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Characterization of an HLA $DR\beta$ pseudogene

(major histocompatibility complex/class II antigen)

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ABSTRACT The class II molecules of the human major histocompatibility complex include the DR, DC, and SB antigens, each composed of an α and a β polypeptide chain. We have isolated a $DR\beta$ gene in overlapping cosmid clones made from genomic DNA of a Dw4/DR4 homozygous individual. This gene consists of six exons and spans >20 kilobases. Upon sequencing, it was found to possess several deleterious mutations, each capable of rendering the gene nonfunctional: (i) four splice junctions deviate from the G-T/A-G rule; (ii) two premature termination codons are present in the first domain exon; (iii) a 2-base-pair insertion causes a translational frame shift in the second domain exon. In addition, several amino acid residues that are conserved in all known expressed β chains have been replaced in the amino acid sequence predicted from the pseudogene. Analysis of the pattern of nucleotide substitutions in the second domain exon suggests that most amino acid replacements occurred after the gene was inactivated. The inactivation may have been caused by insertion of a Kpn I repeat 5' to the promoter region, thereby interfering with transcription of the gene through removal of transcriptional enhancer elements. The $DR\beta$ pseudogene seems to be present also in other DR4 individuals.

The class II antigens of the major histocompatibility complex are expressed on some cell types involved in the immune response, such as B lymphocytes and macrophages (1). They participate in the presentation of foreign antigens in cellular interactions (2). In man, associations between class II antigen alleles and a large number of diseases have been described (3).

The human major histocompatibility complex contains the genes of at least three types of class II antigens—i.e., DR, DC, and SB (4, 5). All three are cell-surface glycoprotein heterodimers, consisting of an α chain of $\approx 35,000$ Da noncovalently associated with a β chain of $\approx 28,000$ Da (6). A characteristic property of the class II antigens is their extensive genetic polymorphism. The variability of the DR antigens is provided exclusively by the β chains (7) whose polymorphism resides primarily in the amino-terminal domains (8, 9).

Cloning of cDNA (10) and protein sequencing (11, 12) have shown that in some cell lines, $DR\beta$ -like genes of at least two distinct loci are expressed. Hybridizations to genomic DNA with DR β probes reveal even further complexity, inasmuch as individuals of different DR specificities seem to display distinct numbers of $DR\beta$ genes, some having at least three $DR\beta$ -like genes (J. Böhme, personal communication).

Class II antigen DNA probes are now being used in hybridizations to genomic DNA in search for more specific correlations of various diseases with class II loci and alleles, detected as restriction fragment length polymorphisms (13, 14). However, the hybridization patterns obtained often reveal a more complex genetic organization than expected from phenotypic observations (15). Molecular characterization of the many class II genes should help simplify interpretations of the genomic hybridization data.

To study the features of the individual $DR\beta$ -like genes, we have isolated several cosmid clones containing $DR\beta$ genes from genomic libraries prepared from DNA of an HLA-homozygous individual. In the present study, we describe the exon organization of a $DR\beta$ gene and we present several features of the nucleotide sequence, indicating that it is a pseudogene. The implications for HLA-DR typing through DNAhybridization techniques are discussed.

MATERIALS AND METHODS

Isolation and Characterization of Genomic Clones. Two genomic libraries were constructed (unpublished data) from DNA donated by an HLA-homozygous offspring of a consanguineous mating. The DNA donor has been typed to be Dw4/DR4 (cell L in ref. 16). The libraries were made in the cosmid vectors pHEP and pNNL, respectively (17), and they were screened with a 790-base-pair Sac I/HindIII fragment corresponding to the entire coding part of the DR β cDNA clone pII- β -3 (9). Screening of the cosmid libraries and growth of cosmid clones were carried out as will be described elsewhere (unpublished observations). The isolated cosmids were characterized by restriction mapping and hybridization to exon-specific DR β cDNA probes. The clone cosII-3301 was in the vector pNNL and cosII-801 was in pHEP. Subclones were prepared in the vector pUC9.

DNA Sequence Determination. Nucleotide sequences were determined with the chemical degradation procedure (18) and with the dideoxy-chain termination procedure (19) using exonuclease III and synthetic oligonucleotide 12-mer primers.

