Volume 11 Number 15 1983 Nucleic Acids Research

The complete nucleotide sequence of the I-Eα^d immune response gene

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Received 9 June 1983; Accepted 7 July 1983

ABSTRACT

We have isolated and sequenced the complete murine I-Eα immune response gene of the H-2^d haplotype. The I-Eα^d gene consists of 5300 basepairs and is organized into five or possibly six exons that correspond to different domains of the α chain. The amino acid sequence deduced from the I-Eα gene shows 75% homology to its human counterpart, the HLA-DR α chain. The absence of I-E antigen in H-2^b mice is due to lack of Eα chain synthesis. We show here that this defect is caused by a deletion in the 5' end of the I-Eα^b gene.

INTRODUCTION

Production of antibodies against defined antigens is a complex procedure controled by several sets of genes (1). Thus, immunoglobulin variable genes giving rise to antibodies specific for a certain antigen must be present in the germ-line genome. Moreover, regulatory genes, called immune response (Ir) genes, affect the production of antibodies. One set of Ir genes has been localized to the Major Histocompatibility Complex (MHC)(2). Several lines of evidence indicate that these MHC gene products are equivalent to the class II transplantation antigens. (3). The class II molecules are present on the surface of macrophages and participate in the presentation of environmental antigens to regulatory T-cells (3, 4).

The Class II transplantation antigens are heterodimers consisting of two membrane-integrated chains denoted α and ß (5). The linear structures of several human class II antigen chains have been elucidated (6-11). Both α and ß chains are composed of two extracellular domains, a membrane-spanning portion and a short cytoplasmic tail. Sequence comparisons have shown that both chains belong to the same protein superfamily

as the immunoglobulins (6-9, 12, 13). The genetic polymorphism, which is a characteristic trait of all transplantation antigens, resides predominantly in the ß chain (14-16).

Mouse strains of the haplotypes b, f, q and s do not express I-E antigens due to inability to produce the I-Eα chain (17). We decided to investigate whether the lack of expressed I-Eα chains is due to abberant transcription or translation of the gene, since a specific regulation of the class II antigens may be important for certain aspects of the immune responsiveness. In order to examine this issue, we have determined the complete structure of a functional I-Eα gene, which has then been compared with the I-Eα^{b} gene. Partial structures of the human DRα gene (13, 19) and the murine I-Eα^{d} gene have been reported (18). Recently Mathis et al. described the sequence of the exons and most of the introns of the I-Eα^{k} gene (19). We report here the first complete structure of a class II transplantation antigen gene. Moreover, we demonstrate that the inability of H-2^{b} mice to produce I-Eα chains is due to a deletion of the 5' end of the I-Eα^{b} gene.

EXPERIMENTAL PROCEDURES

Isolation of cosI^{d}-α-1

A library was constructed with DNA from the Balb/c myeloma cell line X63-5-3-1 (20) using the cosmid vector pOPF (21). Approximately 240,000 colonies were obtained from 3 µg of size fractionated DNA. The cosmid library was screened with a restriction fragment from a human HLA-DR α-chain cDNA clone as the probe (9). Three positive clones were isolated. Hybridizations of several different restriction fragments from these cosmids to restriction enzyme digested Balb/c spleen genomic DNA showed that one of the three cosmids, cosI^{d}-α-1, was colinear with Balb/c spleen genomic DNA. This cosmid was accordingly chosen for further analyses.

Nucleotide sequence determinations

The major part of the sequence of the I-Eα^{d} gene was determined with the chemical degradation method (22) or with the dideoxy chain termination technique (23) with M13 mp7, mp8 and mp9 as cloning vectors (23, 24). Part of the sequence work

was carried out with a modification of the Exo III method (25), whereby synthetic 12 bp-oligonucleotides were used as primers in the chain termination reactions (J.J. Hyldig-Nielsen, in preparation). In the assembly of the nucleotide sequences, computer programs developed by Staden were used (27).

Southern blotting

Genomic DNA was isolated from the livers of Balb/C, C3H, B10, B10.A, B10.D2, B10.A(2R), B10.A(3R), B10.A(4R) and B10.A(5R) mice (28). DNA samples of 10 µg were digested with restriction enzymes. After agarose gel electrophoresis, the DNA was transferred onto nitrocellulose filters and hybridized to nick-translated probes (29) according to Southern (30).

