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Sequencing of an upstream region of the human HLA-DRA gene containing X′ and Y′ boxes

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ABSTRACT

In this paper we report the characterization of a newly sequenced 5′ upstream region of the human HLA-DRA gene. We performed (i) search for transcription factor motifs, (ii) analysis of CpG display and observed/expected frequency ratios, (iii) search for regions homologous to the 5′ upstream sequences of the murine EA gene, (iv) DNase I footprinting experiments and (v) electrophoretic mobility shift assays. Our results demonstrate the existence, in the HLA-DRA gene, of Y′ and X′ boxes highly homologous to the Y and X boxes present in MHC class II genes, but oriented in the opposite direction. These Y′ and X′ boxes have been conserved during the molecular evolution of both human HLA-DRA and murine EA genes. DNase I footprinting and gel retardation experiments suggest that the X′ and Y′ boxes of the HLA-DRA upstream gene region are specifically recognized by nuclear proteins that also bind to the X and Y boxes of the HLA-DRA proximal promoter, respectively.

INTRODUCTION

Tissue-specific expression of class II molecules encoded by the major histocompatibility complex (MHC) is crucial to the regulation of antigen-specific immune responses (1,2). Because interactions between transcription factors and DNA elements present in eukaryotic promoters is a prerequisite for the regulation of constitutive and induced gene expression (3–5), considerable efforts have been devoted, over the past few years, to the elucidation of the DNA sequences of the promoters of class II MHC genes, especially the HLA-DRA gene (6,7), which encodes the heavy chain subunit (DRα) of the main subset of human class II antigens (7).

The sequences of the proximal promoter of class II MHC genes contain a number of *cis*-elements recognized by DNA-binding proteins (8–12). Among the DNA regulatory elements so far identified and demonstrated to be functional, are the X, Y and Z(W)-boxes (8,9), the octamer motif (13), the ICS region (11) and the TATA box (9). In addition to the signals present in the proximal promoter, intragenic enhancer regions have also been described in DRA, DQA and DQB genes (8,9).

With respect to the regulatory *cis*-elements of class II genes, the largest number of published reports are concerned with the biological functions of X and Y boxes. These evolutionarily conserved elements are specifically recognized by a variety of transcription factors, such as NF-Ya, NF-Yb, YB1, hXBP-1, mXBP and RF-X (9,10,14). Recently published results clearly indicate that most of these factors are capable of generating homodimeric and/or heterodimeric complexes (15–20).

Although the proximal promoter has been the most studied regulatory region of the human HLA-DRA gene, the analysis of upstream gene regions also appears to be of great interest, since (i) Y- and X-like boxes are present in upstream control regions (between −1906 and −1180) of the murine EA gene (8–10), and (ii) the deletion of these regions in transgenic animals carrying a truncated EA gene alters its tissue-specific transcription (8,9,21–23). An intriguing observation is that the Y and X boxes present within the EA upstream control region are located in the opposite orientation (8,9), suggesting stem–loop arrangements of the proximal promoter and upstream region, possibly mediated by the dimerization of Y- and X-binding proteins. Although this region is supposed to play a functional role in regulating the expression of murine EA gene, no information is available for the human HLA-DRA gene.

In view of the possible regulatory functions of upstream sequences of the HLA-DRA gene (8,9,24–26), we have determined the 5′ nucleotide sequence of the *Eco*RI–*Eco*RI fragment of recombinant plasmid T9C (2), carrying a complete functional HLA-DRA gene. Previously published results from another (27) and our laboratory (28–30) demonstrate that transgenic mice carrying the *Sal*I–*Sal*I fragment of the T9C cosmid are able to express the DRA transgene in a tissue-specific, interferon-induced fashion (27–30).

MATERIALS AND METHODS

Subcloning of the *Eco*RI–*Eco*RI fragment of T9C and sequencing strategy

The 4.5 kb DNA fragment, generated by digestion of T9C cosmid (2) with *Eco*RI, was inserted in the pUC18 sequencing vector (31). Six clones were sequenced by the dideoxynucleotide chain

