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Complete primary structures of the Eβ chain and gene of the mouse major histocompatibility complex

(major histocompatibility complex I-E molecule/exon–intron/control of gene expression/cDNA/cloning)

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ABSTRACT Using the cross-hybridization with plasmid pDCβ-1, containing the cDNA coding for the DCβ chain of the human major histocompatibility complex class II molecules, we have cloned and subjected to sequence analysis both the cDNA and genomic gene for the Eβ chain of the BALB/c (d haplotype) mouse. The nucleotide sequences of the cDNA and genomic DNA clones permitted us to deduce the entire primary structure of the Eβ chain and the complete exon–intron structure of the Eβ gene. Unlike α chain genes that contain five exons, the Eβ gene consists of six exons corresponding to the six functional domains—the leader, β1 and β2 domains, transmembrane peptide, intracytoplasmic peptide, and 3′ untranslated region. In addition, two short blocks of sequences common to α and β chain genes were identified in the 5′ flanking regions. We propose that these sequences are involved in the coordinate expression of α and β chains.

The I region of the major histocompatibility complex (MHC) contains Ir (immune response) genes that control the level of immune responsiveness to particular antigens (1, 2). These genes encode the class II molecules of the MHC referred to as Ia (immune associated) antigens, which are a set of glycoproteins present on the surface of the antigen-presenting B lymphocytes and macrophages (for a review, see ref. 3). The function of the class II molecules is to provide certain types of T lymphocytes (helper- and delayed-type hypersensitivity T cells) with the specific molecular context in which to recognize the antigens (MHC restriction; for a review, see ref. 4). The molecular analysis of the organization, structure, and expression of Ir (or Ia) genes is important for understanding MHC restriction and the control of immune responsiveness.

In mice two class II molecules, both heterodimers composed of α and β chains and designated as Aα Aβ and Eα Eβ, have been well characterized by serological and biochemical methods (3). However, information concerning the primary amino acid sequences of these four chains has been limited. Although the entire sequences of an Aα and an Eα chain were recently deduced from the analysis of cDNA clones (5, 6), only partial NH_2-terminal sequences have been reported to date for the other two chains (for $E\beta^d$ chain, see refs. 7, 8). In man three class II molecules designated DC, DR, and SB, each consisting of an α and a β chain, have been identified (9, 10). The amino acid sequences of some of these chains have been determined either directly (11) or deduced by the analysis of cDNA clones (12–19). This sequence information suggests that the mouse I-A and I-E molecules correspond to the human DR and DC molecules, respectively (20). The mouse counterpart of the third human class II molecule, SB, has not yet been identified.

Studies on the structure of the class II (or Ir) genes began only recently. To date the complete exon–intron structure has been reported only for the α chain genes—namely, those for the Eα and DRα chains (6, 12). We report here the complete exon–intron structure of a β chain gene, specifically the Eβ gene. The results show that the β gene is organized somewhat differently from the α genes and contains six exons, each of which corresponds to a specific functional domain.

MATERIALS AND METHODS

Escherichia coli strains K803 and χ1776 and phage λ Charon 4A are from our laboratory collection. BALB/c B-cell lymphoma L10A2J (I-A^d and I-E^d positive) was obtained from Philippa Marrack (National Jewish Hospital, Denver, CO). Preparations of mouse embryo (BALB/c) *Eco*RI partial and *Eco*RI* libraries by using Charon 4A as a vector were described previously (21, 22). A cDNA library of L10A2J mRNA was constructed according to Okayama and Berg (23), except that we used χ1776 as a host strain. These libraries were screened by the methods of Benton and Davis (24) and Hanahan and Meselson (25). The human DCβ cDNA clone used as the hybridization probe was previously designated as pDRβ1 (14). Because we now know that the clone in fact contains DCβ rather than DRβ cDNA (18), we refer to this clone as pDCβ1 to avoid any possible confusion. Nucleotide sequences were determined according to Maxam and Gilbert (26).

