A TISSUE-SPECIFIC TRANSCRIPTIONAL ENHANCER IS FOUND IN THE BODY OF THE HLA-DRα GENE

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Major histocompatibility complex class II antigens (MHC class II, Ia, murine I-A and I-E, human HLA-DP, DQ, DR antigens), are expressed on cell surfaces as noncovalently associated heterodimers. They consist of an α (34 kD) and a β (28 kD) chain. They act as restriction elements in both antigen presentation to T cells and in interactions between T and B lymphocytes. MHC class II molecules are constitutively expressed in B cells, T lymphoblasts, and some virally infected T cells. Other cells do not express these determinants; however, their expression can be induced by γ interferon (IFN-γ) in antigen presenting cells (APCs) (1, 2).

MHC class II genes have been investigated for the presence of *cis*-acting regulatory elements. A tissue-specific transcriptional enhancer has been described in the murine I-Eβ gene (3). Plasmid constructions containing a 2 kb 5' flanking region of the I-Eβ gene transfected into a variety of cells increased the number of stable transfectants when introduced into B lymphoma cells, but not when introduced into Ia$^-$ Ltk$^-$ fibroblasts or plasmacytoma cells. When I-Eα genes were introduced into I-Eα^- mice, >1.4 kb of 5' flanking I-Eα DNA was necessary for tissue-specific and IFN-γ-inducible expression of the transgene (4). Truncations of the 5' flanking DNA led to I-Eα expression in inappropriate tissues (5).

Previously, we reported on DNA methylation and chromatin structure of the HLA-DRα gene, the human homologue of I-Eα gene (6). There was no correlation between hypomethylation and HLA-DRα gene expression. All cells studied contained a hypomethylated CpG-rich island around the HLA-DRα promoter and methylated 5' and coding/3' regions (6). However, we found differences in the HLA-DRα chromatin structure. Cells not expressing HLA-DRα, including those that could be induced with IFN-γ, contained only one DNase I–hypersensitive site, which mapped into the promoter region. In cells constitutively expressing HLA-DRα, two additional DNase I–hypersensitive sites were present. These mapped into the first intron of the HLA-DRα gene and contained sequences homologous to the core transcriptional enhancer elements, potential topoisomerase II binding sites, and nuclear matrix–associated regions (7).

Here, we extend our studies on the chromatin structure of the HLA-DRα gene. Fragments of the HLA-DRα gene were cloned into plasmid vectors

containing the Herpes simplex thymidine kinase (tk)[1] promoter linked to the bacterial chloramphenicol acetyltransferase (CAT) gene (8). These plasmid constructions were transfected into lymphoid and nonlymphoid cells. We show that DNA fragments from the body of the HLA-DRα gene, which contain the intronic DNase I–hypersensitive sites, function as tissue-specific transcriptional enhancer elements (9).

Materials and Methods

Plasmid Constructions. Plasmids pRSVCAT, pTE1, and pTE1ΔS/N (10); MSVp-CAT3MSP and pCAT3MSP (11) have been described. pSP64-αtkCAT is a plasmid construction (CAT3MSP digested with Eco RI and Pvu II and the resulting 230 bp fragment ligated into Eco RI and Sma I sites of SP64) that can be transcribed with SP6 polymerase to yield the complementary strand to the 5′ end of pCAT3MSP. Fragments of the HLA-DRα gene were obtained from two different genomic clones, PDG019 (12) and pH-6A (13). They were subcloned into pTE1 or pTE1ΔS/N vectors at polylinker sites. Constructions A, F2, G, H, and I1 were made by inserting the HLA-DRα subfragments into a polylinker site and into the Nru I site in plasmid pTE1ΔS/N. Therefore, these clones do not contain the 598 bp pBR322-derived fragment between the pTE1 Sal I and Nru I sites. Plasmid constructions B, D, D, E, F1, and I2 were subcloned into the polylinker sites of vector pTE1, which contains the 598 bp pBR322-derived spacer. The direction of HLA-DRα fragments A, B, C, D, E, F2, G, H, and I2, but not of fragments F1, I1, with respect to HLA-DRα transcription is opposite to that of the tkCAT fusion gene. Fragment I1 was also placed 3′ of the CAT gene by inverting the tkCAT transcriptional unit (excisable with Bam H1) in plasmid pTE1ΔS/N-I1. Plasmids pCAT3MSP-G1 and G2 were constructed by ligating fragment G in both normal (G1) and opposite (G2) orientations (with respect to HLA-DRα transcription) into the polylinker of vector pCAT3MSP (see Fig. 1). All plasmids were prepared by two cycles of CsCl gradient centrifugation.