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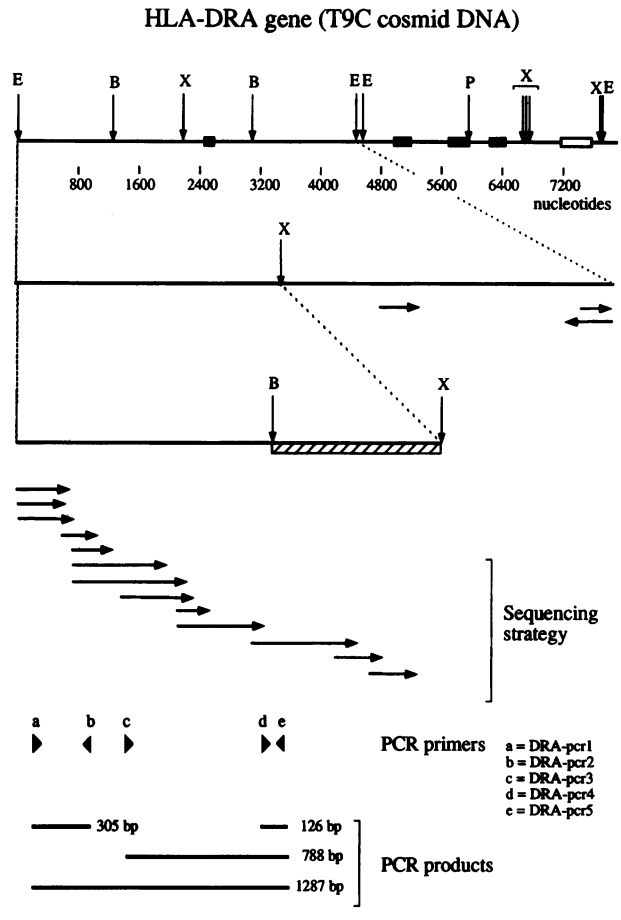

Figure 1. Structure of the human HLA-DRA gene, showing the location of *Eco*RI (E), *Bgl*II (B), *Xba*I (X), *Pst*I (P) restriction enzyme sites. Black boxes = translated exons; open box = 3′ untranslated exon. This sequence was deduced from restriction fragment length analysis of T9C cosmid DNA (2). The cloned *Eco*RI–*Eco*RI DRA fragment is indicated. Horizontal arrows indicate the sequences performed. The shaded segment indicates the sequences elsewhere described (37) of the *Bgl*II–*Xba*I fragment of HLA-DRA gene. In the lower part of the panel black triangles indicate the location of the primers used for polymerase chain reaction (PCR) and the expected sizes of the PCR products. bp = base pairs.

termination method (32) and analysed using the A.L.F. DNA™ Sequencing System (Pharmacia, Uppsala, Sweden).

Nested sets of deletions of these clones were generated by controlled exonuclease III-mung bean nuclease digestions (31). Figure 1 represents the direction and the extent of the individual sequences obtained.

Polymerase chain reaction

In vitro DNA amplifications by the polymerase chain reaction (PCR) (33) were performed in 50 µl of 10 mM KCl, 10 mM Tris–HCl pH 8.3, 2 mM $MgCl_2$ by using 2 U/reaction of *Taq* DNA polymerase (Perkin-Elmer), template DNA and 150 ng of the primers represented in Figures 1 and 2. For template DNA, 30 ng of T9C cosmid or 1 µg of human genomic DNA from different donors were used. Amplification conditions were as follows: denaturation, 1 min, 92°C; annealing, 1 min, 51°C. Elongation times at 72°C were: 45 s for DRA-pcr4/DRA-pcr5 (126 bp) and DRA-pcr1/DRA-pcr2 (305 bp) and 2.5 min for DRA-pcr3/DRA-pcr5 (788 bp) and DRA-pcr1/DRA-pcr5 (1287 bp) PCR products (Fig. 1).

Cloning and sequencing of PCR products obtained by amplification of 5′ upstream HLA-DRA gene regions from human DNA

DRA-pcr1/DRA-pcr2 and *Hin*dIII-digested DRA-pcr1/DRA-pcr5 PCR products obtained by using DNAs from two different individuals were inserted in the pUC18 vector. A number of clones were sequenced in both orientation by using the A.L.F. DNA™ Sequencing System (Pharmacia, Uppsala, Sweden).

Direct sequencing of 5′ upstream HLA-DRA gene regions amplified by PCR

In order to further confirm sequencing data, purified DRA-pcr1/DRA-pcr2 305 bp PCR products from both genomic DNA and T9C cosmid were subjected to direct sequencing by the dideoxynucleotide chain termination method (34) using [α^{32}P]dATP.

Computer-assisted analyses

For computer-assisted analysis, we used the MacVector Sequence Analysis Software (IBI, International Biotechnologies, Inc., New Haven, CT, USA) for Macintosh Computers. The genomic sequences analysed were from GenBank®, release 70.0 (Intelli-Genetics Incorporated, Mountain View, CA).

Pustell DNA Matrix Analyses (35,36) were performed with the following parameters: window size = 30; strand = both; min % score = 65; jump = 1; hash value = 6. The 5′ region of the human HLA-DRA gene was compared with the 5′ region of the mouse MHC class II H2-IE-alpha-d gene (GenBank entry MUSHIEDA, accession number M18158, ID number 199522) (23).

For analysis of the observed/expected frequency ratios of the CpG, TpG and CpA dinucleotides, we used the option *Base composition* of the MacVector Sequence Analysis Software (IBI, International Biotechnologies, Inc., New Haven, CT, USA). The sequence analysed was derived by the fusion of the newly sequenced 5′ region of the human HLA-DRA gene (assigned accession number X83114, European Bioinformatics Institute) and the GenBank entries HUMMHDRHA (accession number X00274, ID number 32136) (7) and HUMMHDRS2 (accession number J00204, ID number 188426 and 188427) (37).