Biosafety

This work was carried out in accordance with NIH guidelines for recombinant DNA research.

RESULTS

Isolation of a cosmid containing the H-2^d I-Eα gene

A cosmid clone, cosI^d-α-1, containing the I-Eα^d gene was isolated by hybridization to a probe corresponding to the translated portion of a human HLA-DR α-chain cDNA clone (9). The hybridizing portion of the cosmid was located close to one end of the insert (Fig. 1). No other α- or ß-chain genes were found in the cosmid by hybridization to HLA-DR and HLA-DC α- and ß-chain probes (6-8, Gustafsson et al., unpublished).The cosmid was mapped to the I-E region by Southern blot hybridizations to genomic DNA from parental and recombinant mouse strains of haplotypes b, d and k. A Pvu I - Sma I restriction fragment of the cosmid, corresponding to the 5' end of the I-Eα^d gene (see fig. 4A) was hybridized to Pvu II-digested genomic DNA from B10 (H-2^b) and B10.D2 (H-2^d) mice. A strongly hybridizing 5.8 kb fragment was found for the H-2^d haplotype while the H-2^b haplotype gave rise to a 5.1 kb fragment (see fig. 4B lanes 1, 2). The latter fragment was also found in Pvu II digested DNA of B10.A(4R) mice but not in DNA from B10.(2R), B10.A(3R) and B10.A(5R) mice. Furthermore, hybridization of a 3.0 kb Bam HI restriction fragment, derived from the 5'end of the cosmid, to Pst I digested DNA of C3H (H-2^k) and B10.A (H-2^a)

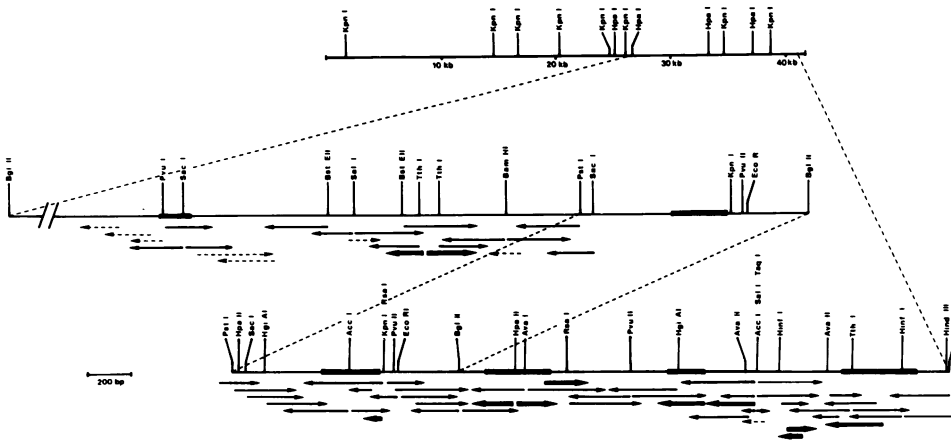


Figure 1
Maps and sequencing strategy. Restriction maps of the cosI^{d}-α-1 cosmid and the Bgl II and Pst I subclones. Hatched lines indicate the relationship between the clones. The 5' ends of the coding strands are to the left. Exons are indicated in the maps of the subclones by bold lines. The nucleotide sequencing strategy is displayed by the arrows.
Fine arrows: Sequences determined by chemical degradation.
Bold arrows: Sequences obtained with dideoxy chain termination technique after cloning of restriction fragments into M13 vectors.
Hatched arrows: Sequences derived by the use of a modified Exo III method.

mice gave rise to a band of 5.4 kb. In contrast, Pst I digested Balb/c (H-2^{d}) DNA displayed a 4.3 kb fragment hybridizing with the same probe. The combined results of these experiments unequivocally map cosI^{d}-α-1 to the I-E region of the MHC (31).