Cell Culture and DNA Transfections. BJAB, Jurkat, and HUT-78 cells have been described previously (7). Ltk⁻ cells and HeLa cells were obtained from American Type Culture Collection, Rockville, MD. 2pk-3 is a murine B cell lymphoma (14). All adherent cells were maintained in Dulbecco's modified Eagle's medium, whereas all nonadherent cells were grown in RPMI-1640 medium (both from UCSF tissue culture facility), supplemented with 10% FCS and antibiotics, at 37°C, in a humidified atmosphere containing 95% air and 5% CO_2.

Nonadherent cells were transfected by the DEAE-dextran (Pharmacia Fine Chemicals, Piscataway, NJ) procedure essentially as described (15). 3×10^7 cells were incubated in 1.5 ml of 500 μg/ml DEAE-dextran in TS buffer (8 g/liter NaCl, 0.38 g/liter KCl, 0.1 g/liter $Na_2HPO_4 \cdot 7H_2$)), 3 g/liter Tris-HCl, 0.1 g/liter $MgCl_2$, 0.1 g/liter $CaCl_2$, pH 7.4) containing 10 μg of supercoiled plasmid DNA for 20 min at room temperature. This mixture was then diluted 10-fold with media containing 0.1 mM chloroquine diphosphate (Sigma Chemical Co., St. Louis, MO) and incubated for an additional hour at 37°C. Cells were centrifuged, washed once, resuspended in media, and grown for 48–60 h in the CO_2 incubator, after which CAT assays were performed and/or RNA isolated. Adherent cells were transfected using the $CaPO_4$ procedure (16).

CAT Assays and RNA Analysis. CAT assays were performed as described (17). After 48–60 h incubation, cells were lysed by three freeze-thaw cycles, and the lysates heated for 5 min at 65°C (18). The lysates (0.125 M Tris-HCl, pH 7.5) containing 1 mM acetyl coenzyme A (Sigma Chemical Co.) were incubated at 37°C for 4 h in the presence of 0.2 μCi of [14C]chloramphenicol (Amersham Corp., Arlington Heights, IL). After thin-layer chromatography, the silica plates were exposed to film and the 1-acetyl and 3-acetyl chloramphenicol spots were excised and counted in a liquid scintillation counter. All

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[1] *Abbreviations used in this paper:* CAT, chloramphenicol acetyl transferase; tk, thymidine kinase.

transfections and CAT assays were repeated at least three times and standardized to amounts of protein in the lysates.

RNA was isolated from transfected cells by the guanidinium isothiocyanate/CsCl procedure (19). 50 μg of total RNA was incubated with 10^6 cpm of [^{32}P]UTP-labelled Eco RI–linearized pSP64-αtkCAT probe for 12 h in 80% formamide, 0.4 M NaCl, 20 mM Tris-HCl (pH 7.0), 1 mM EDTA, and 0.1% SDS. The volume was increased 10-fold with 10 mM Tris-HCl (pH 7.5), 5 mM EDTA, 300 mM NaCl. RNase A (10 μg/ml final) and T1 (1 μg/ml final) were added for 1 h at room temperature. The reactions were stopped with SDS and proteinase K, organically extracted, ethanol precipitated, electrophoresed on 6% urea-acrylamide sequencing gels, and autoradiographed as described (20).

Results

A Transcriptional Enhancer Is Found in the Body of the HLA-DRα Gene.   To test whether any part of the HLA-DRα gene can increase transcription from a heterologous promoter, plasmid vectors containing elements of the HLA-DRα gene fused to the tkCAT transcriptional unit were transfected into BJAB and Jurkat cells. 48–60 h after transfection, cell lysates were prepared and assayed for CAT enzymatic activity. BJAB are Ia$^+$ human B cells, whereas Jurkat are Ia$^-$ human T cells. Their phenotypes were confirmed by Northern blot hybridization using HLA-DRα cDNA probes. That the population of cells was homogeneous was determined by FACS analysis of tissue culture cells labelled first with anti-Ia monoclonal antibodies and second with fluoresceinated goat anti–mouse Ig antiserum (data not shown) (7). Similar analyses were done for all other cells used in this study.

