



MULTIPLE TRANSCRIPTS OF THE MURINE IMMUNOGLOBULIN ϵ MEMBRANE LOCUS ARE GENERATED BY ALTERNATIVE SPLICING AND DIFFERENTIAL USAGE OF TWO POLYADENYLATION SITES

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Abstract—The human $C\epsilon$ gene produces a number of alternatively spliced heavy chain transcripts of which some encode functional IgE isoforms. We now show that differentially processed ϵ mRNA variants also exist in the mouse and are generated by differential polyadenylation and alternative splicing of primary ϵ chain transcripts. The two poly(A) sites of the mouse membrane transcripts were identified in the present study by RACE-PCR analysis. The first poly(A) site is located 743 nt downstream from the beginning of the second membrane exon (M2) and contains the same non-consensus AGTAAA signal sequence as the single poly(A) site of the human membrane transcripts. The second poly(A) site is located almost 500 nt further downstream and is characterized by an AAGAAA hexamer. This poly(A) site contains a (G+T) rich element downstream to the site of cleavage and polyadenylation and is preferentially utilized by the membrane ϵ transcripts. Additional diversity of ϵ transcripts is generated by alternative splicing between the last constant region exon (CH4) and the two membrane exons (M1 and M2). The alternatively spliced transcripts include two variants that skip the first membrane exon and encode ϵ heavy chains that lack the transmembrane domain. The third variant is generated by splicing to an internal site in M2 and codes for a membrane isoform that is 10 amino acids shorter in the cytoplasmic domain than the classical membrane IgE. Although little amino acid sequence homology exists between the murine ϵ chain isoforms and their human counterparts, the pattern of splicing is rather conserved between the two species. © 1997 Published by Elsevier Science Ltd. All rights reserved.

Key words: alternative splicing, polyadenylation, IgE heavy chain, mouse immunoglobulin.

INTRODUCTION

The $C\epsilon$ gene is the penultimate gene from the immunoglobulin (Ig) heavy (H) chain locus in both mice and humans, and encodes the constant region part of the IgE H-chain molecules. It is composed of four constant region exons (CH1–CH4) and two membrane exons that encode the membrane-proximal, transmembrane and cytoplasmic parts of membrane IgE (Max *et al.*, 1982; Peng *et al.*, 1992; Zhang *et al.*, 1992; Batista *et al.*, 1995b). In humans, the second membrane exon (M2) contains a long 3' untranslated region (1046 bp) and an AGTAAA polyadenylation signal sequence which differs from the consensus AATAAA found in the second membrane exon of all other human Ig H-chain genes (Batista *et al.*, 1995b; Dorai and Gillies, 1989; Bensmana and Lefranc, 1990). This rather inefficient poly(A) signal could in part determine the low levels of expression of membrane ϵ

transcripts and the extremely low number of human peripheral blood lymphocytes (PBL) expressing surface IgE.

The human $C\epsilon$ gene also appears to be peculiar in its capacity to produce a number of ϵ mRNAs that are generated by alternative splicing between the last two constant region exons and the two membrane exons (Batista *et al.*, 1995a,b; Diaz-Sanchez *et al.*, 1994; Efremov *et al.*, 1993; Hellman, 1993; Peng *et al.*, 1992; Zhang *et al.*, 1994, 1992). Many of these isoforms are apparently just aberrant by-products of alternative splicing which are unable to form properly assembled IgE molecules (Batista *et al.*, 1995a). However, one of this variants, referred to as the ϵ_{S2} , was found to be secreted with the same efficiency as the classical secreted ϵ_{S1} H-chain (Batista *et al.*, 1996b). This isoform contains only six additional amino acids which are added by splicing to an internal acceptor splice site in the M2 exon.

More recently, we have investigated the two putative membrane isoforms and found that both of them form functional B-cell antigen receptors which are capable of transducing different cellular responses upon their crosslinking (Batista *et al.*, 1996a). These two isoforms

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differ only in the extracellular membrane proximal domain which is encoded by the 5' part of the first membrane exon. The longer variant of this exon (M1') uses an acceptor splice site which is located 156 nucleotides upstream of the splice site for the short exon (M1). Thus, the two membrane proteins have the same ϵ constant region and the same transmembrane and cytoplasmic domains, but differ in a 52 amino acids segment which is present only in the long membrane IgE variant.

Considering the possible functional differences of the human IgE isoforms, it would be important to investigate whether similar isoforms exist in other species. If so, these species could serve as useful animal models to determine the biological effects of the various IgEs. We focused our study on the mouse $C\epsilon$ gene whose sequence had previously been characterized until the stop codon of the second membrane exon (Ishida *et al.*, 1982). This gene shares considerable homology with the human $C\epsilon$ gene in the 3' part (the CH4, M1 and M2 exons), although to a lower extent than the other genes from the Ig H-chain locus (Ishida *et al.*, 1982). Northern blot analysis of IgE-producing mouse hybridoma cells has shown that the mouse $C\epsilon$ gene is transcribed into a major RNA species corresponding to the secretory form and two minor species corresponding to membrane form(s) of the ϵ chain (Ishida *et al.*, 1982; Sitia, 1985). The latter species could have been generated by alternative splicing of membrane-exon sequences or by differential usage of two poly(A) sites.