Cell cultures and purification of nuclear factors

The human H9 cell line (38), expressing at high level both DRA mRNA and DRα protein (data not shown) was grown in RPMI medium, supplemented with 10% FBS at 37°C in 5% CO_2 humidified atmosphere (34). Nuclear extracts were prepared according to the procedure described by Dignam *et al.* (39).

DNase I footprinting

A DNA fragment mimicking a region of the 5′ upstream HLA-DRA gene containing both X′ and Y′ boxes (see Fig. 1 for location of the primers) was prepared by PCR (40) using 1 µg of human genomic DNA as template and 150 ng of cold DRA-pcr1 and ^{32}P-end-labelled DRA-pcr2 primers. The ^{32}P-labelled 305 bp amplified PCR product was purified by low-melting gel electrophoresis and phenol–chloroform extraction, ethanol precipitated

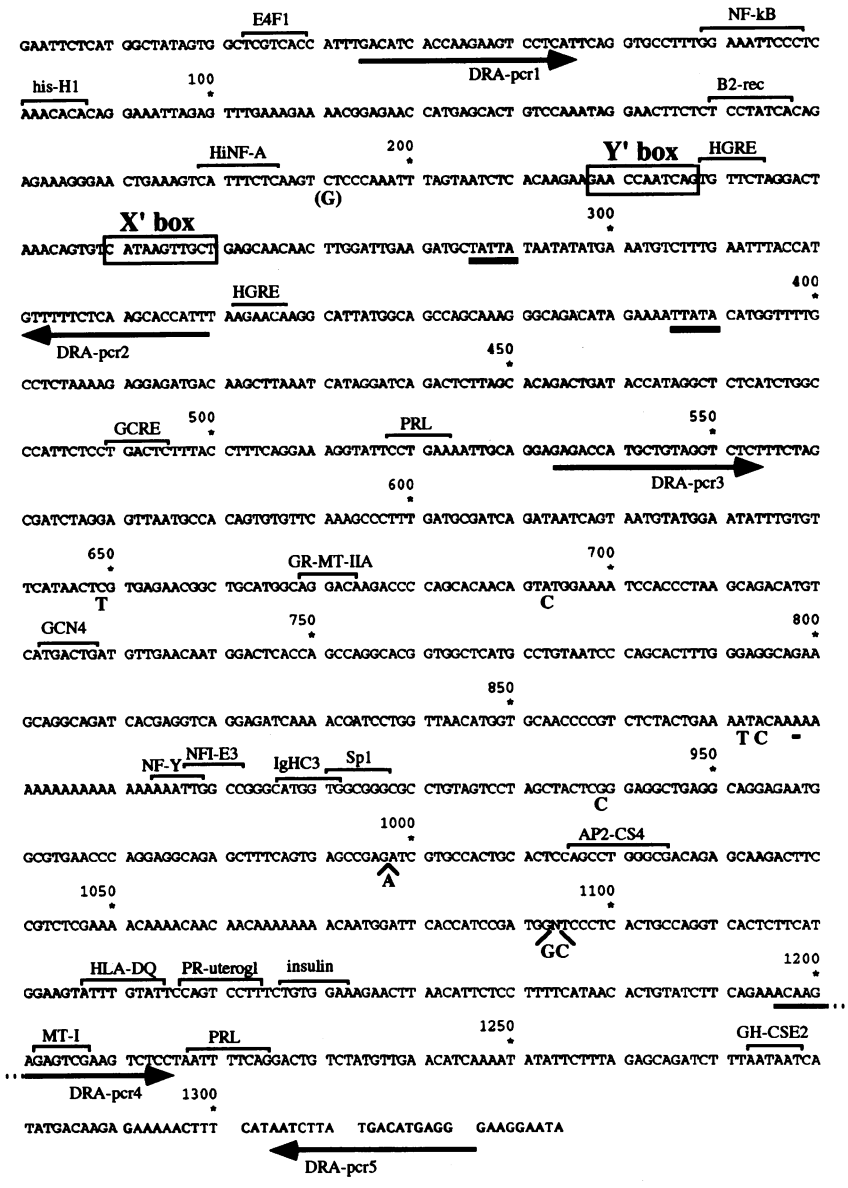

Figure 2. Nucleotide sequence of the 5′ region of the cloned *Eco*RI–*Eco*RI fragment of T9C cosmid DNA (2). Bases reported below this sequence represent nucleotide variations with respect with sequenced human DRA-pcr1/DRA-pcr5 products (individual a of Fig. 3). The C→G base substitution (in parenthesis) corresponding to nucleotide position 191 refers to sequenced DRA-pcr1/DRA-pcr2 product from DNA of subject b (Fig. 3). The hyphen corresponding to nucleotide position 878 represents a 1 nt deletion. TATA-like boxes are underlined. Y′ and X′ sequences are boxed. A partial list of transcription signals is also indicated (4,46–48). Horizontal arrows indicate location and nucleotide sequences of the PCR primers used for PCR-mediated amplification of upstream HLA-DRA sequences of normal human individuals (see Fig. 1 for lengths of PCR products and Fig. 3 for results of PCR amplifications).