RESULTS AND DISCUSSION

Isolation of Cosmids Containing $DR\beta$ Genes. The cosmid libraries were screened with a DR β cDNA probe under low stringency conditions (unpublished data). Several cosmids were isolated and hybridized to a DR β cDNA probe corresponding to the 3'-untranslated region of the mRNA, since this probe does not cross-hybridize to DC and SB β -chain clones (20). The cosmids hybridizing to the DR β -specific probe were restriction-mapped, whereupon two clones were found to overlap by 18 kilobases (kb) (Fig. 1A). The exons of the gene contained in these cosmids were found to be distributed over a region of >20 kb. After nucleotide sequencing as shown in Fig. 1B, the gene was found to display several deleterious mutations that prevent expression of a DR β polypeptide (see below) and will, therefore, be referred to as $DR\beta\psi$ (for $DR\beta$ pseudogene).

Exon-Intron Organization of the $DR\beta$ **Pseudogene.** The exon boundaries were localized after alignment of the $DR\beta\psi$

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Abbreviation: kb, kilobase(s).

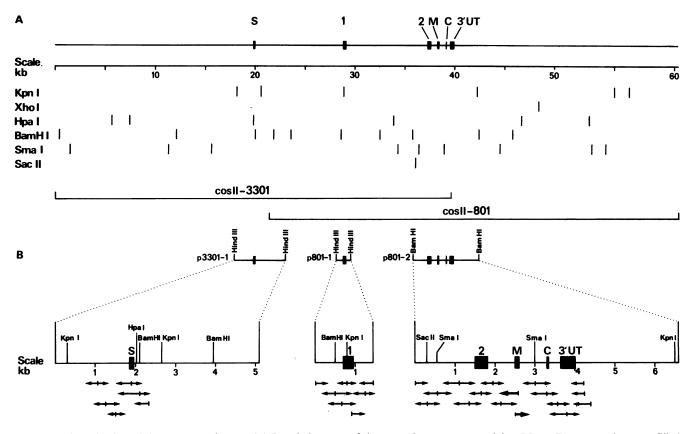


FIG. 1. Organization of the $DR\beta$ pseudogene. (A) Restriction map of the genomic segment containing $DR\beta\psi$. Exons are shown as filled boxes. Positions of restriction sites are indicated by vertical bars. No restriction sites were found for the enzymes Cla I, Mlu I, Nru I, Pvu I, and Sal I. Inserts of the two overlapping cosmid clones are shown by horizontal bars. (B) Restriction maps of subclones utilized for nucleotide sequencing. Sequencing strategy is shown by light-face arrows for the chemical degradation procedure and bold-face arrows for the dideoxychain termination procedure. Exons are denoted with S for signal sequence; 1, first domain; 2, second domain; M, transmembrane segment; C, cytoplasmic tail; and 3'UT, 3'-untranslated region.

sequence with the sequences of previously described DR β cDNA clones (9) and a $DC\beta$ gene (21) (Fig. 2). The first exon corresponds to the 5'-untranslated region, the signal sequence, and four amino acids of the first extracellular domain. The main part of the first domain and the second domain are encoded by exons 2 and 3, respectively. The fourth exon contains information for the connecting peptide, the transmembrane region, and four amino acids of the cytoplasmic tail. The core of the cytoplasmic segment is encoded by a separate exon of 24 nucleotides, whereas the remaining three amino acids of the cytoplasmic tail are encoded by the sixth exon together with the 3'-untranslated region.

Two of the introns of $DR\beta\psi$ are very large; the first intron is ≈ 9 kb and the second intron is ≈ 8 kb. However, such large introns have previously been found in expressed genes (e.g., see ref. 22) and, therefore, need not correlate with the fact that the gene is a pseudogene. It is not known whether $DR\beta\psi$ is transcribed.

Two Alu type 1 repeats are present in intron 4 and the 3'flanking region, respectively (Fig. 2C). These repeats are ubiquitous in vertebrate genomes, and they have been found in introns and flanking sequences of several genes. The role of the Alu repeats is unknown (see ref. 23).