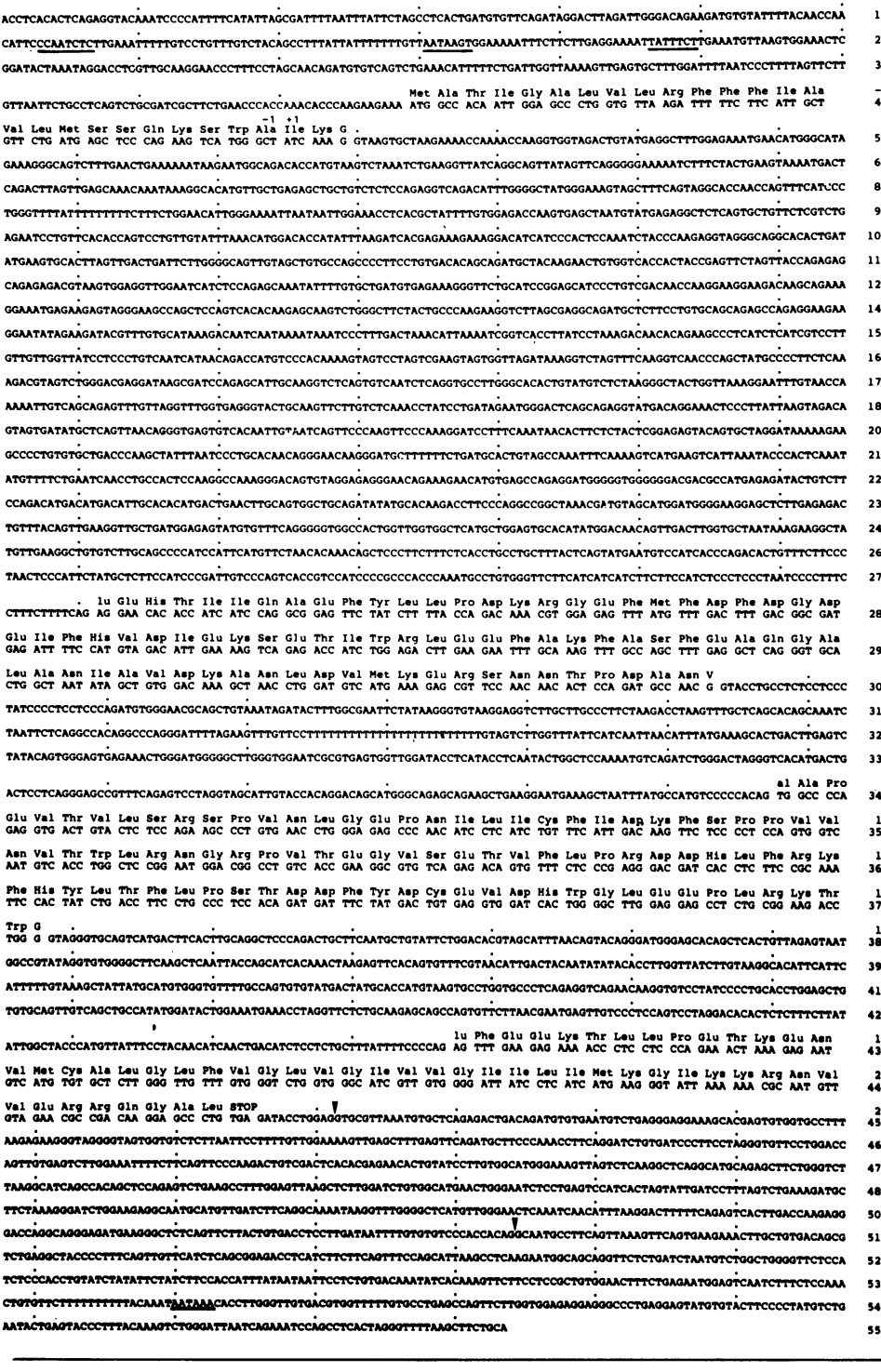
Structure of the I-Eα^{d} gene

Two overlapping subclones were isolated from the insert of cos I^{d}-α-1, a Pst I clone containing the coding information for the 3'-part of the gene and a Bgl II clone containing its 5' part (Fig. 1). The Pst I clone whose insert comprises 3.3 kb, was restriction site mapped by single and double digestions (Fig. 1). A restriction map of the partially overlapping Bgl II clone was also constructed. The nucleotide sequences of the complete Pst I clone insert and of a portion of the Bgl II clone insert were determined. The sequencing strategy is outlined in Fig. 1 and the complete nucleotide sequence is

shown in Fig. 2.