and washed through microcon 30 (Amicon Inc-Grace Company, Beverly, MA, USA) with 200 µl of water. DNA was recovered in water and diluted at 10 000 c.p.m./µl. Footprinting reactions were carried out as described (40). Molecular weight markers were obtained by a G+A sequencing reactions (40) of the ^{32}P-labelled 305 bp amplified PCR product. Quantitative analyses of DNase I protection patterns were performed using the Molecular Analyst™/Macintosh Image Analysis Software version 1.1 for the Model GS-670 Imaging Densitometer (Bio-Rad Laboratories).

Electrophoretic mobility shift assay

The electrophoretic mobility shift assay (EMSA) (41) was performed by using double-stranded synthetic oligonucleotides containing the X, Y, X′ and Y′ boxes as well as the target DNA sequences of transcription factors NF-κB and Sp1. The nucleotide sequences of the sense strand of the oligonucleotides used were the following: 5′-AGAAGAACCAATCAGTGTTCT-3′ (Y′ mer), 5′-AGTGTCATAAGTTGCTGAGCA-3′ (X′ mer), 5′-TTTTTCTGATTGGCCAAAG-3′ (Y mer), 5′-ACCCTTCC-CCTAGCAACAGATGCGTCATCT-3′ (X mer), 5′-ATTCGAT-CGGGGCGGGGCGAG-3′ (Sp1 mer) and 5′-AGTTGAGGG-ACTTTCCCAGGC-3′ (NF-κB mer). Binding reactions were set up as described elsewhere (40,41) in the presence of the indicated concentrations of poly(dI:dC)·poly(dI:dC) (Pharmacia, Uppsala, Sweden), 2 µg of crude nuclear extract (39) and 0.25 ng of [γ-^{32}P]ATP labelled oligonucleotide, in a total volume of 25 µl.

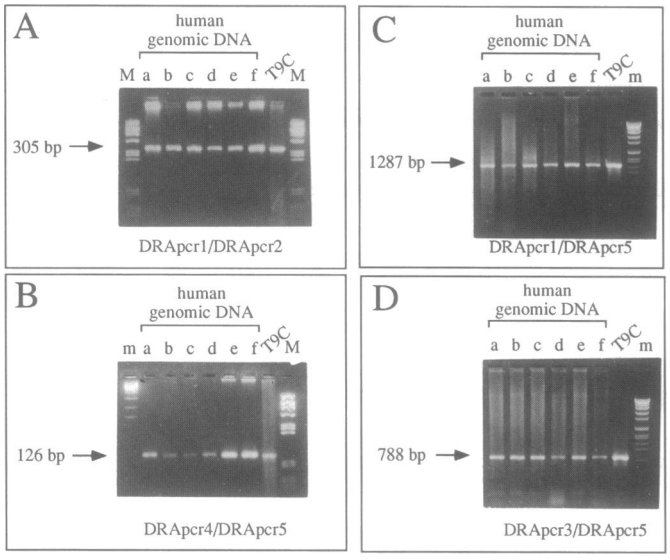

Figure 3. Polymerase chain reactions (PCRs) performed on T9C cosmid or DNAs from white blood cells of six normal human subjects (a–f). PCRs were performed with the primers indicated. Sizes of PCR products are arrowed. M and m = molecular weight markers; M = *Alu*I digested pBR322; m = *Eco*RI digested SPP1 DNA.

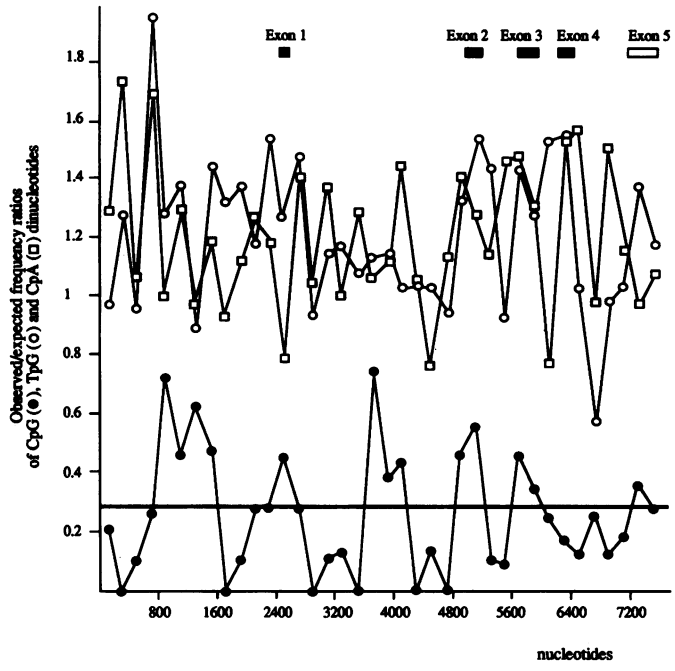

Figure 4. Observed/expected frequency ratios of CpG, TpG and CpA dinucleotides within 200 bp segments of the human HLA-DRA gene. The sequence analysed was obtained after fusion of the new 5′ upstream HLA-DRA sequence (Fig. 2) with HUMMHDRS2 (37) and HUMMHDRHA (7) GenBank®™ entries. Boxes represent exons.