 $DR\beta\psi$ Has Several Deleterious Mutations. Although the exon organization of $DR\beta\psi$ is identical to that of expressed class II β genes (21, 24, 25), proper processing of a primary RNA transcript is unlikely to occur, because four of the splice junctions deviate from the G-T/A-G rule (26). The first splice donor site following the signal sequence exon has mutated from G-T to G-C. The other anomalous splice junctions occur after the first domain exon, after the transmembrane exon, and before the separate cytoplasmic exon.

Two premature termination signals have arisen through point mutations in codons 26 and 76, precluding synthesis of a β chain even if a correctly spliced mRNA were to be made.

Two nucleotides have been inserted in codon 183 of $DR\beta\psi$ as compared to the sequences of three $DR\beta$ cDNA clones (9, 10) (see Fig. 2C), thereby altering the translational reading frame. However, the frame used in the $DR\beta$ cDNA clones has been retained in Fig. 2 to facilitate comparison with the amino acid sequences of the expressed $DR\beta$ chains.

Comparisons of Nucleotide and Amino Acid Sequences. $DR\beta\psi$ displays an average of 90 nucleotide differences from the sequences of three DR β cDNA clones (9, 10). The cDNA clones, on the other hand, differ from each other at <60 positions. Nevertheless, $DR\beta\psi$ clearly belongs to the DR type, since both DC and SB cDNA clones have unique 3'-untranslated segments.

In coding nucleotide sequences, the proportions of potential replacement substitutions and potential silent substitutions depend on the codon composition of any given sequence (27). Gustafsson et al. (9) have previously found that for randomly distributed nucleotide substitutions in the DR β exons, \approx 75% should result in amino acid replacements (Table 1). Values close to this figure are indeed observed for the first domain exon (9). However, in the second domain exons of the expressed $DR\beta$ genes the observed proportion of replacement substitutions is only 50% of the total number of substitutions, probably because of phenotypic selection against amino acid replacements. In contrast, the proportion of replacement substitutions observed in the $DR\beta\psi$ second domain exon, as compared to the corresponding sequences of three DR β cDNA clones, is 71% (Table 1). Thus, the proportion of replacement substitutions in $DR\beta\psi$ does not differ Genetics: Larhammar et al.