The HLA-DRα sequences tested in this study are covered by fragments A (Pst I/Xba I; 2.5 kb), B (Xba I/Cla I; 1.8 kb), C (Bgl II/Hind III; 1,079 bp), D (Hind III/Cla I; 3.8 kb), and E (Hind III/Hind III; 2.5 kb) (see Fig. 1A). They span 7 kb of HLA-DRα DNA and extend from 2.8 kb upstream of the RNA cap site to 3.2 kb into the body of the gene. The CAT activity in cells transfected with fragments A and E was baseline, but it was increased 2–18-fold with fragments B, C, and D (Fig. 2 and Table I). Therefore, the DNA segments that comprise the promoter, exon 1, and part of intron 1 contain transcription-enhancing activity. Furthermore, fragments B and D, which contain all three elements, resulted in three- to fivefold higher CAT activities than fragment C, which contains only the intronic sequences, and fragment B was twofold more active than fragment D (Table I). In fragment D, HLA-DRα regulatory elements are 2 kb further upstream from the tk promoter than they are in fragment B. Therefore, the reduction in CAT activity could be due to distance. However, no differences in CAT activities were observed between constructions using the pTE1 or the pTE1ΔS/N plasmid vectors (Table I). Unlike pTE1ΔS/N, pTE1 contains a 598 bp spacer derived from pBR322 between the tk promoter and the polylinker into which HLA-DRα fragments were inserted. The pTE1 (tkCAT) control plasmid (without any insert to be tested) occasionally resulted in weak and visually undetectable autoradiographic signals for the monoacety-lated chloramphenicol spots (Fig. 2A, lane 4; Fig. 2, *B* and *C*, lane 3). However, CAT activities were measured by counting excised spots in a liquid scintillation counter. Here, the tk promoter could be shown to function in all cells and resulted in comparable but low CAT enzymatic activities (Table I).

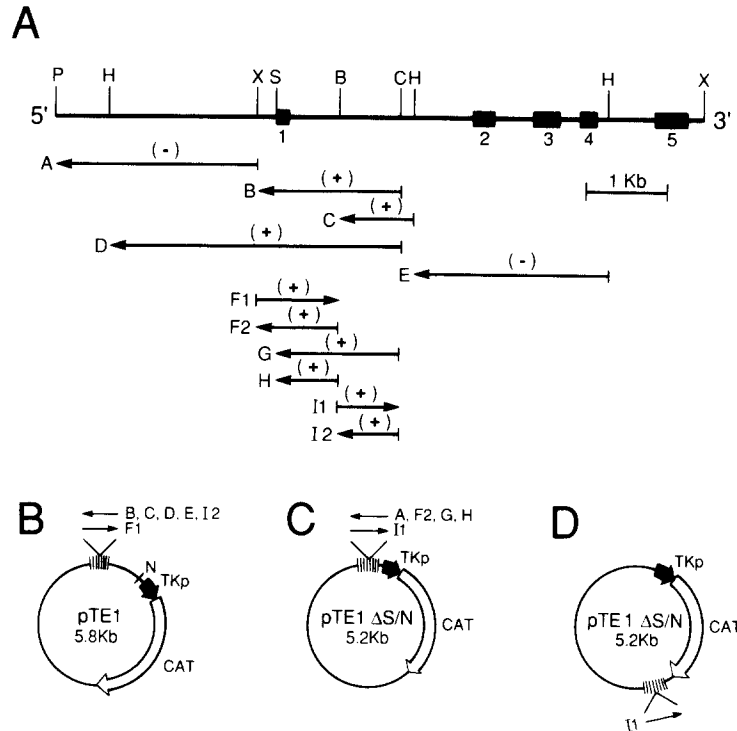To map the *cis*-acting regulatory elements more precisely, we cloned DNA

FIGURE 1.   Schematic representation of the HLA-DRα constructions used in transient trans-fection experiments. A: The informative restriction sites and the exons of the HLA-DRα gene are shown. Restriction endonucleases are: P, Pst I; H, Hind III; X, Xba I; S, Sac I; B, Bgl II; C, Cla I. The direction of HLA-DRα transcription is from left to right. Fragments A–E, F1, F2, G, H, I1, and I2 of the HLA-DRα gene were subcloned 5' or 3' to the tk promoter-initiated CAT gene in the vectors pTE1 or pTE1ΔS/N. The arrows indicate the orientation of the HLA-DRα fragments relative to the tkCAT gene. Fragments are: A, Pst I/Xba I; B, Xba I/Cla I; C, Bgl II/Hind III; D, Hind III/Cla I; E, Hind III/Hind III; F1 and F2, Xba I/Bgl II: G, Sac I/Cla I; H, Sac I/Bgl II; I1 and I2, Blg II/Cla I. The activity of each fragment is represented by symbols (+ or −) placed above the arrow denoting that fragment. B and C: Fragments B–E, I2, and F1 were cloned 5' of the tkCAT gene in the vector pTE1. Fragments A, F2, G, H, and I1, were cloned 5' of the tkCAT gene in the vector pTE1ΔS/N. D: Fragment I1 was cloned 3' of the tkCAT gene in the vector pTE1ΔS/N.

segments of fragment B into pTE1 or pTE1ΔS/N plasmid vectors and obtained fragments F (Xba I/Bgl II; 1 kb), G (Sac I/Cla I; 1.5 kb), H (Sac I/Bgl II; 750 bp), and I (Bgl II/Cla I; 750 bp). All these fragments contained reproducible transcriptional enhancing activities in BJAB and Jurkat cells; however, none of them increased CAT activities as much as fragment B. Fragments F and H increased CAT activities two- to threefold, and fragment I (from the middle of intron 1) three- to sixfold, which is statistically significant (Fig. 2 and Table I).