Addition of the reagents was as follows: (i) poly(dI:dC)·poly(dI:dC); (ii) cold double-stranded DNA competitor; (iii) labelled double-stranded oligonucleotide; (iv) binding buffer (41) containing crude nuclear extracts.

RESULTS

Nucleotide sequence of the upstream region of the human HLA-DRA gene

Figure 1 shows the sequencing strategy and the sequencing reactions performed (horizontal arrows) using the A.L.F.™ automated Sequencing System. We have performed 13 partially overlapping sequencing reactions corresponding to regions comprised between the upstream *Eco*RI and the *Xba*I restriction enzyme sites. Figure 2 shows the nucleotide sequence of the 5′ region of the human HLA-DRA gene, starting from the 5′ *Eco*RI site. Sequences between *Bgl*II and *Xba*I sites overlap with already published DRA sequences (GenBank HUMMHDRS2, accession number J00204, ID numbers 188426 and 188427) (37). Sequencing of the intronic portion identified by the 3′ *Eco*RI site confirms sequences elsewhere published (7,37) and it is therefore not reported here. Figure 3 shows polymerase chain reaction analyses performed on human genomic DNA (see Fig. 1 for location of the primers) and showing that the amplified products are identical to those obtained using T9C cosmid as target DNA.

In addition, DRA-pcr1/DRA-pcr2 and *Hin*dIII-digested DRA-pcr1/DRA-pcr5 PCR products obtained by using DNAs from the individuals a and b (see Fig. 3 for analysis of PCR products) were cloned and sequenced confirming the sequence analysis of T9C cosmid with exception of a few nucleotides (Fig. 2 and data not shown).

Analysis of distribution of CpG dinucleotides: evidence for a CpG rich island

Figure 4 shows the analysis of the observed/expected ratios of CpG, CpA and TpG dinucleotides, performed on the complete sequence of the human HLA-DRA gene, obtained by fusing the sequence reported in Figure 2 with two previously published sequences (GenBank entries HUMMHDRS2 and HUMMHDR-HA, accession numbers J00204 and X00274, respectively), one of which (HUMMHDRS2) partially overlaps with that sequenced by us. The analysis of observed/expected ratios was conducted in each 200 nt-long portion of the complete sequence of the HLA-DRA gene. This analysis demonstrates that the *Eco*RI–*Xba*I 5′ upstream HLA-DRA region displays CpG suppression, with the exception of the region comprised between nucleotides 850 and 1600. While the finding of CpG suppression is not unexpected (the HLA-DRA gene is known as extensively methylated and CpG suppressed) (42–45), the presence of this CpG-rich island is of interest, since it is well known that CpG rich regions oftenly contain transcription signals (for instance Sp1 binding sites) and play functional roles.

Identification of motifs for transcription factors

Computer-assisted analysis of the nucleotide sequence shown in Figure 2 was performed in order to identify further putative signals for transcription factors. The MacVector search program was used with the compilation of transcription factor signals (4,46–48). This analysis evidentiates the presence of some

transcription control signals (underlined or boxed), including a NF-κB site and a Sp1 site at positions 69–78 and 911–917, respectively (Fig. 2). The Sp1 site is located within the CpG rich region previously reported.

Examples of the identified motifs for transcription factors are reported in Figure 2. It should be underlined the presence in the newly sequenced upstream HLA-DRA region of two boxes, Y′ and X′, homologous to the Y and X boxes present in the proximal promoters of class II MHC genes, but oriented in the opposite direction. This finding is of particular interest, since the Y and X boxes are crucial to class II MHC gene expression.

In agreement with this interpretation, we have confirmed the presence of the Y′ and X′ boxes in human HLA-DRA gene from eight normal unrelated individuals by amplification of the upstream region using the DRA-pcr1 and DRA-pcr2 primers and direct sequencing of the PCR products (data not shown).

Homology between the murine EA upstream sequences and the upstream region of the human HLA-DRA gene

Studies on the nucleotide sequence homology between the newly sequenced upstream region of the human HLA-DRA gene and the known sequence of the murine EA upstream promoter clearly show that the DRA-pcr1/DRA-pcr2 region exhibits extensive homologies to the so-called B-cell control region of the murine EA gene (Fig. 5A), in particular with three distinct regions (see Fig. 5B).

Among these regions, of most interest is region C, between nucleotides 218 and 228 and 250 and 260, displaying 100% homology with the Y′ and X′ boxes of the EA gene.

