidence of polymorphism in the interconsensus regions of the *BoLA-DRB3* upstream regulatory region. The functional role of the polymorphic sites in bovine needs to be analyzed and confirmed by means of gene expression assays.

Acknowledgements

We thank Dr. Patricia Mirol for her many helpful comments on the manuscript. This research was supported by grants PICT 99 No 0805216 of the Secretaría de Ciencia y Técnica de la Nación and the Japan International Cooperation Agency (JICA).

References

Altschul, S.F., Madden, T.L., Schaffer, A.A., Zhang, J., Zhang, Z., Miller, W., Lipman, D.J., 1997. Gapped BLAST and PSI-BLAST: a new generation of protein database search programs. Nucleic Acids Res. 25, 3389–3402.

Andersen, L.C., Beaty, J.S., Nettles, J.W., Seyfried, C.E., Nepom, G.T., Nepom, B.S., 1991. Allelic polymorphism in transcriptional regulatory regions of HLA-DQB genes. J. Exp. Med. 173, 181–192.

Benoist, C., Mathis, D., 1990. Regulation of major histocompatibility complex class II genes: X, Y, and other letters of the alphabet. Annu. Rev. Immunol. 8, 681–715.

Cowell, L.G., Kepler, T.B., Janitz, M., Lauster, R., Mitchinson, N.A., 1998. The distribution of variation in regulatory gene segments, as present in MHC Class II promoters. Genome Res. 8, 124–134.

Emery, P., Mach, B., Reith, W., 1993. The different level of expression of HLA-DRB1 and -DRB3 genes is controlled by conserved isotopic differences in promoter sequence. Hum. Immunol. 38, 137–147.

Giovambattista, G., Ripoli, M.V., Peral García, P., Bouzat, J.L, 2001. Indigenous domestic breeds as reservoirs of genetic diversity: the Argentine Creole cattle. Anim. Genet. 32, 240–247.

Glimcher, L.H., Kara, C.J., 1992. Sequences and factors: a guide to MHC class II transcription. Annu. Rev. Immunol. 10, 13–49.

Hake, S.B., Tobin, H.M., Steimie, V., Denzin, L.K., 2003. Comparison of the transcriptional regulation of classical and non classical MHC class II genes. Eur. J. Immunol. 33, 2361–2371.

Heldt, C., Listing, J., Sozeri, O., Blasing, F., Frischbutter, S., Muller, B., 2003. Differential expression of HLA class II genes associated with disease susceptibility and progression in rheumatoid arthritis. Arthritis Rheum. 48 (10), 2779–2787.

Kumar, S., Tamura, K., Nei, M., 1994. MEGA: molecular evolutionary genetics analysis software for microcomputers. Comput. Appl. Biosci. 10, 189–191.

Lewin, B., 2000. Genes VII: Chap. 9: Transcription. Oxford University Press and Cell Press, Oxford.

Maillard, J.C., Kemp, S.J., Leveziel, H., Teale, A.J., Queval, R., 1989. Le complexe majeurd'histocompatibilitie de bovins ouest africains. Typage dántigenes lymphocytaires (BoLA) de taurins Baule (*Bos taurus*) et de Zebu Soudaniens (Bos indicus) du Burkina Faso (Afrique Occidentale). Zootechnie 42, 275–281.

Mikko, S., Andersson, L., 1995. Extensive MHC class II DRB3 diversity in African and European cattle. Immunogenetics 42, 408–413.

Mitchinson, N.A., Roes, J., 2002. Patterned variation in murine MHC promoters. Proc. Natl. Acad. Sci. U. S. A. 99 (16), 10561–10566.

Nei, M., 1987. Molecular Evolutionary Genetics. Columbia University Press, New York.

Pascale, L., Eliaou, J.F., Kerlan-Candon, S., Pinet, V., Vincent, R., Clot, J., 1993. Polymorphism in the regulatory region of HLA-DRB genes correlating with haplotype evolution. Immunogenetics 38, 21–26.

Reichstetter, S., Krellner, P.H., Meenzen, C.M., Kalden, J.R., Wassmuth, R., 1994. Comparative analysis of sequence variability in the upstream regulatory region of the HLA-DQB1 gene. Immunogenetics 39, 207–212.

Ripoli, M.V., Díaz, S., Peral-García, P., Giovambattista, G., 2002. Nucleotide sequence of the upstream regulatory region of the BoLA-DRB. Eur. J. Immunogenet. 29, 537–540.

Ripoli, M.V., Lirón, J.P., De Luca, J.C., Rojas, F., Dulout, F.N, Giovambattista, G., 2004. Gene frequencies distribution of the BoLA-DRB3 locus Saavedreño in Creole Dairy Cattle. Biochem. Genet. 42 (7/8) (In press).

Russell, G.C., 2002. Bos taurus partial BLA-DRB3 gene for MHC class II antigen, DRB3*2502 allele, promoter region and exon 1. Unpublished.

Schneider, S., Roessli, D., Ecoffier, L., 2000. Arlequin, Ver. 2.000. A software for population genetic data analysis. University of Geneva, Geneva, Switzerland.

Servenius, B., Rask, L., Peterson, P.A., 1987. Class II genes of the human major histocompatibility complex. The DO beta gene is a divergent member of the Class II beta gene family. J. Biol. Chem. 262, 8759–8766.

Singal, D.P., Qiu, X., 1994. Polymorphism in the upstream regulatory regions and level of expression of HLA-DRB genes. Mol. Immunol. 31, 1117–1120.

Singal, D.P., Qiu, X., 1996. Polymorphism in both X and Y motifs controls level of expression of HLA-DRB1 genes. Immunogenetics 43, 50–56.

Singal, D.P., Qiu, X., D'Souza, M., Sood, S.K., 1993a. Polymorphism in the upstream regulatory regions of HLA-DRB genes. Immunogenetics 37, 143–147.

Singal, D.P., D'Souza, M., Sood, S.K., Qiu, X., 1993b. Sequence-specific interactions of the nuclear proteins with polymorphic upstream regulatory regions of HLA-DRB genes. Transplant. Proc. 25, 180–182.

Takeshima, S., Saitou, N., Morita, M., Inoko, H., Aida, Y., 2003. The diversity of bovine MHC class II DRB3 genes in Japanese Black, Japanese Shorton, Jersey and Holstein cattle in Japan. Gene 316, 111–118.

Thompson, J.D., Higgins, D.G., Gibson, T.J., 1994. CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, positions-specific gap penalties and weight matrix choice. Nucleic Acids Res. 22, 4673–4680.

Ting, J.P.Y., Trowsdale, J., 2002. Genetic control of MHC class II expression. Cell 109, 521–533.

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, 117–128.