To further characterize the informative HLA-DRα intronic fragments F and I, they were tested in both orientations 5' to the CAT gene, and in a selected case, 3' to the CAT gene. In both orientations, these fragments resulted in increased CAT activities in BJAB and Jurkat cells. Transfections with fragment I resulted in consistently higher CAT activities than did those with fragment F
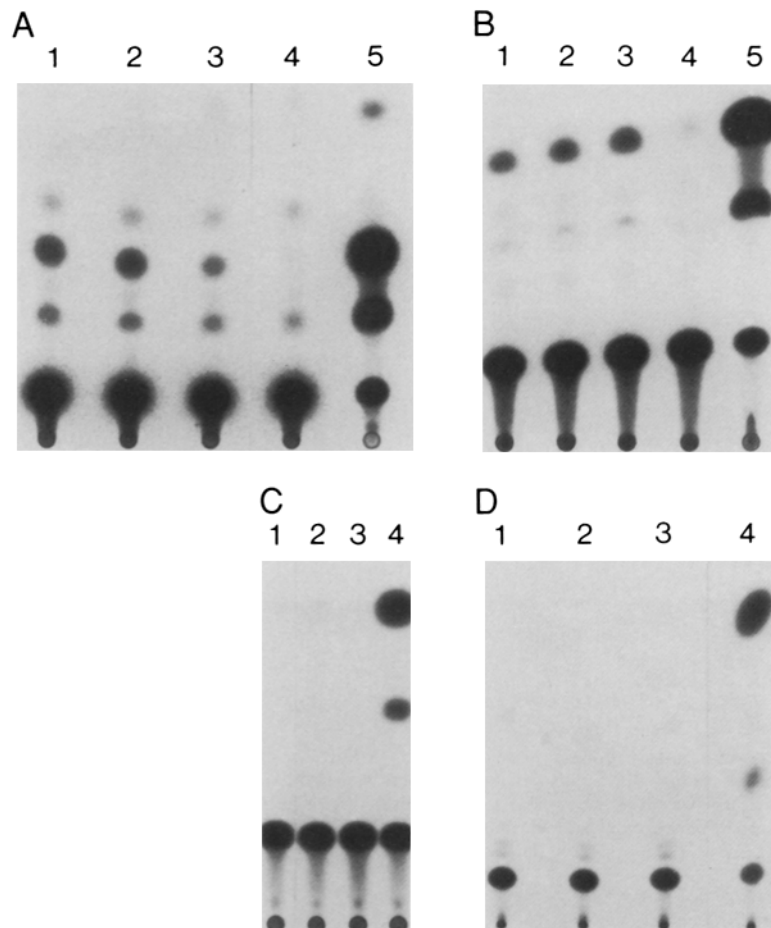
FIGURE 2. Autoradiograph of CAT assays in BJAB (*A*), Jurkat (*B*), Ltk (*C*), and HeLa (*D*) cells. Transfections and CAT assays were carried out as described in Materials and Methods. *A* and *B*: lanes *1–5* represent transient transfections with pTE1-I2, pTE1ΔS/N-I1, pTE1ΔS/N-H, pTE1, and pRSVCAT, respectively. *C* and *D*: lanes *1–4* represent transient transfections with pTE1-I2, pTE1-B, pTE1, and pRSVCAT, respectively. In *A*, above the 3-monoacetylated chloramphenicol spots are seen contaminant bands found in some batches of commercially available [^{14}C]chloramphenicol.

(Table I). Fragment I also increased CAT activities in a position 3' to the CAT gene in BJAB and Jurkat cells (Table I).

The HLA-DRα Transcriptional Enhancer Functions in a Tissue-specific Manner. To determine whether HLA-DRα *cis*-acting regulatory elements function in a tissue-specific manner, the plasmid constructions that were positive in BJAB and Jurkat cells were also tested in HUT-78, HeLa, and Ltk⁻ cells. HUT-78 are human Ia⁺ T cells. HeLa are human endometrial carcinoma cells that are Ia⁻ in their resting state; however, HLA-DRα expression can be induced with IFN-γ. Ltk⁻ are Ia⁻ murine fibroblasts. Fragments B and I2 increased CAT activities 20 and 6.5-fold in HUT-78 cells, but not in HeLa and Ltk⁻ cells (Table I). Thus, fragments B and I2 function in lymphoid cells but not in human or murine