We now provide the complete 3' untranslated region sequence of the mouse $C\epsilon$ gene and show that it contains two poly(A) sites. We also show that the mouse $C\epsilon$ gene generates alternatively spliced molecules which share some homology with the human with respect to splice site usage, but no homology with respect to amino acid sequence.

MATERIALS AND METHODS

RNA and DNA samples

The murine IgE producing B cell lymphoma cell line I29 ϵ^+ was maintained in DMEM supplemented with 5% (v/v) fetal calf serum, penicillin (100 U/ml), streptomycin (100 μ g/ml) and 50 μ M 2-mercaptoethanol. Spleens were excised from BALB/c mice, mechanically disrupted and the cells were dispersed in cold PBS.

Total cellular RNA was isolated using the acid guanidinium thiocyanate procedure (Chomczynski and Sacchi, 1987). High molecular weight genomic DNA was isolated from mouse splenocytes by standard methodology (Sambrook *et al.*, 1989).

Identification of poly(A) sites by RACE-PCR

The 3' ends of ϵ mRNAs were amplified using the RACE technique (Frohman *et al.*, 1988). Briefly, 3 μ g of total cellular RNA from I29 ϵ^+ cells were reverse transcribed with the Gene Amp RNA PCR kit (Perkin Elmer Cetus, Norwalk, CT) using an oligo(dT)₁₅-adaptor (dT-Ad, 5'-GACTCGAGTCGACATCGATTTTTTTTTT-

TTTTT-3') as primer. Thirty-five cycles of PCR were next performed on the RT sample with the M23/Ad (M23, 5'-TCCACACCGATGCAGGATACA-3'; Ad: 5'-GACTCGAGTCGACATCGA-3') and the M25/Ad primer pairs (M25, 5'-AGACCTTCCAAGACTATGCCA-3'). The PCR reactions were carried out with 1 min of denaturation at 95°C, 1 min of annealing at 58°C and 2 min of extension at 72°C. The specificity of the products obtained was confirmed by Southern blot analysis with the M22 oligonucleotide (M22, 5'-CCTATGCCCTGGTCTGGAGGAT-3').

Cloning and sequencing

PCR fragments obtained by amplification of cDNA from I29 ϵ^+ cells and mouse splenocytes were purified from 1% agarose gels by electroelution. The recovered DNA fragments were ligated in the SmaI site of pUC18 (Pharmacia LKB, Uppsala, Sweden) and used to transform *E. coli* strain DH5- α . At least three positive clones from each transformation were sequenced using the T7 Sequencing kit (Pharmacia LKB).

PCR amplification of unknown 3' flanking DNA

The sequence of the 3' flanking region of the membrane locus poly(A) site was determined by analysing "mis-primed" or "jumping" PCR artefacts which were generated using primers complementary to sequences from the M2 exon and several non-specific primers unrelated to the mouse $C\epsilon$ gene (Batista *et al.*, 1995b; Struck and Collins, 1994). Briefly, PCR reactions were performed with 1 μ g genomic DNA, 50 pmol of M2 exon primer (M23), 50 pmol of non-specific primer, 200 μ mol of each dNTP, 0.5 U Taq polymerase and 10 μ l of 10 $\times$ PCR buffer in a total volume of 100 μ l. Thirty-five cycles of denaturation at 95°C for 1 min, annealing at 58°C for 1 min and extension at 72°C for 2 min were performed. The PCR artefacts containing the sequence of interest were selected in semi-nested PCRs in which 2 μ l of the first PCRs were reamplified with the internal primer M25 and the same non-specific primer from the first reaction. The specific PCR products were identified by Southern blot analysis with the internal oligonucleotide M22, and were cloned and sequenced as described above. The obtained sequences were confirmed by PCR amplification of genomic DNA with primers from the known and new sequence, followed by cloning and sequencing of both strands of the PCR products.

Northern blot analysis of ϵ transcripts

Samples (20 μ g) of total I29 ϵ^+ RNA were separated by electrophoresis in 1% agarose gels containing 6% formaldehyde, transferred to nylon membranes (Hybond-N, Amersham, U.K.) by overnight capillary blotting in 20 $\times$ SSC and fixed with u.v. light. The filters were hybridized with three different probes generated by restriction enzyme digestion or PCR amplification of cloned $C\epsilon$ gene fragments. The h α C probe was a 543 bp (HindIII/BstXI) fragment containing part of the CH3

and CH4 exons. The M1 probe was a 135 bp PCR fragment encompassing the complete M1 exon. The M2 probe was a 399 bp BstXI/AvaI fragment starting 66 nucleotides downstream of the first poly(A) signal. The probes were labeled with [α - ^{32} P]dCTP (Amersham, U.K.) using the Oligolabeling kit (Pharmacia, LKB). Hybridizations were carried out overnight at 42°C in 5 × SSPE/0.5% SDS/5 × Denhardt's/50% formamide/100 µg/ml salmon sperm DNA. Filters were washed in 2 × SSC/0.1% SDS at 42°C and autoradiographed.