As no cDNA clone corresponding to the I-Eα chain was available, we compared the I-$E\alpha^d$ gene sequence with the nucleotide sequence of a human HLA-DR α chain cDNA clone, pII-α-4 (Gustafsson et al. submitted). The pII-α-4 is a nearly full length clone containing 57 bp of the 5' untranslated region, the complete translated portion and 397 bp of the 3' untranslated part. Four nucleotide stretches of the I-Eα gene nucleotide sequence displayed significant homology to the pII-α-4 sequence; i.e. regions corresponding to the 5' untranslated part and the signal peptide, the first domain, the second domain and the membrane-spanning region.

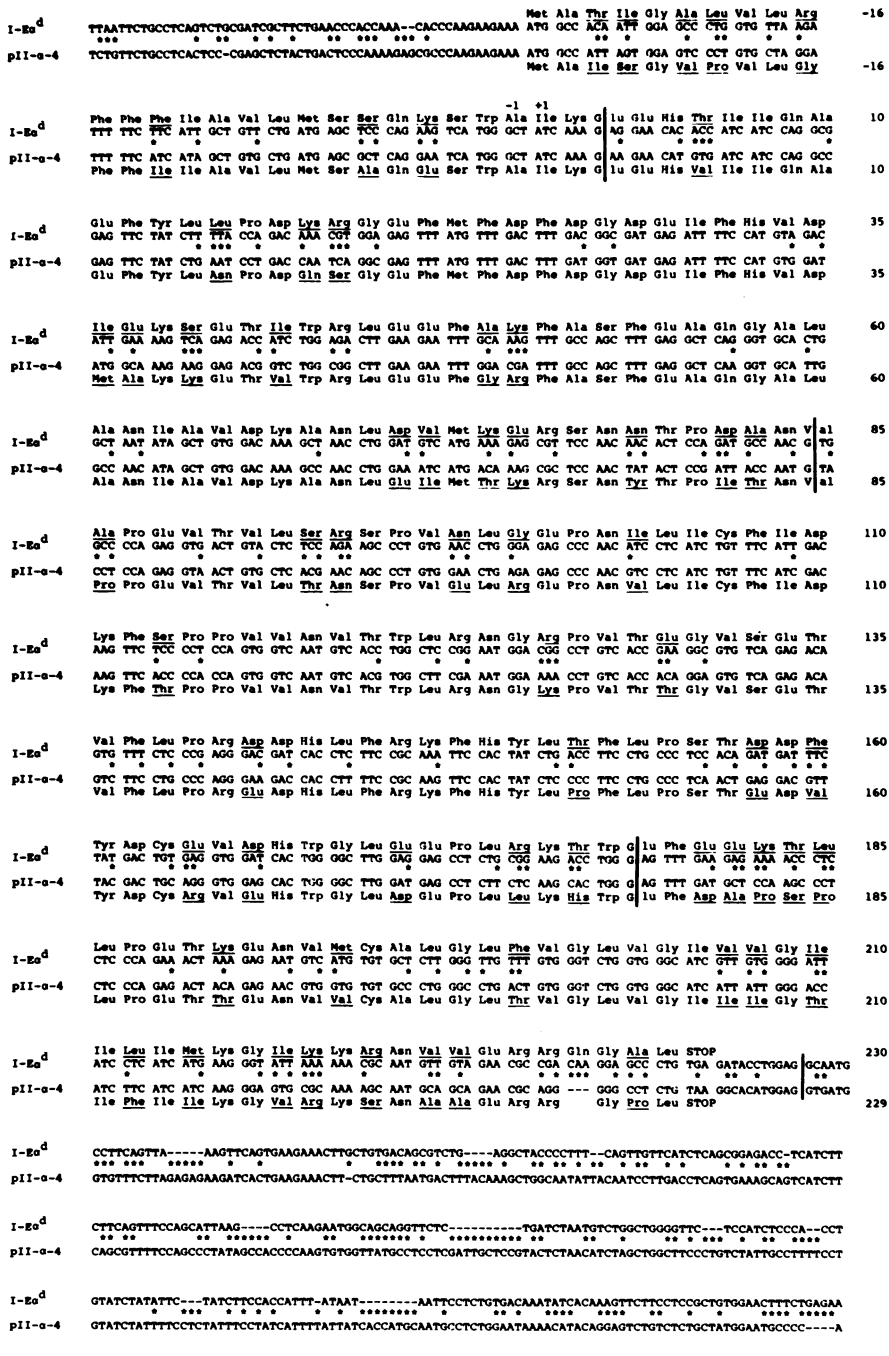
Consequently, the signal peptide-encoding nucleotide sequence of the I-Eα gene is contiguous with the 5' untranslated sequence and this exon also contains the coding information for the two NH_2-terminal amino acids of the first domain. The signal peptide exon is separated from the exon encoding the first extracellular domain by a long intron consisting of 2242 bp. This exon, encoding 82 amino acids is followed by an intron of 487 bp separating the coding sequences of the first and second extracellular domains. The connecting peptide, the membrane spanning segment, the cytoplasmic tail and 11 nucleotides of the 3' untranslated sequence are encoded in another exon, separated from the second domain exon by a 537 bp intron. The last intron of the gene is located within the 3' untranslated sequence and consists of 626 bp. This intron is not as easily defined as the others since the homology between the I-Eα gene and pII-α-4 is lower in the 3' untranslated region than in the translated portions. However, the homology between the nucleotide stretches encoding the membrane-spanning parts of pII-α-4 and the I-Eα gene extends 11 bp into the 3' untranslated region after which a donor splice signal appears. Contiguous with this sequence in the I-Eα gene is a nucleotide stretch of 626 bp which is not found in pII-α-4. The homology to pII-α-4 is resumed 3' to this nucleotide sequence and an acceptor splice signal emerges in the Eα sequence (see Fig. 2). Thus, the last exon of the gene extends to the putative polyadenylation signal, occurring 311 nucleotides downstream