RESULTS AND DISCUSSION

Isolation and Sequence Analysis of Eβ cDNA Clones. We screened the L10A2J cDNA library by using plasmid pDCβ1 as the probe. Independently isolated cDNA clones fell into two distinctive groups on the basis of their restriction maps. The clones of the first group hybridized to the human DCβ probe much more strongly than the clones of the second group. Because the human DCβ gene corresponds to mouse Aβ gene (20) and because Aβ and Eβ genes are expected to cross-hybridize (27), we tentatively concluded that clones in the first group contained the mouse Aβ cDNA and those in the second group, the mouse Eβ cDNA. Among the five putative Eβ cDNA clones, one (pEβ24) contained an insert of about 1.3 kilobases (kb), which was almost as long as the Eβ mRNA. Because the original isolate of pEβ24 tends to spontaneously produce a small deletion in the relatively long poly(dA) tail, we subcloned a plasmid (pEβ24-1) with a shorter poly(dA) tail. We then constructed the restriction map of this clone and determined its nucleotide sequence according to the strategy shown in Fig. 1.

Isolation of the Eβ Gene Clones. We isolated a series of overlapping genomic DNA clones that contain the entire Eβ

Abbreviations: MHC, major histocompatibility complex; TM and CP peptides, transmembrane and intracytoplasmic peptides, respectively; bp, base pair(s); kb, kilobase(s); Ir, immune response; Ia, immune associated.

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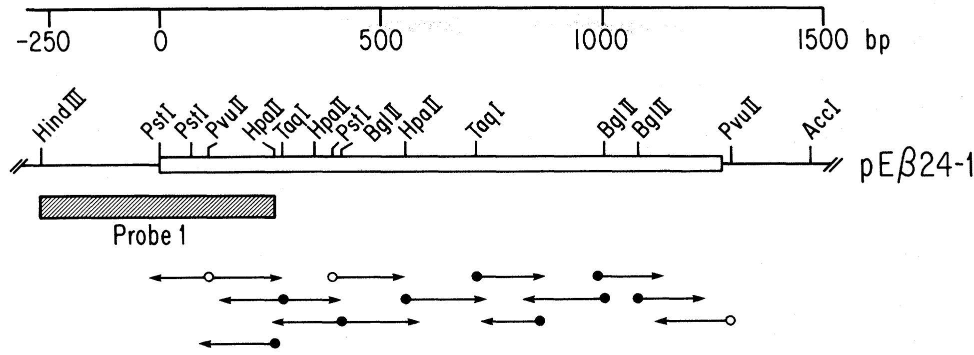

FIG. 1. Restriction map of the mouse $E\beta^d$ cDNA insert of pEβ24-1 and the sequence analysis strategy. The arrows indicate the direction and extent of the sequence determination. Open circles and filled circles indicate 5′- and 3′-end labeling, respectively. bp, Base pairs.

gene and its flanking regions. First we screened the BALB/c embryonic *Eco*RI partial library by using the *Pst* I–*Eco*RI fragment of pDCβ1 as the probe (14) and isolated 12 independent clones. The restriction mapping analysis indicated that 11 of the 12 clones are identical (represented by clone 9.2) and contain two *Eco*RI fragments of 6 and 9 kb. The 12th clone (clone 11.1) was different from the others and contained two *Eco*RI fragments of 1.9 and 12 kb. Comparison of the restriction enzyme cleavage sites contained in each of our two types of clones and the data reported by Steinmetz *et al.* (27) indicated that clone 9.2 contains the Aβ gene, whereas clone 11.1 carries the Eβ gene. This conclusion was supported by the fact that both the 1.9- and 12-kb *Eco*RI fragments of clone 11.1 hybridized strongly with the Eβ cDNA clone pEβ24, whereas clone 9.2 hybridized with this cDNA clone much more weakly (data not shown).

The sequence analysis revealed that clone 11.1 lacks the leader exon (see below). To isolate the genomic clones containing the leader exon, we screened the same *Eco*RI partial library by using the 5′ end probe dissected out from cDNA clone pEβ24-1 (probe 1 in Fig. 1) and obtained clone HS1. This clone contains a 13-kb *Eco*RI fragment carrying the leader exon but does not overlap with clone 11.1. To isolate clones that bridge the sequences contained in the two clones, we screened the BALB/c *Eco*RI* library with the 1.25-kb *Eco*RI–*Pvu* II fragment of clone 11.1 (probe 2 in Fig. 2*A*) as the hybridization probe and isolated clones 6D and 10A. The restriction maps of the four genomic clones showed that they contain overlapping fragments covering a continuous stretch of about 28 kb. Our composite restriction map shown in Figure 2 is, for the most part, in agreement with that of Steinmetz *et al.* (27). However, their map lacks several relatively small restriction fragments such as the 0.7-kb *Eco*RI fragment located in the middle of the map.