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	ACCACAATGAGATACCATCTCATGCCAGTTAGAATGGCGATCATTAAAAAGTCAGGAAACAACAGCGCGCGC	240 360 480 600 720 840	Kpn I repeat
	Met Val Cys AGTAACTTCCTCCC <u>TATAATTTGGAATATGGGTGGAGCAGGGTCATAGTTCTCGCTGAGTGAG</u>	-27 956	Exon 1
	-1 +1 Leu Lys Pro Pro Gly Gly Ser Cys Met Ala Ala Leu Thr Val Thr Leu Met Val Leu Ser Ser Pro Leu Ala Leu Ala Gly Asp Thr Arg P	5 1047	S
	GCAAGTGCACATTGTGGGTGCTGAGCTACTACGAGGTCAGGAAAATAGGGAGTTTTGTTAACACCGTGCCCAGGCAATGCCCCTTAAGAGATTGTGACGTTTTCTTCAAGAGATTGCCCA TCTTTATCACGGGATCCTAAGTTATTTCCACCACAAAAGGAGCTTGGTACTTGCCCTCCATGAGGTTTGTGTAAGGAACTTCCATACAGGCCATTTCTTTTCAAATCTCCAC AACCTTTGCATCACATTTCCTCAGGGTCTTTAGAGGATTTAGAAATAAAGGATGCTAAAATAAAT	1166 1286 1406 1438	
ļ	3 AAGCTITITGTACATGGGATÍTCCTAAACCTGAAAAGTGCĆCCCCGTTTTGTCCCAACAGACAACAGGGCTCCACTCTGĆCCCXTGGCTCACACCTGCTAAGCTGTAAGTCACACTT AACTGACACTCTICAGAGGGTCCTTCTGTGGGACCCTAGTGTAATTGAGATCATCCTAITATCCTCTGTTCTAGAAGTCCACACTTCTGACATTTCTCACATTTCTCACAGTCGTGCTTAAGCTCTTGGGT GTTGGGTTTTTGGCATCGCTTTCACTGCTCTTTAAGCCCCCCCAGTGGAGAGGGTCGATGAGGAGGACCAGAGCTCCACACTTCTGACAGTCGCAGGGCAGGCA	120 240 360 480 600 720	
	To Arg Phe Leu Glu Glu Ala Lys Ser Glu Cys His Phe Phe Asn Gly Thr Glu Arg Tyr Leu (***) Arg Tyr Phe Tyr Asn Gln CCCCACAG CA CGT TTC TTG GAG CAG GCT AAG TCT GAG TGT CAT TTC TTC AAT GGG ACG GAG CGG TAC CTG TAA AGA TAC TTC TAT AAC CAA	32 811	Exon 2
	Glu Glu Tyr Val Arg Phe Asn Ser Asp Met Gly Glu Phe Árg Ala Val Thr Glu Leu Gly Arg Pro Asp Ala Glu Tyr Trp Asn Ser Gln GAG GAG TAC GTG CGC TTC AAC AGC GAC ATG GGG GAG TTC CGG GCG GTG ACT GAG CTG GGG CGG CCT GAC GCT GAG TAC TGG AAC AGC CAG	62 901	Domain 1
	Lys Asp lle Leu Glu Gln Lys Arg Ala Glu Val Asp Asn (***) [Cys] Arg His Asn Tyr Gly Val Val Glu Arg Phe Thr Val Gln Arg Arg I ANG GAC ATC CTG GAG CAG ANG CGG GCC GAA GTG GAC AAC (TAA) TGC AGA CAC AAC TAT GGG GTT GTG GAG AGA TTC ACA GTG CAG CGG CGA A	93 992	
	ATGAGCACGGCGGGGGGGGGGGGGGGGGTGTGAGTCCCTGTGAGCTGGGAATCTGAGTGTTGTGTGTG	1111 1231 1351 1463	
	C GGATCCTACCTTTGTCACTĠGAGTGACCCAGACTGATGCĊTGCACATGAGGAGCCTGGGĠTCCCAGGCCCACGACTTGAĠCCGGGGTGCTGATGCCAGĠĠGCTGTCCCAGGTTAGGCĊ GTGGCCTGGGGGACAGCAGCGCTGCCCAAAGGGAAGCTCCGTGGGGATGGTGGGGGCGCGCGGGAAGGCAGCCGGGATGCTGCGCCAGGGGAGCCAGCGCCAGGCGCCAGGGCCCCGCGCAGGGCCCAGGCCCCCGCGCAGGGCCCCCGCGCAGGGCCCCCC	120 240 360 480 600 720 840 960 1080 1200 1320 1440	
	[†] le Gln Pro Lys Val Thr Val Tyr Pro Ser Lys Asp Gln GACAAACAGCAAAACATGGAGACTTACTCTTCTCCTGACTCATTCCCTCACTTGTTCTTCTCCTAG TC CAA CCT AAG GTG ACT GTG TAT CCT TCA AAA GAC CAG	105 1546	