from the splice site. Accordingly, an intron is located between nucleotides 4463 and 5088 in the I-Eα gene. At all intron/exon boundaries the splice junctions are in accordance with the GT-AG rule (32). This intron/exon organisation is identical to the organisation of the I-Eα gene from the k haplotype recently published by Mathis et al. (19)

Partial sequences covering the central two-thirds of the $I\text{-}E\alpha^d$ gene (18) and the exons and most of the introns of the $I\text{-}E\alpha^k$ gene has been published (19). These results are in good agreement with ours. However, out of the 3450 bp available for comparison with the $I\text{-}E\alpha^d$ gene sequence of McNicholas et al. (18) positions are different. There are 66 nucleotide differences between the part of the $I\text{-}E\alpha^k$ gene sequenced (3608 bp) and our sequence of the $I\text{-}E\alpha^d$ gene. 33 of those differences are due to three insertions or deletions of 8, 5 and 19 bp, respectively. The two largest insertions/deletions are found in connection with two long stretches of T´s in the intron between the exons encoding the first and second domains and in the 3'-untranslated region. The vast majority of the differences between these three sequences are located in introns, but two amino acid substitutions are found in both sequences when compared to ours. Glu-130 and Thr-177 in our sequence are replaced by threonine and alanine, respectively, in the sequence reported by McNicholas et al. (18), while again Thr-177 and Met-194 are replaced by histidine and valine, respectively, in the sequence determined by Mathis et al. (19). In only three positions does our sequence differ from both of the other sequences. Surprisingly enough, the sequence of the $I\text{-}E\alpha^k$ gene is more alike our $I\text{-}E\alpha^d$ gene sequence than the two $I\text{-}E\alpha^d$ gene sequences to one another. It is impossible to asses to which extent the nucleotide differences between the three sequences are the result of sequencing errors or of genetic variability, but the former may account for at least some of

Figure 2
Nucleotide sequence of the $I\text{-}E\alpha^d$ gene and its predicted amino acid sequence. Suggested CAT-sequence, TATA-box, cap sequence and polyadenylation signal are underlined. Arrows denote the location of an intron in the 3' untranslated region. The numbering of the nucleotides extends beyond the gene proper.



the differences. For instance, in the portion of the large intron sequenced by McNicholas et al. (18), 11 out of 21 differences involve C's which have either been deleted or recorded as T's.

The comparison of the I-Eα genes of the d and k haplotypes shows that this gene is highly conserved not only in the exon but also in the intron sequences. The differences in the introns between the two sequences are in the range of 0.6 to 3 % taking also insertions/deletions into account. These figures are considerably lower than those for differences in introns between two alleles of the class I antigen H-2K gene (32). It can therefore not be exluded that in addition to the selective pressure on the protein level, which tend to conserve exons, some constraints also exist on the Eα gene nucleotide sequence.