TABLE I

Relative Transcriptional Activities of DNA Fragments from the HLA-DRα Gene

Constructions	Relative CAT activity		Chloramphenicol Conversion	
	BJAB	Jurkat	BJAB	Jurkat
			%	
pRSVCAT	170	257	32–44	45–60
pTE1 or pTE1ΔS/N	1.0	1.0	0.1–0.30	0.1–0.45
pTE1ΔS/N-A	0.7 ± 0.2	0.7 ± 0.3	0.05–0.2	0.05–0.3
pTE1-B	10.3 ± 0.8	17.0 ± 1.3	1.0–4.0	2.0–8.0
pTE1-C	2.1 ± 0.1	3.6 ± 0.4	0.4–0.7	0.4–1.3
pTE1-D	5.1 ± 0.4	7.8 ± 0.8	0.8–1.6	1.0–3.0
pTE1-E	1.0 ± 0.6	1.6 ± 0.2	0.2	0.4
pTE1-F1	2.1 ± 0.1	2.2 ± 0.2	0.5–0.6	0.6–0.9
pTE1ΔS/N-F2	2.0 ± 0.2	2.3 ± 0.4	0.5	0.8
pTE1ΔS/N-G	3.9 ± 0.9	5.6 ± 0.1	0.8–1.1	1.0–2.2
pTE1ΔS/N-H	2.0 ± 0.1	2.6 ± 0.1	0.6	0.7
pTE1ΔS/N-I1	3.6 ± 0.5	5.9 ± 0.5	0.7–1.2	0.7–2.5
pTE1-I2	3.4 ± 0.8	5.4 ± 0.4	0.4–1.2	0.6–2.0
pTE1ΔS/N-3′I1	2.4 ± 0.4	2.1 ± 0.3	0.3–0.5	0.3–0.8

	HUT-78	HeLa	Ltk⁻	HUT-78	HeLa	Ltk⁻
pRSVCAT	250	170	150	46.0	26.0	30.0
pTE1 or pTE1 S/N	1.0	1.0	1.0	0.2	0.15	0.2
pTE1-B	19.5 ± 0.8	1.0 ± 0.2	1.0 ± 0.4			
pTE1-I2	6.5 ± 0.3	0.9 ± 0.3	1.2 ± 0.2			

Relative CAT activities obtained with different plasmid constrictions in BJAB, Jurkat, HUT-78, HeLa, and Ltk⁻ cells. Cells constitutively expressing HLA-DR determinants are BJAB and HUT-78. All transfections were repeated at least three times. Mean ± SD is shown. The ranges of percent chloramphenicol conversion are given for BJAB and Jurkat cells, and the mean percent chloramphenicol conversions are given for HUT-78, HeLa, and Ltk⁻ cells. All CAT signals were normalized to the number of cells transfected and to amount of total protein. pRSVCAT showed the strongest signals in Jurkat and HUT-78 cells.

fibroblasts. We also tested the effects of IFN-γ on these cis-acting regulatory elements. IFN-γ in concentrations of 100 IU/ml did not increase CAT activity in HeLa cells transfected with fragments B and I2 (data not shown).

CAT Transcripts Initiate from the tk Promoter. To map the precise sites of transcriptional initiation in these plasmid constructions, RNA was isolated from transfected cells and assayed by RNase protection techniques. Because the level of CAT-specific RNA was so low in transiently transfected human lymphoid cells that it could not be detected with conventional techniques, the HLA-DRα genomic fragments were cloned into vectors containing the polyoma early region and the origin of replication. These constructions replicate in rodent cells, yielding high copy numbers of intracellular plasmid DNA (21). After transient transfection into murine 2pk-3 cells, which are Ia⁺ murine B cells, there is no difference in the transcription initiation between tkCAT (pCAT3MSP)-, MSVtkCAT (MSV-pCAT3MSP)-, and fragment G (pCAT3MSP-G1 and G2)-containing plasmids (Fig. 3). However, the murine Moloney sarcoma virus (MSV) transcriptional unit was weaker than the Rous sarcoma virus long terminal repeat in these cells, and resulted in only moderately increased CAT activities and
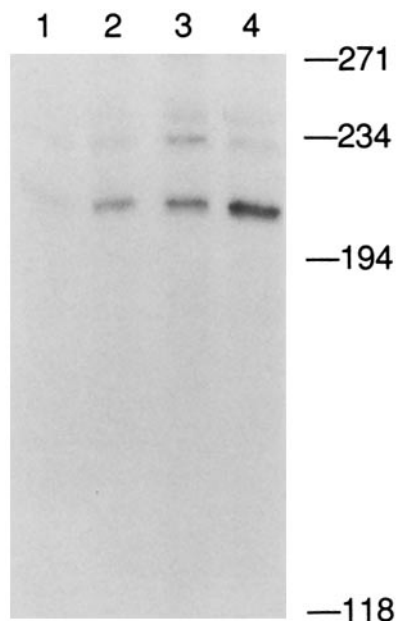
1 2 3 4

—271

—234

—194

—118

FIGURE 3. RNase protection analysis. Lanes _1–4_ represent transient transfections into 2pk-3 cells and with pCAT3MSP, pCAT3MSP-G_1, G_2, and MSVPCAT3MS plasmid constructions, respectively. Size markers (on the right, in basepairs) are from end-labeled Hae III–digested ϕX174. The pSP64-αTKCAT transcript protects a 200-bp-long fragment in all four lanes, which corresponds to initiation of CAT transcription from the usual tk promoter cap site.

transcripts compared with those with fragment G (Fig. 3 and data not shown). These data show that fragments from the body of the HLA-DRα gene increase the levels of appropriately initiated CAT RNA.