Binding of nuclear proteins to the X′ and Y′ boxes

In order to determine whether the X′ and Y′ boxes belong to regions recognized by nuclear factor(s), DNase I footprinting and electrophoretic mobility shift assay (EMSA) have been performed. The DNase I footprinting results are shown in Figure 6, indicating that, when crude nuclear extracts from H9 cells are employed, less efficient cleavage by DNase I occurs at the level of DNA regions containing both X′ and Y′ boxes, suggesting that the X′ and Y′ boxes belong to a HLA-DRA region extensively recognized by nuclear proteins. The electrophoretic mobility shift assay (EMSA) was employed in order to confirm the results of Figure 6 and to determine whether X′ and Y′ boxes are identified by proteins able to interact with X and Y boxes of the HLA-DRA proximal promoter. The results of EMSA are shown in Figure 7 and demonstrate that the electrophoretic mobility of ^{32}P-labelled double-stranded X′ and Y′ mers is shifted when the oligonucleotides are incubated with crude nuclear extracts from H9 cells (Fig. 7, see lanes C, control). Bound X′ and Y′ mers are clearly detected even in the presence of 800 ng of poly(dI)(dC)·poly(dI)(dC) (data not shown); in addition, Figure 7 shows that the binding of nuclear factors to the ^{32}P-labelled X′ and Y′ mers is competed by cold X′ and Y′ mers, respectively, but also by oligonucleotides containing the X and Y boxes (X mer and Y mer) of the HLA-DRA proximal promoter. By contrast, competition was not observed with either Sp1 or NF-κB cold double-stranded oligonucleotides (Fig. 7). We previously demonstrated that (i) these oligonucleotides (the X, Y, Sp1 and NF-κB mers) specifically bind nuclear proteins and (ii) this binding is competed by the same cold oligonucleotides, but not by double-stranded oligonucleotides containing unrelated binding sites (data not shown).

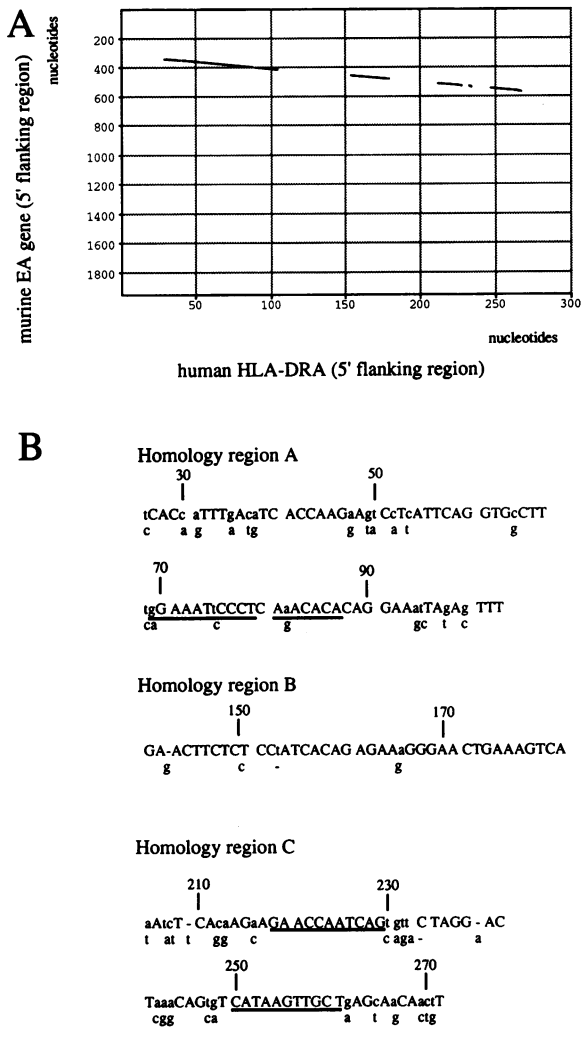

Figure 5. (**A**) Dot-matrix analysis showing the homology between the 1–300 nt region of the 5′ upstream HLA-DRA sequence shown in Figure 2 and the GenBank entry MUSHIEDA, a 1959 bp sequence corresponding to the 5′ flanking region of the murine EA gene (23). The analysis has been performed with the Pustell DNA matrix program of McVector DNA Sequence Analysis Package (35,36). (**B**) Comparison of the human HLA-DRA and murine EA sequences of the homology regions A, B and C. Capital letters: sequence of the human HLA-DRA gene; small letters: murine EA gene. Sequences corresponding to signals for transcription factors are underlined (see also Fig. 2). Homology region C corresponds to the Y′ and X′ boxes. Note that no differences are observed between human and murine sequences, suggesting that Y′ and X′ boxes are evolutionarily conserved sequences.