The promoter region of the I-Eα^d gene

In eukaryotic genes the consensus sequences of the promoter region contain a TATA-box and a CAT-box located 30±4 bp and 77±10 bp, respectively, 5' to the cap-site, which most commonly is an A in a pyrimidin-rich region (33, 34). However, since no full length I-Eα chain cDNA clone is available, we cannot precisely localize the cap-site of the I-Eα^d gene. Consequently, we have chosen to number the nucleotides of the I-Eα^d gene according to the sequence information available. This sequence most likely extends beyond the boundaries of the gene proper (see Fig. 2 and below).

Examination of the I-Eα^d sequence 5' to the initiating Met, reveals a putative cap-site TATTTCT, 204 bp prior to the initiation codon. This site is preceeded by the sequences TAATAAGT and CCAATCTC (underlined in Fig. 2) at distances of 33 and 88 bp, respectively. These sequences and the distance between them are in excellent agreement with the concensus sequences and distances described by Breathnach and Chambon, Efstratiadis et al. (33, 34). This interpretation suggests that

Figure 3
Comparison of the nucleotide sequence and predicted amino acid sequence of the HLA-DR antigen α chain$_d$ cDNA clone pII-α-4 with the corresponding portions of the I-Eα^d gene. Splice junctions are indicated by vertical lines. Nucleotide substitutions are denoted by stars. Amino acid replacements are underlined.

the 5'-untranslated region of the I-Eα gene consists of 205 bp. On the other hand Mathis et al. (19) relying on primer extension experiments with reverse transcriptase, and Mung Bean nuclease mapping, suggest that the 5'-untranslated region of the I-Eα gene is about 50 bp long. Taking these results into account one might consider the possibility of an intron in this region. Introns in the 5'-untranslated region have been described for several genes (35-38). Examination of the sequence of the I-Eα^{d} gene, shows that there are several possible donor and acceptor sites for splicing in this region. The Mung Bean nuclease mapping described by Mathis et al. (19) does not exclude the possibility of an intron in the 5'-untranslated region, since the DNA fragment used is too short to protect the presumed 5'-end of the gene. Also the labeling at the Sst I site, combined with electrophoresis of the protected fragment in a denaturing gel would leave the DNA fragment 5' of a presumed intron unlabeled, and therefore not detectable.

The failure of H-2^{b} mice to express the I-E antigen is caused by a deletion in the 5'-end of the I-Eα^{b} gene

Probes corresponding to the second and third exons of the I-Eα^{d} gene were used in Southern blots of genomic DNA from different mouse strains. It was clearly shown that structures corresponding to the probes are present in the genome of mice of the H-2^{b} haplotype, although such mice do not express the I-Eα^{b} chain (17). With a probe corresponding to most of the signal sequence exon and the promoter region of the I-Eα^{d} gene (Fig. 4 A, probe I), Pvu II digested genomic DNA from B10 (H-2^{b}) and B10.D2 (H-2^{d}) mice was shown to contain two hybridizing fragments each. The size of the strongest hybridizing fragment of the H-2^{d} haplotype corresponds perfectly to the size expected from the restriction map of the cosmid (Fig. 4A). The nature of the weaker hybridizing bands is not known, since the complete nucleotide sequence of this probe has not been determined. Nonetheless, the probe revealed a notable difference between the strongest hybridizing fragments of DNA of the haplotypes H-2^{d} and H-2^{b} (Fig. 4B). The Pvu II fragment of the H-2^{b} haplotype hybridizing to the probe was slightly smaller