DNA Sequences in Intron 1 Share Homologies With Other Eukaryotic Transcriptional Enhancers. Within fragments F and I there are DNA sequences found in other eukaryotic transcriptional enhancers (Fig. 4). These have been described previously and consist of core transcriptional enhancer elements (9), octamer (22), A-T-rich regions (23), and potential topoisomerase II sites (24), as well as nuclear matrix–associated regions (25). In addition, immunoglobulin switch consensus $(TGGGGG)_n$ (26) and 250 bp _Alu_ repeat sequences (27) are found. A diagramatic representation of these sequence motifs is shown in Fig. 4.

Discussion

The data presented map tissue-specific enhancer elements into the body of the HLA-DRα gene. The largest transcriptional effects were observed with fragment B, which contains the HLA-DRα promoter, exon 1, and part of intron 1. When this fragment was divided into smaller subfragments, fragment I from the middle of intron 1 retained most of the activity. Fragment I increased transcription from a heterologous promoter in an orientation- and position-independent fashion. It functioned in lymphoid cells, but not in human or mouse fibroblasts. Thus, fragment I satisfies the definition of a tissue-specific eukaryotic transcriptional enhancer element (9). The adjacent fragment H showed similar but smaller effects. The effects of fragments I and H are not additive, because fragment G, which combines DNA sequences of fragments H and I, resulted in CAT activities similar to those of fragment I alone. However, intronic and promoter elements must be multiplicative, because fragment B resulted in threefold higher CAT activities than fragments G or I alone. Similar cooperativity has been found for
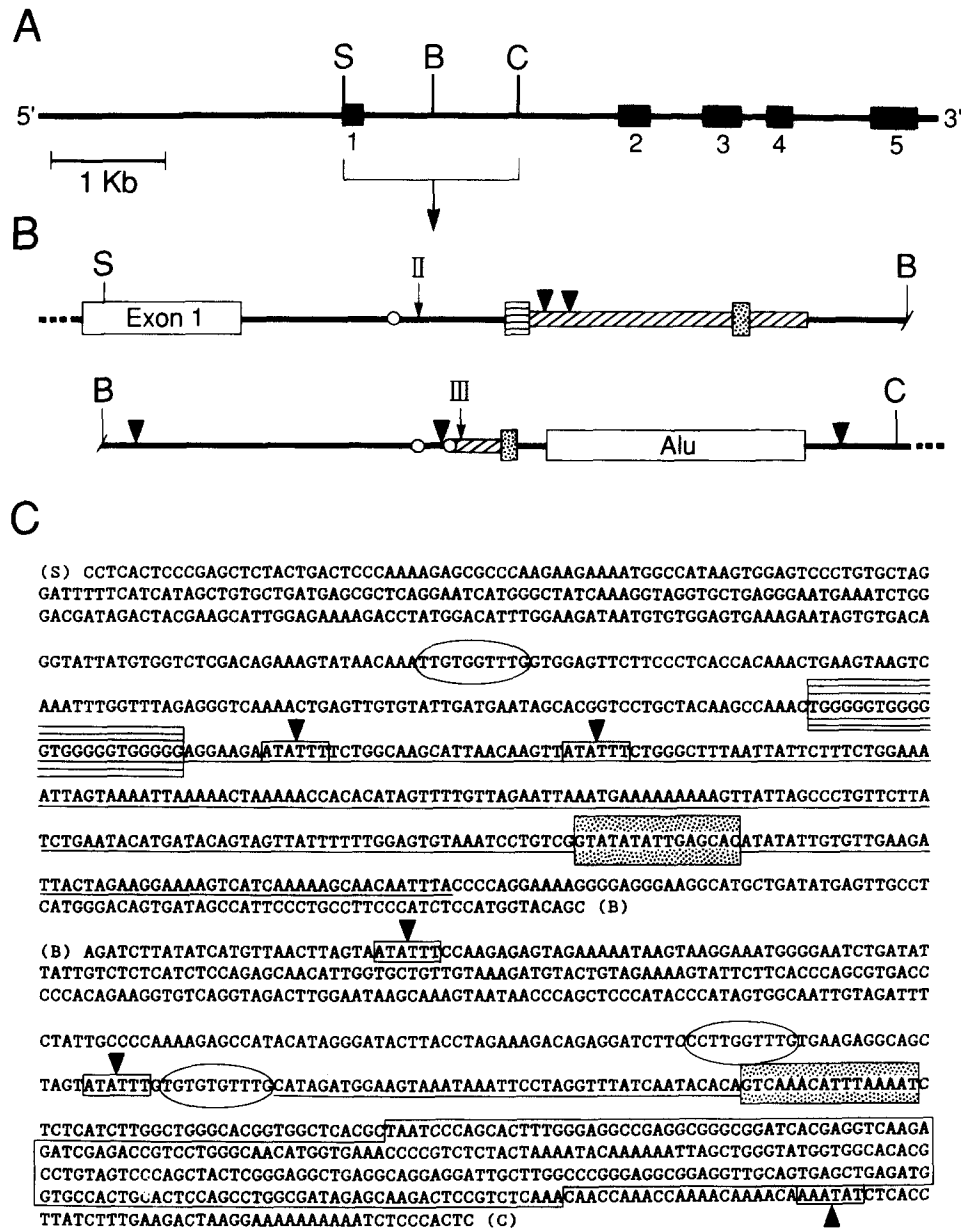
A