	Pro Leu Gln His His Asn Leu Leu Val Cys Ser Val Ser Gly Phe Tyr Pro Gly Ser Ile Glu Val Arg Trp Phe Gln Asn Gly Gln Glu CCC CTG CAG CAC CAC AAC CTC CTG GTC TGC TGC AGT GGT TTC TAT CCA GGC AGC ATT GAA GTC AGG TGG TTC CAG AAT GGC CAG GAA	135 1636	
	Glu Lys Ala Gly Val Val Ser Thr Gly Leu Ile Gln Asn Gly Asp Trp Thr Phe Gln Thr Leu Val Met Leu Glu Thr Val Pro Gln Asn GAG AAG GCT GGG GTG GTG TCC ACA GGC CTG ATC CAG AAT GGA GAC TGG ACC TTC CAG ACC CTG GTG ATG CTG GAA ACA GTT CCT CAG AAT	165 1726	
	Ala Glu Val Tyr Thr Cys Gln Leu Glu His Pro Ser Met Met Ser Leu Leu T hr Val Glu Trp A GCA GAG GTT TAC ACC NOT CAA CTG GAG CAC CCA AGC ATG ATG AGC CTT CTC ATGCA) GTG GAA TGG A GTGAGCAGCTTTCTGACTTCATACATTTCTCACC	187 1826	
	AACCATGAAGGGGTCTTTGGTAATCCCTGAGTGTCAGGTTTCTTCTCTCCCCACATCATGTTTTCATTTGCTCCATGTTCTCATCTCCATTAGGACGGGCAGGCCAGGGGGGGG	1946 2066 2186 2306 2426	5
	rg Ala Trp Ser Glu Ser Ala Gln Ser Lys Met Leu Ser ATCTCAGGGTCTCAATTAAAGAGGTTCCATTTAGGATAAAAATCACTAATCCTTGCTTCCTCTCAG GA GCA TGG TCT GAA TCT GCA CAG AGC AAG ATG CTG AGT	199 253	Exon 2 4
	Gly Val Gly Gly Phe Val Leu Gly Leu Leu Phe Leu Gly Ala Gly Leu Phe Ile Tyr Phe Arg Asn Gln Lys G GGA GTC GGG GGC TTC GTG GGC CTG GTC TTC CTT GGG GCC GGG CTG TTC ATC TAC TTC AGG AAT CAG AAA G CTGAGGAGTCTTTTGTATCGGC	224 262	
	TCTCTCCATAGACTTATCTGGAGGAGGAAAATATGGCCTTTGCTGAGGTTAGTCTCAGTATATGAGTGGCCCTAGATAAAGCC <u>TTTCTT</u> TCCGCAAATGACCTCCAAATGACCTCCAATGACCTCGAGATAATAC CAGAAATCATCAGTGCATGGTTACTATGTCAAAGCATAATAGCTTTAGGCCTGCGAGAGAAGGATAAGAGAAAGGTTAATAAC <u>BAAGAGTCCTTAGGCCAAGGTGGCGAGGCGAGGCGAGGCG</u>	274 286 298 310 322 334	7 7 7 7 7
	↓ Iy His Ser Gly Leu Gln Pro Thr G TTAAGTGTATATCTTTTCCCTGTGTGG GA CAC TCT GGA CTT CAA CCA ACA G GTAATACCTTTTAATTCTCTTTTAGAAACAGATTCAATTTCCCTAGAATGATAGT	23 345	
	GGAGGTGATAAGGCATGAGACAGAAATAATAAAAAGACTTTGGATTCAAATTTCTGATCAGGCAATTTACACCAAAACTCCTTCTCTCCACATAGAAAAGGCCTGTGCTCTGCAGGAGAA CTGGCTCATGGAGACTTAAGAACTTGTTTTTTCTTCCTGCAGTGCTCTCAATCTGAGTCCTTGAAAGAGGGGAAAGGAGGCGGTTAGTAGAGACGCGGATCTGAAAACAACAC	357 369	7
	UN LEU SET TEM GTCTTTTGCAG GA CTC CTG AGC TGA AGTGAAGATGACCACATTCAAGGAGGAACCTTCTTCCCCAGCTTTGCAGGATGAAAAGCTTTCCCACCTGGCTGTTATTCTTCCATAAGA	23 381	
	GAGGGCTTTCTCAGGATCTGGTTGGTTCAGGAACTCTGCAGAAAATGTCCTCCCCTTGTGGCTTCAGCCCCTGCCTTTGGCCTGAAGTCCTGGCATTGATGGCAGCACCTCA TCTTCAACTTTTGTGCTCCCCTTACCTAACCCTTCCTCCCCCCGCATCTGTACTCCCCCCTTGTGCCACAACACTTACATTAAATGTTTTCTCACACACGAGATAAAAATC	393 405	2
	<u>ATCINGTCCAGCCAGGCACTOTGCCTCATGCCTCATATCCCAGCATUTTGGAGGCCGGCGAGACGCGAGGTCAATAGATCACTACCATCCCTGCAGGTGAAACCCCCCT</u> CTCTACTAAAAATATAAAAATTAGTIGGCGTGGGGTGGAGCTACCACCCCTGAGGCCAGGCC	417 429 439	2 2