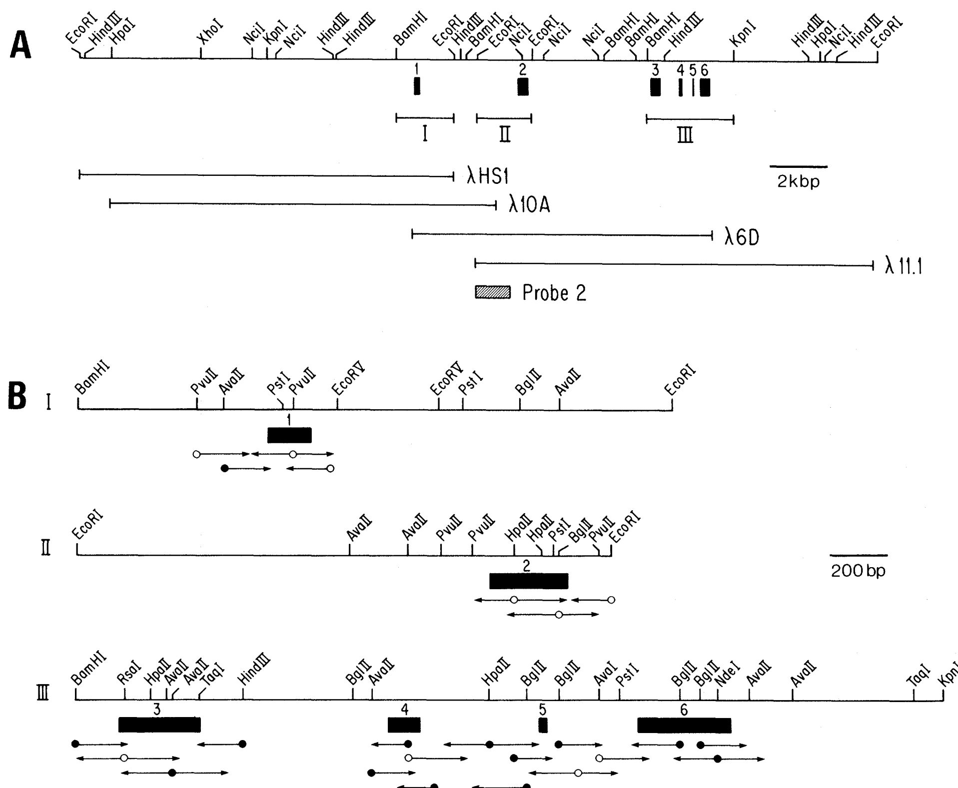

FIG. 2. (*A*) Restriction map of the mouse genomic DNA segment containing the $E\beta^d$ gene. The inserts in the four overlapping λ phage clones are shown by horizontal bars. The three regions (I, II, and III) that hybridized to the cDNA clone pEβ24-1 are enlarged in *B*. Positions and approximate sizes of exons are shown by filled boxes. The horizontal arrows indicate the sequence analysis strategy. kbp, Kilobase pairs.

To locate the exons we analyzed the four genomic clones by Southern gel blotting using various subfragments of clone pEβ24-1 as the probes. These studies lead to the identification of three regions (I, II, and III shown in Fig. 2A) that showed homology to the cDNA. By comparing the detailed restriction maps of these areas (Fig. 2B) with that of pEβ24-1 (Fig. 1), we could identify five distinct exons—namely, exons 1, 2, 3, 4, and 6. One additional exon (exon 5) was identified by DNA sequence analysis (see below).

Nucleotide Sequence and the Complete Exon–Intron Structure of the Eβ Gene. The nucleotide sequences of the six exons and the flanking regions of the Eβ gene are shown in Fig. 3. Comparison of these sequences with the nucleotide sequence of the cDNA clone pEβ24-1 permitted the identification of the exact exon–intron boundaries, except for the 5′ end of the first exon. Because we do not know whether the cDNA is a full transcript of the Eβ mRNA, we could not precisely identify this end. Within the exons the cDNA sequence matches exactly with the genomic DNA sequence. Thus, in Fig. 3 those sequences contained in the cDNA clone are indicated simply by underlines.