B

C

(S) CCTCACTCCCGAGCTCTACTGACTCCCAAAAGAGCGCCCAAGAAGAAAATGGCCATAAGTGGAGTCCCTGTGCTAG
GATTTTTCATCATAGCTGTGCTGATGAGCGCTCAGGAATCATGGGCTATCAAAGGTAGGTGCTGAGGGAATGAAATCTGG
GACGATAGACTACGAAGCATTGGAGAAAAGACCTATGGACATTTGGAAGATAATGTGTGGAGTGAAAGAATAGTGTGACA

GGTATTATGTGGTCTCGACAGAAAGTATAACAAATTGTGGTTTGGTGGAGTTCTTCCCTCACCACAAACTGAAGTAAGTC

AAATTTGGTTTAGAGGGTCAAAACTGAGTTGTGTATTGATGAATAGCACGGTCCTGCTACAAGCCAAACTGGGGGTGGGG

GTGGGGGTGGGGCAGGAAGAATATTTTTCTGGCAAGCATTAACAAGTTATATTTCTGGGCTTTAATTATTCTTTCTGGAAA

ATTAGTAAAATTAAAAACTAAAAACCACACATAGTTTTGTTAGAATTAAATGAAAAAAAAAGTTATTAGCCCTGTTCTTA

TCTGAATACATGATACAGTAGTTATTTTTTGGAGTGTAAATCCTGTCGGTATATATTGAGCAGATATATTGTGTTGAAGA

TTACTAGAAGGAAAAGTCATCAAAAAGCAACAATTTACCCCAGGAAAAGGGGAGGGAAGGCATGCTGATATGAGTTGCCT
CATGGGACAGTGATAGCCATTCCCTGCCTTCCCATCTCCATGGTACAGC (B)

(B) AGATCTTATATCATGTTAACTTAGTAATATTTCCAAGAGAGTAGAAAAATAAGTAAGGAAATGGGGAATCTGATAT
TATTGTCTCTCATCTCCAGAGCAACATTGGTGCTGTTGTAAAGATGTACTGTAGAAAAGTATTCTTCACCCAGCGTGACC
CCCACAGAAGGTGTCAGGTAGACTTGGAATAAGCAAAGTAATAACCCAGCTCCCATACCCATAGTGGCAATTGTAGATTT

CTATTGCCCCAAAAGAGCCATACATAGGGATACTTACCTAGAAAGACAGAGGATCTTCCCTTGGTTTGTGAAGAGGCAGC

TAGTATATTTGTGTGTGTTTGCATAGATGGAAGTAAATAAATTCCTAGGTTTATCAATACACAGTCAAACATTTAAAATC

TCTCATCTTGGCTGGGCACGGTGGCTCACGCTAATCCCAGCACTTTGGGAGGCCGAGGCGGGCGGATCACGAGGTCAAGA
GATCGAGACCGTCCTGGGCAACATGGTGAAACCCCGTCTCTACTAAAATACAAAAAATTAGCTGGGTATGGTGGCACACG
CCTGTAGTCCCAGCTACTCGGGAGGCTGAGGCAGGAGGATTGCTTGGCCCGGGAGGCGGAGGTTGCAGTGAGCTGAGATG
GTGCCACTGCACTCCAGCCTGGCGATAGAGCCAAGACTCCGTCTCAAACAACCAAACCAAAACAAAACAAAATATCTCACC
TTATCTTTGAAGACTAAGGAAAAAAAAAAATCTCCCACTC (C)