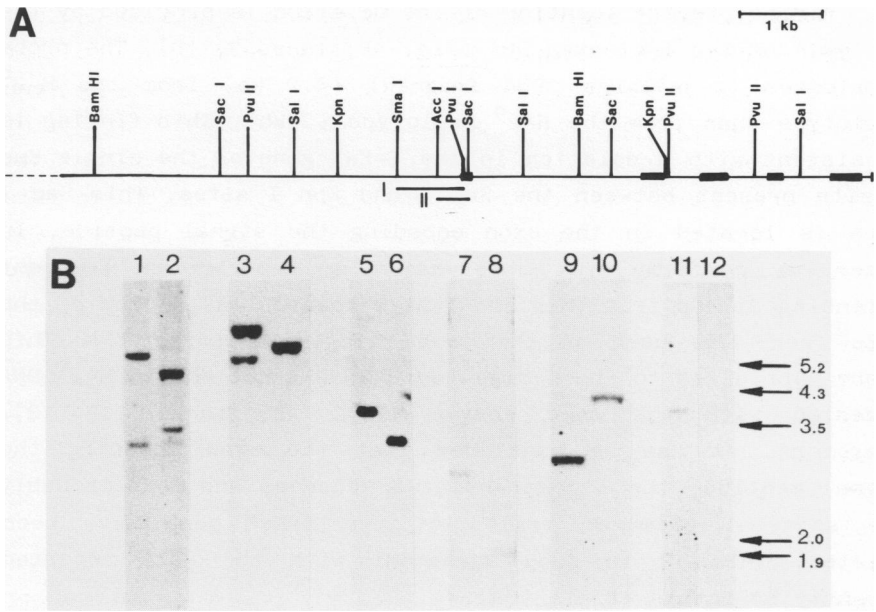


Figure 4
Comparison of the I-Eα^d and the I-Eα^b genes. Genomic hybridizations with probes corresponding to the 5' portion of the I-Eα^d gene and its 5' flanking sequence.

A. Partial restriction map of the I-Eα^d gene. Exons are indicated by bold lines. The two restriction fragments used as probes in the hybridizations are shown below the map.

B. Hybridization of the Pvu I-Sma I restriction fragment (probe I in A) to genomic DNA from B10.D2 (H-2^d) (lanes 1, 3, 5, 7, 9) and from B10 (H-2^b) mice (lanes 2, 4, 6, 8, 10) and of the SacI-AccI restriction fragment (probe II in A) to DNA from B10.D2 (lane 11) and B10 mice (lane 12). Genomic DNA was digested with Pvu II (lanes 1, 2), Bam HI (lanes 3, 4), Sal I (lanes 5, 6) Kpn I (lanes 7, 8, 11, 12) and Sac I (lanes 9, 10), respectively. A deletion of approximately 680 bp is located in the 2200 bp stretch between the Kpn I and Sal I sites framing the signal peptide exon in the H-2^b haplotype. The deletion involves the Sac I site in the signal peptide exon and extends at least 200 bp towards the 5' end of the gene.

than that of the H-2^d haplotype. Similar differences were noted when Pvu II was substituted by Bam HI, Sal I, and Kpn I, respectively. Therefore, we conclude that a deletion of approximately 680 bp has occurred in the I-Eα^b gene within the 2200 bp nucleotide stretch between the Sal I and Kpn I sites flanking the first exon (Fig. 4).

A more precise location of the deletion is provided by the analysis of Sac I cleaved DNA (Fig. 4B, lanes 9, 10). The probe hybridizes to a larger DNA fragment (4.2 kb) from the H-2^b haplotype than from the H-2^d haplotype (3 kb). This finding is consistent with a deletion in the I-Eα^b gene of the single Sac I site present between the Sal I and Kpn I sites. This Sac I site is located in the exon encoding the signal peptide. In order to confirm this observation a restriction fragment extending 317 bp from this Sac I site towards the 5' end of the I-Eα^d gene was used as the probe (Fig. 4A, probe II). This probe hybridized to Kpn I digested H-2^d DNA but not to H-2^b DNA digested with the same enzyme (Fig. 4B, lanes 11, 12). Therefore, it can be concluded that the exon encoding the signal peptide, the 5' untranslated sequence and most probably the adjacent promoter region of the I-Eα^b gene have been deleted. This is in good agreement with the data reported recently by Mathis et al. (39).

DISCUSSION

Comparison of the I-Eα^d gene with the homologous human gene

Molecular cloning of the I-region in overlapping cosmids has shown the existence of a single I-Eα gene (40). The cosmid cosI^d-α-1 containing the α gene we have sequenced was mapped to the I-E region. Furthermore, the amino acid sequence predicted from this gene agrees perfectly with available partial NH_2-terminal sequences of I-Eα chains (3). We therefore conclude that the sequenced gene is the expressed I-Eα^d gene (17).

The overall organization of the I-Eα^d gene, including the 3' untranslated part, is very similar to that of the HLA-DR α chain gene (9, 13). In both the murine and the human genes, exons correspond to domains of the α chains. The homologies between the nucleotide sequences of the I-Eα gene exons and the corresponding portions of pII-α-4 (or the DRα gene exons (9, 13) are 82, 80, 82 and 74% for the exons encoding the signal peptide, first domain, second domain, and the membrane spanning and cytoplasmic portions, respectively (see Fig. 3). The homology between the 3' untranslated regions is considerably lower. The alignment obtained by use of the computer program

ALIGN (41) corresponded to a homology of 50% provided 15 insertions/deletions were introduced (Fig. 3).