The six exons indeed code for the Eβ chain. In the cDNA sequence the first methionine codon ATG is located at the nucleotide positions 53–55. This methionine codon is followed by an open reading frame for translation of 792 nucleotides that spans the six exons. The predicted amino acid sequence (264 residues in total) is indicated in Fig. 3. The methionine is followed by a hydrophobic peptide composed of 26 residues, which is reminiscent of a signal peptide. This peptide is followed by a peptide having a high degree of sequence homology with the previously determined partial NH_2-terminal amino acid sequence of the mouse $E\beta^d$ chain (7, 8): of the 15 determined amino acid residues of the 28 NH_2-terminal positions, 13 matched with the residues predicted by the nucleotide sequence (Fig. 4). In addition to these matches, the predicted amino acids from the positions 15–19 correspond exactly to the pentapeptide Glu-Cys-His-Phe-Tyr, which was shown to compose the corresponding region of the mouse $E\beta^k$ chain (27). Altogether, the

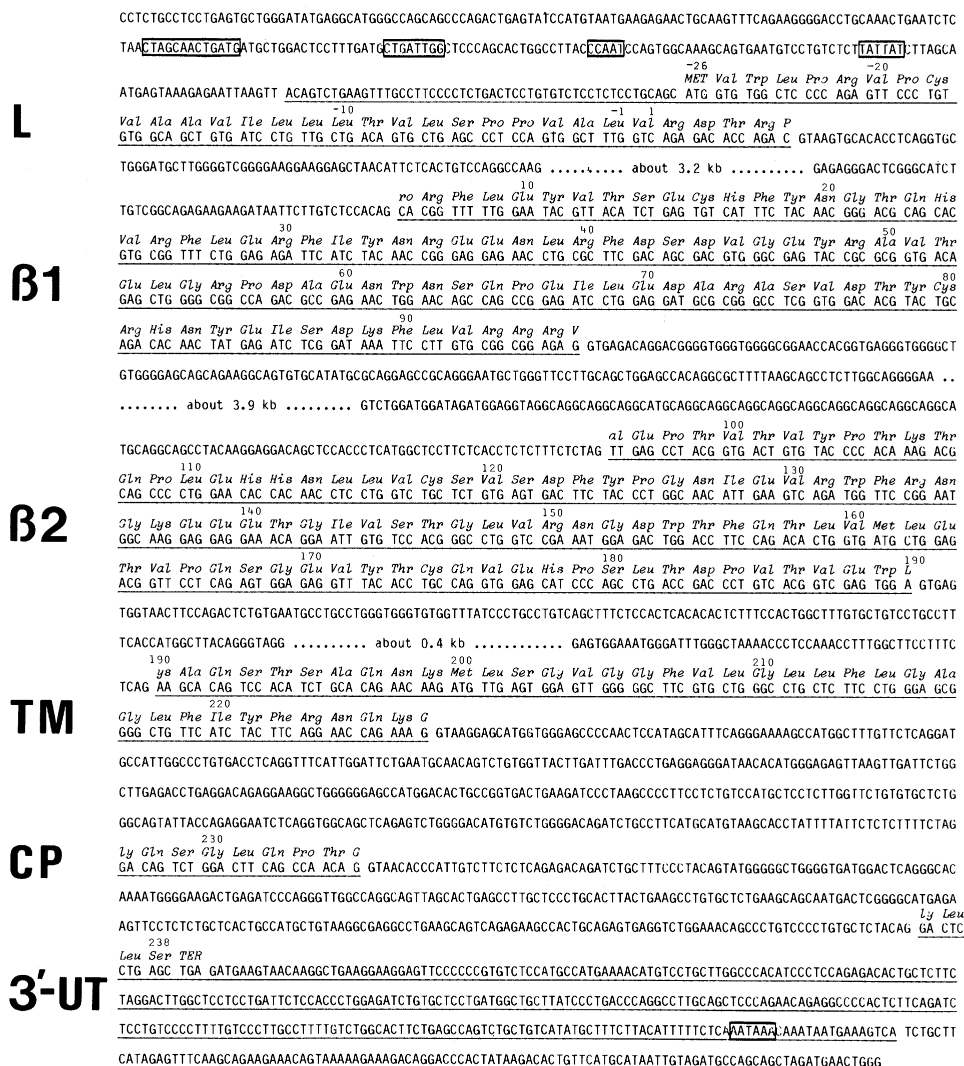

FIG. 3. Nucleotide sequences of the Eβ gene and the predicted amino acid sequences of the Eβ chain. The nucleotide sequences of the six exons [i.e., leader (L), β1, β2, transmembrane (TM), intracytoplasmic (CP), and 3′ untranslated (3′-UT)] and their immediate flanking regions are shown. The nucleotide sequence of the cDNA insert in pEβ24-1, which is indicated by underlining, matches exactly the exon portions of the genomic DNA sequences. The predicted amino acid sequences are shown above the nucleotide sequences. The numbers refer to the amino acid positions in the mature Eβ chain. Several possible controlling DNA elements involved in the transcription are enclosed in boxes; these are: two 5′ upstream sequences common among the Eβ, Eα, and DRα genes (see text and Fig. 5A), "CCAAT" and "TATA" boxes, and the poly(A) addition signal A-A-T-A-A-A.