FIG. 2. Nucleotide sequence and predicted amino acid sequence of $DR\beta$ pseudogene. Exons are denoted with S for signal sequence; M, transmembrane region, C, cytoplasmic segment; and 3'UT, 3'-untranslated region. Splice junctions inferred from $DR\beta$ cDNA clones (9) and a $DC\beta$ gene (21) are shown by arrows. The conserved cysteine residues are within boxes. Circles enclose the two premature termination codons (26 and 76) and the frame-shift mutation (183). Underlined in the 5' part of the gene (A) are the four putative control and promoter elements. Kpn I repeat in the 5'-flanking region is within a box (A). The 3'-untranslated region is underlined, and the polyadenylylation signal (A-T-T-A-A-A) is within a box (C). Two Alu type 1 repeats are underlined by double lines, and their flanking 11-mer repeats are within boxes (C).

Table 1.	Potential and observed substitutions in nucleotide sequences encoding first and second domains of expressed $DR\beta$ genes (9)	9) and
$DR\beta$ pset	ogene	

	First domain (1-94)		Second domain (95-188)	
	Expressed $DR\beta$	DRβψ	Expressed $DR\beta$	DRβψ
Potential replacement sites in % of total sites	76		77	
Observed number of replacement substitutions	28	34	9	20
Observed number of substitutions	35	44	18	28
Observed replacement substitutions in % of observed substitutions	80	77	50	71

significantly from what would be expected in a phenotypically unselected coding nucleotide sequence, suggesting that $DR\beta\psi$ was relieved from phenotypic constraints before most of the nucleotide substitutions in the second domain exon occurred. This finding is further corroborated by the presence of replacements in the amino acid sequences predicted from $DR\beta\psi$ at positions that are well conserved in all other β chains known or even in other members of the immunoglobulin-histocompatibility antigen superfamily of proteins (28) (position 173, L for V; 178, M for L; and 181, L for P).

Two complete codons have been deleted after codon 23 in $DR\beta\psi$, as compared to other $DR\beta$ and $DC\beta$ sequences, as well as their murine homologues (Fig. 3). Deletions of short nucleotide segments have been proposed to be generated by "slipped mispairing" at short direct repeats (29). Indeed, a short repeat can be observed in the sequences of the DR β cDNA clones (Fig. 3). Interestingly, the corresponding codons are absent also in the presumably expressed $SB\beta l$ gene previously isolated from the same genomic library (ref. 20; K. Gustafsson, personal communication). The deletion may have occurred independently in the $DR\beta$ and $SB\beta$ genes or, alternatively, a nucleotide segment that had lost the two codons may have been copied from one gene to the other by microgene conversion, as has been reported for class I as well as class II histocompatibility genes (30-34). It will be interesting to see whether the two-codon deletion is found in all SBB1 alleles and in the SBB2 gene, and also whether it is present in some expressed $DR\beta$ alleles.

Promoter Region. Two nucleotide stretches in the 5'-flanking regions of genes have been implicated in the regulation of transcription. These are C-C-A-A-T at position -70 from the mRNA start site, and the "TATA" box at position -25 (26). Both of these elements can be identified in the 5' region of $DR\beta\psi$, as well as in the murine $E\beta$ gene (25) (Fig. 2A).

Two segments of conserved nucleotides have previously been identified (25) in the 5' flanking regions of the human $DR\alpha$ gene (35) and the murine $E\alpha$ (36, 37) and $E\beta$ genes (25). The conserved elements are also present in human $DC\alpha$ (A.-K. Jonsson, personal communication) and $DC\beta$ genes (21), as well as in the murine $A\beta$ gene (24). These nucleotide segments are remarkably well conserved also in $DR\beta\psi$. However, alignment of the conserved elements from different class II genes (Fig. 4) shows that $DR\beta\psi$ alone differs at the last

CLONE DESIGNATION	Locus	20 25 30
Р11-в-3	DRø	GLYTHRGLUARGVALARGTYRLEUASPARGTYRPHE gggacgga <u>gcgg</u> tg <u>cgg</u> tacctggacagatacttc
РII-в-4	DRB	TGACG-G
DRBI	DRß	TGCA
cosiI-801	DR₿ψ	T-A
cosII-412	SB\$1	ACC++++++TGA
cosII-102	DCß	CTCTTGACA-

FIG. 3. Alignment of class II β nucleotide sequences. The six nucleotides missing in the $DR\beta\psi$ and the $SB\beta l$ sequences are indicated by asterisks. Amino acid sequence is deduced from pII- β -3. The direct repeat proposed to be involved in the deletion event is underlined. Sequences were obtained from the following sources: pII- β -3 and -4 (9), DR β I (10), cosII-412 (20), cosII-102 (21).

two positions of the otherwise almost invariant 8-mer element. If the conserved nucleotide segments are involved in the regulation of class II gene expression, this mutation might have contributed to the inactivation of the $DR\beta$ gene.