DISCUSSION

In this paper we report the nucleotide sequence of the 5′ *Eco*RI–*Bgl*II region of the human HLA-DRA gene. The finding of transcription factor motifs (among which putative NF-κB and Sp1 binding sites), the presence of a CpG-rich stretch and, most importantly, its homology to the B-cell control region of the murine EA gene (8–10), indicate that the newly sequenced region of the HLA-DRA gene may have a functional role.

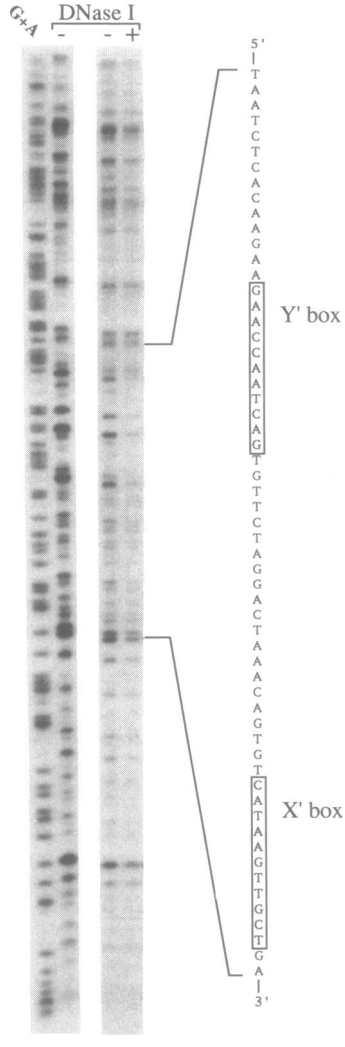

Figure 6. DNase I footprinting of the 5′ upstream region of the HLA-DRA gene. DNase I reactions were carried on in the absence (–) or in the presence (+) of 8 μg of crude nuclear extracts from human H9 cells (38). The footprinting probe was a PCR product obtained by using 1 μg of human genomic DNA as template and 150 ng of cold DRA-pcr1 and ^{32}P-end labelled DRA-pcr2 primers. G+A = molecular weight markers obtained by sequencing reaction of the ^{32}P-labelled 305 bp amplified PCR product (40). The sequence containing the Y′ and X′ boxes is shown in the right side of the panel and correspond to a large footprint.

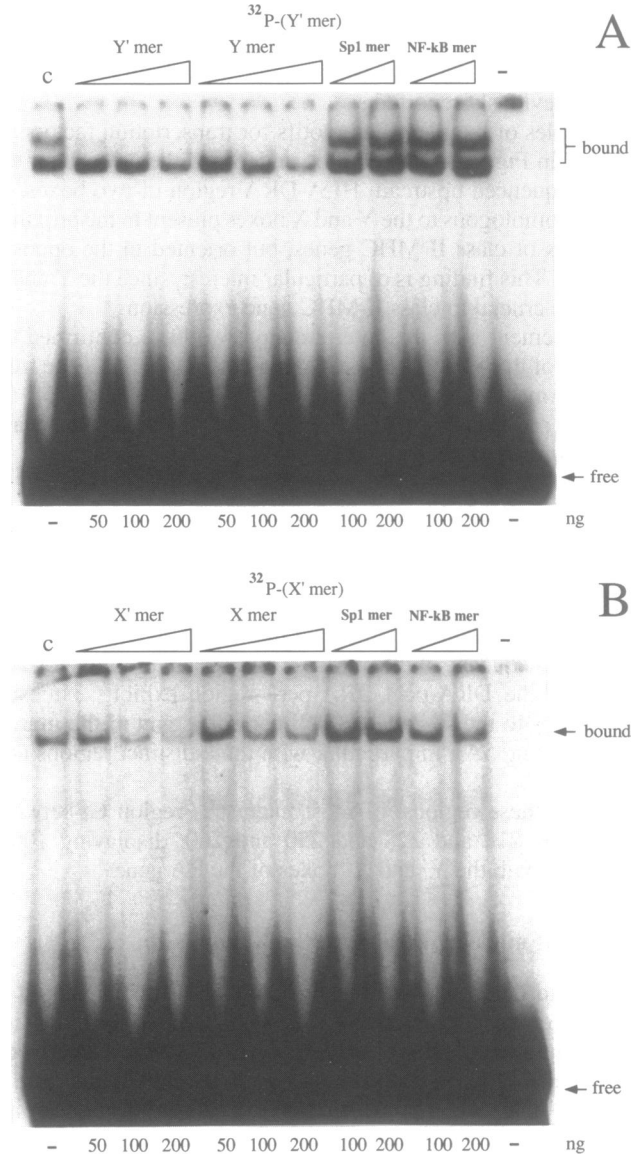

Figure 7. Electrophoretic mobility shift assay (EMSA). ^{32}P-labelled Y′ (**A**) and X′ (**B**) mers were incubated in the presence of 2 μg of nuclear extracts from H9 cells (38) and 600 ng of poly(dI:dC)·poly(dI:dC) in the absence (c = control) or in the presence of the indicated amounts of cold double stranded X, Y, X′, Y′, NF-κB and Sp1 mers. Bound and free ^{32}P-labelled oligonucleotides are indicated.