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
Protein	Val	Arg	---	Ser	Arg	Pro	---	Phe	Leu	---	Tyr	Val	Lys	Ser	---
cDNA	Val	Arg	Asp	Thr	Arg	Pro	Arg	Phe	Leu	Glu	Tyr	Val	Thr	Ser	Glu

	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30
Protein	---	---	Phe	Tyr	---	---	---	---	---	---	---	Phe	Leu	---	---
cDNA	Cys	His	Phe	Tyr	Asn	Gly	Thr	Gln	His	Val	Arg	Phe	Leu	Glu	Arg

FIG. 4. Comparison of the determined partial amino acid sequence of the NH_2-terminal of the $E\beta^d$ chain (7, 8) with the amino acid sequence predicted from the nucleotide sequence. A dash indicates that the amino acid residue at that position has not been determined. Matching residues are underlined.

number of matches between the predicted and determined NH_2-terminal amino acid residues are 16 out of 18. The discrepancies occur at amino acid positions 4 (threonine vs. serine) and 13 (threonine vs. lysine). These discrepancies may result from errors in either the amino acid or the nucleotide sequence determination, although consistent data have been repeatedly obtained by nucleotide sequence determination. Alternatively, the discrepancies could be attributed to microheterogeneity within an inbred strain because both substitutions could be explained by single base changes in the corresponding codons. Ultimately, we believe that it is highly unlikely that this gene codes for a hitherto unidentified Eβ-like chain. The reasons are that the amino acid sequence match is good, all five independently isolated cDNA clones give indistinguishable restriction maps and therefore should represent the major Eβ mRNA species expressed in L10A2J, and four genomic clones clearly contain the genomic Eβ gene on the basis of its restriction map (compare Fig. 2 with the restriction map of ref. 27).

Exons correspond to the functional domains. As was first shown for an immunoglobulin heavy chain gene (28) and subsequently confirmed by numerous examples, including the Eα (6) and DRα (12) genes, the Eβ gene is split into multiple exons, each of which corresponds approximately to a functionally distinct domain. Thus, exon 1 corresponds to the 5′ untranslated region and the signal peptide. Exons 2 and 3 code for the external domains β1 and β2, respectively. Each of these exons contains a pair of codons for cysteine that will probably form intradomain disulfide bonds. Exon 4 contains codons for a stretch of 23 hydrophobic amino acids (positions 200–222) that comprises the expected TM peptide. Unlike the Eα or DRα gene, the core portion of the CP peptide of the Eβ gene is encoded by its own exon (exon 5). Also unlike Eα or DRα gene, where the 3′ untranslated region is split into two exons, the sixth exon of the Eβ gene encodes the entire 3′ untranslated region and the COOH terminus of the CP peptide. In these two respects the structure of the Eβ gene is more similar to those of MHC class I genes than to the Eα or DRα gene (29).

Human DCβ gene may lack the independent CP exon. The nucleotide sequences of the human DCβ and DRβ cDNAs have been reported (14, 18). In the translated regions, Eβ is highly homologous to both DCβ and DRβ (the nucleotide sequence homology of Eβ and DCβ is 72%, and that of Eβ and DRβ is 81%), except for a deletion of a 24-bp stretch near the 3′ end of DCβ (Fig. 5A). Interestingly, the core part of the CP peptide encoded by the exon 5 of the Eβ gene is precisely the peptide deleted in the DCβ chain (compare Fig. 3 and Fig. 5A). This immediately suggests the possibility that the human DCβ gene lacks the corresponding exon. This is reminiscent of the demonstrated absence of one of the two CP exons in the human class I genes in comparison with the mouse counterparts (30). However, it is also possible that the DCβ gene does have the exon 5 but the pDCβ-1 cDNA clone arose from a mRNA that was generated by the direct splicing of the donor splice site at the 3′ end of the exon 4 with the acceptor splice site at the 5′ end of the exon 6. It is unknown whether such skipping of the exon 5 sequence might occur as a normal or abnormal event in