The introns of the I-Eα and DRα genes (13) are of similar lengths. Nevertheless, the nucleotide sequences of the introns of the two genes are quite different. Only short stretches of homology occur in the introns separating the extracellular domain exons and the exons encoding the membrane-spanning and cytoplasmic portions of the α chains. The significance of this homology is, however, questionable.

The amino acid sequences of the human HLA-DR α chain and the I-Eα^d chain, including the signal peptides, are of almost the same size, i.e. 254 and 255 amino acid residues, respectively (Fig. 3). The extra amino acid occurs in the cytoplasmic tail of the I-E α chain, which consists of 16 residues in contrast to 15 of the HLA-DR α chain. The cysteine residues and the amino acid triplets specifying the two glycosylation sites occur in identical positions in the two chains.

The overall amino acid homology between the two chains is 75%. The two extracellular domains display greater homology (79% for the first and 81% for the second domain) than the signal sequences (70%) and the membrane-spanning segments including the cytoplasmic tails (62%).

The second extracellular domain of the HLA-DR α chain is homologous to the second domain of human class II antigen ß chains, to the third extracellular domain of human class I transplantation antigen chains, to $ß_2$-microglobulin and to immunoglobulin constant domains (7, 9, 13). Likewise, a computer analysis with the program ALIGN (41) showed that the second domain of the I-E α chain is homologous to the third domain of murine class I transplantation antigen chains, murine $ß_2$-microglobulin, the second domain of the I-Aß^b chain and murine immunoglobulin constant domains (data not shown). The functional significance of these similarities remains to be investigated.

Overall organization of genes encoding class I and II antigens.

With the completion of the I-Eα gene sequence, the detailed organization of genes encoding murine class I and

class II antigen chains can be compared. Three murine class I genes (or pseudogenes)(42-45), the $ß_2$-microglobulin gene (46) and the I-Aß^b gene (47) have been characterized in detail. The general outline of all these genes is similar. However, the membrane-spanning and cytoplasmic portions of murine class I antigen chains and of the I-A^b ß chain are encoded by several exons. The functional implication of this differenceto the I-E^d α chain is unknown. All genes encoding class I and class II antigen chains are located in the MHC, except the $ß_2$-microglobulin gene, which is present on a separate chromosome (48, 49). In spite of that, the $ß_2$-microglobulin gene displays several features in common with the I-Eα^d gene. The signal sequence is separated by a long intron from the rest of the gene and an intron is present in the first part of the 3' untranslated region. It is thus, possible that the I-E α chain is more closely related to $ß_2$-microglobulin than to the class I antigen heavy chains.

A deletion in the I-Eα^b gene accounts for its lack of expression

The present data clearly demonstrate that mice of the H-2^b haplotype contain the Eα gene, although its transcription or translation is impeded. Using the I-Eα^d gene as the prototype we could convincingly show that the I-Eα^b gene contains a deletion encompassing the first exon and most probably also the promoter region. Thus, the failure of mice of the H-2^b haplotype to express the I-Eα chain is probably accounted for by the lack of transcription of the gene.

Consequently, this implies that no specific regulation of gene expression is responsible for the failure of H-2^b mice to synthesize the I-Eα chain. To which extent deletions or point mutations have inactivated the I-Eα gene in other haplotypes which do not express this gene remains to be investigated. Inactivation of class II antigen genes may well take place also in man. It cannot be excluded that such a mechanism may operate in some class II antigen related diseases (50)

ACKNOWLEDGEMENTS

The technical assistance of Ms Pia Ågren, Ms Anita Morén and Mr Peter Wenkler is highly appreciated. Ms Elisabetta Rossi

kindly assisted with the computer analyses. Dr. Roger Staden is gratefully acknowledged for providing computer programs for nucleotide sequence analyses. This work was supported by grants from the Swedish Cancer Society, King Gustaf V:s 80-years´fund, Marcus Borgström´s fund and Centrala Försöksdjursnämnden. P.H. Schreier and F. Sablitzky were supported by the Deutsche Forschungsgemeinschaft through SFB 74. P. H. Schreier acknowledges a short term EMBO fellowship.

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