Of interest is the finding of Y′ and X′ boxes, highly homologous to the Y and X boxes of class II MHC genes, but positioned in the opposite orientation. A similar arrangement of Y′ and X′ boxes is present in the upstream gene sequence of the murine EA gene (10). As recently reviewed (8,9), both Y and X boxes are necessary for the correct expression of class II MHC genes; this was conclusively demonstrated by transfection experiments with deletion mutants (49,50), *in vitro* transcription (8), studies of class II negative cell lines (51), experiments employing antisense oligonucleotides to mRNA encoding for X-binding factors (18). Accordingly, homo- and hetero-dimers complexes were demonstrated (i) between nuclear proteins binding to the Y and X boxes of the MHC class II genes (8,9,15–19) and (ii) between the X-box binding proteins and other transcription factors belonging to the jun/fos and AP1 families (17,18).

Our analysis, showing that the sequences of Y′ and X′ boxes are conserved during molecular evolution of both human HLA-DRA and murine EA genes, further supports the central role of these regions for the regulation of HLA-DRA gene expression. Interestingly, although no conclusive information is available on far upstream DNase I hypersensitive sites of the human HLA-DRA gene, for the murine counterpart it is known that a DNase I hypersentive site is close to the upstream region containing the X′ and Y′ boxes (52).

The possibility to explore alterations of the upstream HLA-DRA gene regions could bring new information concerning the

issue of the relationship between gene structure and expression of class II MHC genes. The results of the footprinting and EMSA are in this respect of interest, as they suggest that the HLA-DRA 5′ upstream region containing X′ and Y′ boxes is recognized by nuclear proteins, and that nuclear proteins interacting with the X′ and Y′ boxes are also able to bind to the X and Y boxes of the proximal promoter.

The results presented in this paper are of interest when considered together with the data published by Dorn *et al.* (10) describing nuclear protein binding to the X′ and Y′ boxes of the murine EA gene. The finding that proteins interacting with the proximal promoter region of HLA-DRA and EA genes are also able to bind upstream regions containing the X′ and Y′ boxes (Fig. 7 and ref. 10), support the hypothesis of stem–loop arrangements of the proximal promoter and upstream region, possibly mediated by the homo and/or hetero-dimerization of these binding proteins (8,9,15–19,53–56).

In addition, it should be pointed out that recently published results on EA transgenic mice demonstrate that deletions of the EA upstream gene region carrying the X′ and Y′ boxes alter the expression of the EA transgene. When the 5′ flanking sequence of the EA transgene was shortened to only 1.3/1.2 kb, the phenotype of the transgenic mice produced was peculiar, with little or no expression of the truncated EA transgenes in B lymphocytes (12,20–23). By contrast, normal expression was found in the thymus, as well as in peripheral macrophages and dendritic cells (20–23).

The strong homology between the murine and human X′ and Y′ boxes (Fig. 5) strongly supports the use of HLA-DRA transgenic mice to study the biological functions of X′ and Y′ boxes. Tissue specific and γ-interferon regulated expression of the HLA-DRA gene has been correctly reproduced in transgenic mice (27–30). Accordingly, transgenic mice carrying 5′ truncated HLA-DRA gene are expected to be a suitable model system to further investigate the role of upstream sequences on regulation of HLA-DRA gene expression.

Knowledge of the upstream sequences of the HLA-DRA gene is also of interest when considering the alterations of class II MHC gene expression in a variety of human pathologies. It is well established that polymorphism and/or aberrant expression of class II MHC genes (57–61) is associated with a variety of diseases (62), including type-I diabetes, rheumatoid arthritis, celiac disease, pemphigus vulgaris, multiple sclerosis, systemic lupus erythematosus, Greaves disease, Bare-lymphocyte syndrome (see ref. 63 for a comprehensive listing including over 70 diseases associated with HLA specificities). Of interest, with respect to the data reported in our paper, is the evidence that alteration of transcription of HLA class II genes is linked with predisposition to autoimmunity (62).

In addition, it should be noted that an interdependence between the polymorphism of the coding regions and the polymorphism of regulatory regions of HLA-DRA proximal promoter has been suggested to influence both capacity of peptide binding and transcriptional activity (58).

With respect to this last point, we would like to underline that DNA polymorphism of the proximal promoter of the HLA-DRA (57,58) and HLA-DRB genes have been described (59–61), although it is unclear whether these nucleotide variations correlate with alterations of gene expression. In fact, while nucleotide substitution(s) of the regulatory regions (such as those affecting the X box) of HLA-DRB genes might be responsible for variation of transcriptional activity, the reported polymorphisms of the proximal promoter of the HLA-DRA gene seem to have no functional consequence (58).

The results presented in our paper could be relevant for further studies focusing on the relationship between nucleotide variation of the upstream sequences of HLA-DRA gene and HLA-related diseases.

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