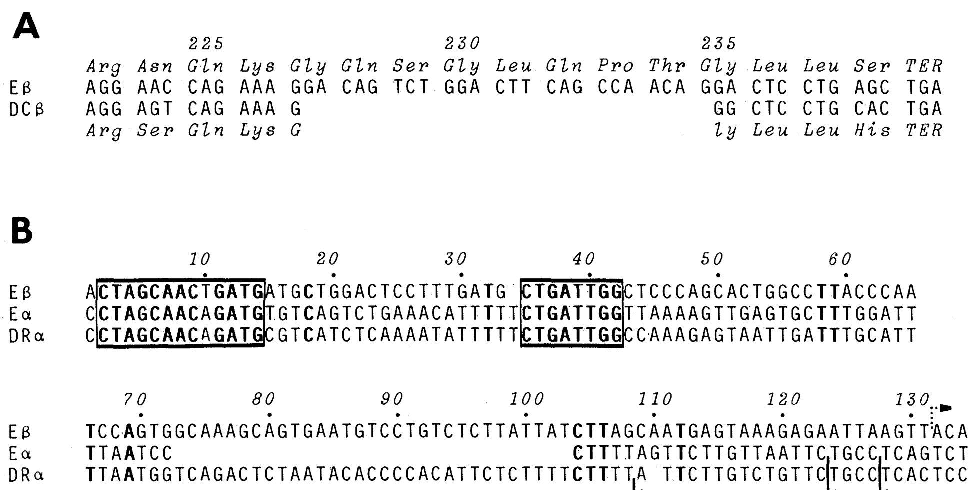

FIG. 5. (*A*) Comparison of the determined nucleotide sequences and the predicted amino acid sequences in the COOH-terminal regions of the mouse Eβ chain (this work) and the human DCβ chain (14). Note that the 24-bp stretch deleted in the DCβ gene corresponds exactly to the region encoded in the CP exon of the Eβ gene (see Fig. 3). (*B*) Comparison of the nucleotide sequences of the 5′ flanking regions of the Eβ, Eα, and DRα (6) genes. Nucleotides common to all three genes are shown in boldfaced type, and the two blocks of homologous sequences discussed in the text are enclosed in boxes. The transcription start sites of the Eα and DRα genes are indicated by the solid arrows. The 5′ end of the Eβ cDNA insert that is thought to be in close vicinity of the transcription initiation site (see text) is indicated by the dotted arrow. The gaps are placed in order to maximize the sequence homology.

the processing of the primary RNA transcript. An interesting possibility is that both RNA forms are physiologically functional and that the two corresponding forms of the polypeptide chain that differ in the CP peptide structure have different effector functions. The determination of the exon–intron structures of the DCβ and DRβ genes and more extensive analysis of the mRNA encoded by these and Eβ genes in different cells will resolve this issue.

Conserved sequences in the 5′ flanking regions of Ia genes. Like many eukaryotic genes, the Eβ gene seems to carry a TATA box (31) and a CCAAT box (32) in the 5′ flanking region (Fig. 3 and Fig. 5*B*). The TATA box plays a critical role in determining the precise transcription initiation site (33).

The comparison of the nucleotide sequences in the 5′ flanking regions of three Ia genes—Eα, DRα (6), and Eβ—revealed two short conserved sequences further upstream (Fig. 5*B*). These are C-T-G-A-T-T-G-G and C-T-A-G-C-A-A-C-$\frac{A}{T}$-G-A-T-G located 89 and 116 bp upstream of the 5′ end of the pEβ24-1 insert, respectively. The locations of these sequences relative to the cap sites vary somewhat depending on the individual Ia gene, but the length of the spacer separating the two sequences is remarkably conserved and is 19 (Eβ) or 20 bp (Eα and DRα). Recently two upstream DNA elements (beside the TATA and CCAAT boxes) have been identified that are essential for high level transcription of the thymidine kinase gene. These elements are located around 50 and 100 bp upstream of the cap site (34). Although no sequence homology can be found between these DNA elements and the elements associated with the Ia genes, it is tempting to speculate that the latter play a critical role in the expression of the Ia genes. In light of the conservation of these sequences between α and β chain genes, one possibility is that these sequences are involved in the coordinate expression of the two types of chains. This hypothesis can be experimentally tested by applying the DNA-mediated gene transfer technique to these gene systems.

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