The presence of tissue-specific transcriptional enhancer elements in the region preceding the putative promoter elements of a murine $E\beta$ gene, the structural homologue of $DR\beta$, has recently been described (38). However, the corresponding region of $DR\beta\psi$ is occupied by a member of the Kpn I family of repeated sequences (39). The repeat terminates with a polyadenylylation signal 80 base pairs 5' of the conserved 13-mer (Fig. 2A). Thus, the juxtaposition of the Kpn I repeat close to the DR $\beta\psi$ promoter region might interfere with transcription by removing the enhancer elements. The role of the Kpn I repeats is obscure, but it is known that some family members are transcribed (e.g., see ref. 39).

Possible Importance of $DR\beta\psi$ in the Generation of β -Chain Polymorphism and in Disease Associations. Individuals of the DR4 specificity have at least three $DR\beta$ -related genes (J. Böhme, personal communcation). $DR\beta\psi$ corresponds to some of the strongly hybridizing fragments in the restriction enzyme-digested genomic donor DNA when using DR β cDNA probes. Hybridizing fragments of the same sizes are present also in the genomic DNAs of other DR4 individuals (not shown), suggesting that $DR\beta\psi$ is present in most DR4 individuals. Considering the large number of pseudogene criteria, it is unlikely that this locus may encode a functional β chain in other DR4 individuals.

The genomic DNA restriction fragments corresponding to the $DR\beta$ pseudogene of the DR4 donor are not present in the genomic DNAs of individuals of other DR specificities except DR7. This suggests either that $DR\beta\psi$ is located on polymorphic restriction fragments, or that it is absent in individuals of other DR specificities. These two alternatives cannot be easily distinguished, as the pseudogene and two presumably expressed $DR\beta$ genes isolated from the same libraries (unpublished observations) hybridize equally strongly to $DR\beta$ exon probes. Preliminary attempts to isolate $DR\beta\psi$ specific intron probes have been unsuccessful. Even if such probes can be isolated, absence of hybridization signals from DNAs of other DR specificities will have to be interpreted with caution, because intron sizes can vary considerably between alleles (40).

Although $DR\beta\psi$ does not give rise to a functional β chain,

Gene	13-MER	DISTANCE	8-MER	DISTANCE TO CAP SITE	DISTANCE TO AUG CODON
CONSENSUS SEQUENCE	CTAGCAACAGATG	- 19 -	CTGATTGG		
DRβΨ	-c	19	CA		148
Еβ		19		89	141
DRa		20		66	130
Eα		20		51	99
DCB	——AG———T	19			133
Ав	-C-AG	19			125
DC∝	T GA	20	— A ——		116

FIG. 4. Alignment of the putative control elements in the 5' regions of class II genes. Sequences were obtained from the following sources: $E\beta$ (25), $DR\alpha$ (35), $E\alpha$ (36), $DC\beta$ (21), $A\beta$ (24), and $DC\alpha$ (A.-K. Jonsson, personal communication).

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it may contribute to the generation of polymorphism in the expressed β genes by accumulating mutations that are subsequently transferred to other β genes through microgene conversion. Transferred segments containing deleterious mutations will probably be selected against, whereas others may spread and become fixed. The contribution of $DR\beta\psi$ to β -chain polymorphism may be significant if it is present in individuals of different DR specificities.

The identification of one of the $DR\beta$ genes in DR4 individuals as a pseudogene makes this gene unlikely to be responsible for DR4-linked diseases (e.g., insulin-dependent diabetes mellitus; see ref. 3). The mere absence of an expressed $DR\beta$ allele at this locus is less likely to cause disease susceptibility. Nevertheless, $DR\beta\psi$ may serve as a useful marker in DNA hybridizations, since it may be linked to expressed disease-associated β -chain alleles whose polymorphic nucleotide positions do not affect restriction sites.

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