FIGURE 4. A map of the HLA-DRα gene. A: 8.0 kb of HLA-DRα DNA composed of 2.5 kb of 5' flanking and 5.5 kb of coding information. S, B, and C stand for Sac I, Bgl II, and Cla I restriction endonucleases. B: enlargement of the area between Sal I and Cla I sites. II and III denote DNase I–hypersensitive sites (7). Open squares denote exons and Alu repeat sequences. Open circles denote positions of core transcriptional enhancer consensus elements. Dotted squares represent potential topoisomerase II binding sites. Squares with slanted lines represent A-T-rich regions of DNA, and black arrows denote the locations of ATATTT sequences. The square with horizontal lines denotes the location of the sequence TGGGGG repeated four times. C: DNA sequences found between Sac I (S) and Cla I (C) sites marked as in B.

fragments of the immunoglobulin κ gene, where the intronic transcriptional enhancer and the promoter associated octamer result in transcriptional effects which are multiplicative (11, 28, 29). It is of interest that an octamer in the immunoglobulin κ orientation is also found in the promoter of the HLA-DRα gene. Thus, these regions flanking and including exon 1 form a transcriptional unit that facilitates the tissue-specific transcription of the HLA-DRα gene.

Previously, we described DNase I–hypersensitive sites in the HLA-DRα gene (7). In cells that do not express Ia determinants, and in those that can be induced with the administration of IFN-γ, there is only one promoter associated DNase I–hypersensitive site. However, in cells constitutively expressing high levels of HLA-DRα, two additional DNase I–hypersensitive sites were found in intron 1. One site maps into fragment I and the other into fragment H. Both contain sequences found in other eukaryotic transcriptional enhancers. It is therefore not surprising that these fragments function to increase transcription in lymphoid cells. However, it is unexpected that they act in both B and T cells, even though in resting T cells, HLA-DR α gene is not expressed, and Jurkat cells did not contain the intronic DNase I–hypersensitive sites. The immunoglobulin heavy chain transcriptional enhancer also functions in some T cells (30). These anomalies may be particular to transient-expression assays, where the introduced plasmid DNA does not reproduce the environment and the chromatin structure of endogenous genes. Alternatively, T cells may contain *trans*-acting factors that prevent initiation of HLA-DRα transcription. Recently, we have observed that the upstream promoter elements block HLA-DRα transcription in Jurkat but not in HUT-78 and BJAB cells (Peterlin, B. M., S. Y. Tsang, and M. Nakanishi, manuscript in preparation). Thus, the transcriptional enhancer elements in the body of the HLA-DRα gene would still function, but transcription from the promoter is blocked. In HeLa and Ltk⁻ cells neither the transcriptional enhancer elements nor the HLA-DRα promoter show detectable activity.

Both DNA sequences in fragments I and H contain core transcriptional enhancer elements similar to the immunoglobulin κ B element, which shows tissue-specific protein–DNA interactions (31). Fragment I also contains the octamer, with one base mismatch, GTTTGCAT. Different orientations of the octamer ATTTGCAT are found in the immunoglobulin and the HLA-DRα promoters and in the immunoglobulin heavy chain enhancer (22). It also shows tissue-specific DNA–protein interactions (23). In addition, potential topoisomerase II binding and cleavage sites, as well as A-T-rich regions that may form nuclear matrix attachment sites are found in fragments H and I (23, 24). Such sequence motifs are found adjacent to the immunoglobulin κ enhancer and may introduce torsional stress into the chromatin loops of the HLA-DRα gene (24). Different types of torsional stress are thought to help determine the transcriptional activity of eukaryotic genes.

The comparison of the HLA-DRα *cis*-acting regulatory sequences with those of the immunoglobulin genes invite speculation that some *trans*-acting factors will be tissue specific. Some of these will bind DNA directly and will be revealed by studies where proteins bound to DNA either retard gel migration of labeled DNA fragments, or protect the underlying DNA sequences from chemical or enzymatic degradation. These studies are now in progress. Because the HLA-

DRα transcriptional enhancers do not function in one distinct genetic complementation group of the MHC class II–negative Raji cell mutants whereas they do function in other independently derived mutants and in parental Raji cells, we have designed direct genetic approaches to rescue the gene(s) coding for HLA-DRα tissue-specific transcriptional enhancer *trans*-acting factors.

Summary

We mapped *cis*-acting regulatory elements in the HLA-DRα gene, which encodes the monomorphic subunit of the HLA-DR heterodimer. Genomic fragments of HLA-DRα were placed 5' or 3' to the chloramphenicol acetyltransferase reporter gene, the transcription of which was initiated from the Herpes simplex thymidine kinase promoter. In transient expression assays, fragments from the body of the HLA-DRα gene were able to increase chloramphenicol acetyltransferase activity in a position-, orientation-, and promoter-independent yet tissue-specific fashion. These HLA-DRα *cis*-acting regulatory elements contain previously identified DNase I–hypersensitive sites and DNA sequences homologous to those found in other eukaryotic transcriptional enhancers.

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