RT-PCR analysis of ϵ transcripts

Two micrograms of splenocyte or I29 ϵ^+ RNA were reverse transcribed with the Gene Amp RNA PCR kit using oligo-dT₍₁₅₎ as primer. Thirty-five cycles of PCR were done on each RT sample with the E41 (5'-AAGTGATCCATGAGGCACTTCA-3') and the M28 (5'-TCCATCCCATGAAGAGGTCT-3') or M30 (5'-CCAGAGACAAAGTAGGACTG-3') oligonucleotide under the following conditions: 1 min denaturation at 95°C, 1 min annealing at 64°C and 2 min extension at 72°C. The RT and PCR analyses were performed in accordance with the manufacturer's instructions. The specificity of the obtained PCR products was confirmed by Southern blot analysis with an oligonucleotide probe (M27, 5'-AACTCAGAGTCTAGAGCT-3') or a PCR probe from the M2 exon. The PCR probe was obtained by amplifying a cloned M2 exon fragment with oligonucleotides M29 (5'-GCTTCAGACCTCTGGTC-3') and M26 (5'-TTCCTGAGACAGTGTACAT-3'). Positive bands were cloned and sequenced as described above.

The efficiency of amplification of the alternatively spliced ϵ mRNA species was investigated by performing experiments with known quantities of corresponding cloned fragments. Maxi preparations of the plasmid DNAs were purified with a QIAGEN plasmid kit (Qiagen, Germany) and the concentrations were determined by spectrophotometry. The three plasmids were mixed together in different relative amounts and PCR amplified as described above.

RESULTS

Characterization of the two murine membrane ϵ mRNA polyadenylation sites

The poly(A) sites of the mouse membrane ϵ transcripts were identified by RACE-PCR analysis of total cellular RNA isolated from the IgE expressing B-cell lymphoma I29 ϵ^+ . The cDNA resulting from the reverse transcription was amplified with the Ad primer and two oligonucleotides located in the M2 exon (primers M23 and M25 from Fig. 1). The PCR products were analysed by Southern blot with an internal radiolabeled oligonucleotide from the same exon (primer M22). Two strong bands of ~700 bp and ~1200 bp and a weak band of ~900 bp hybridized to this oligonucleotide, indicating that the transcripts containing the M2 exon use more than one polyadenylation site. Cloning and sequencing of the above fragments showed that the two major bands

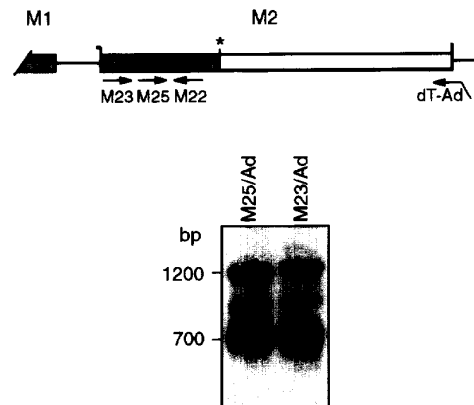


Fig. 1. RACE-PCR analysis of ϵ transcripts containing M2 exon sequences. The location of the RT-PCR primers (M23, M25 and dT-Ad) and the oligonucleotide that was used for the hybridization (M22) are indicated. Asterisk indicates the stop codon in the M2 exon. Filled and open boxes represent coding and 3' untranslated regions, respectively. The lower panel shows an autoradiogram from the RACE-PCR analysis of I29 ϵ^+ mRNA.

represent differentially polyadenylated ϵ transcripts, whereas the weak ~900 bp fragment was a heteroduplex of the two species. This analysis also yielded the complete sequence of the 1.17 kb 3' untranslated region of the M2 exon (shown in Fig. 2).

Analysis of the 3' untranslated region sequence showed that the M2 exon contains two polyadenylation signals. The first one is an AGTAAA hexanucleotide located 743 bp downstream from the beginning of the M2 exon and 9 nt upstream from a CA dinucleotide where mRNA cleavage and poly(A) addition had occurred. The second polyadenylation signal is an AAGAAA hexanucleotide located 486 nt further downstream. The transcripts that used this polyadenylation signal were cleaved at a GA dinucleotide located 17 nt after the hexamer.

Using a modification of a PCR-based chromosome walking technique (Struck and Collins, 1994), we also determined 836 nt of M2 exon 3' flanking region sequence (Fig. 2). The sequence was determined by sequencing both strands of three independent PCR products. Only a few discrepancies were noted between the sequences, which were apparently due to Taq polymerase errors. The consensus sequence (from two identical sequences) was subsequently confirmed by cloning and sequencing a PCR product obtained by amplification of mouse genomic DNA with a primer located in the M2 exon (M23) and a primer derived from the 3' end of the new sequence (M26 in Fig. 2). This sequence has been submitted to the EMBL database under Accession No. X99259. Analysis of this sequence showed that a GT rich region is located 14 nt downstream to the site of cleavage and polyadenylation of the second poly(A) signal.

Both poly(A) signals are used by the membrane ϵ transcripts

The ϵ mRNA species produced by I29 ϵ^+ cells were investigated by Northern blot analysis using three differ-

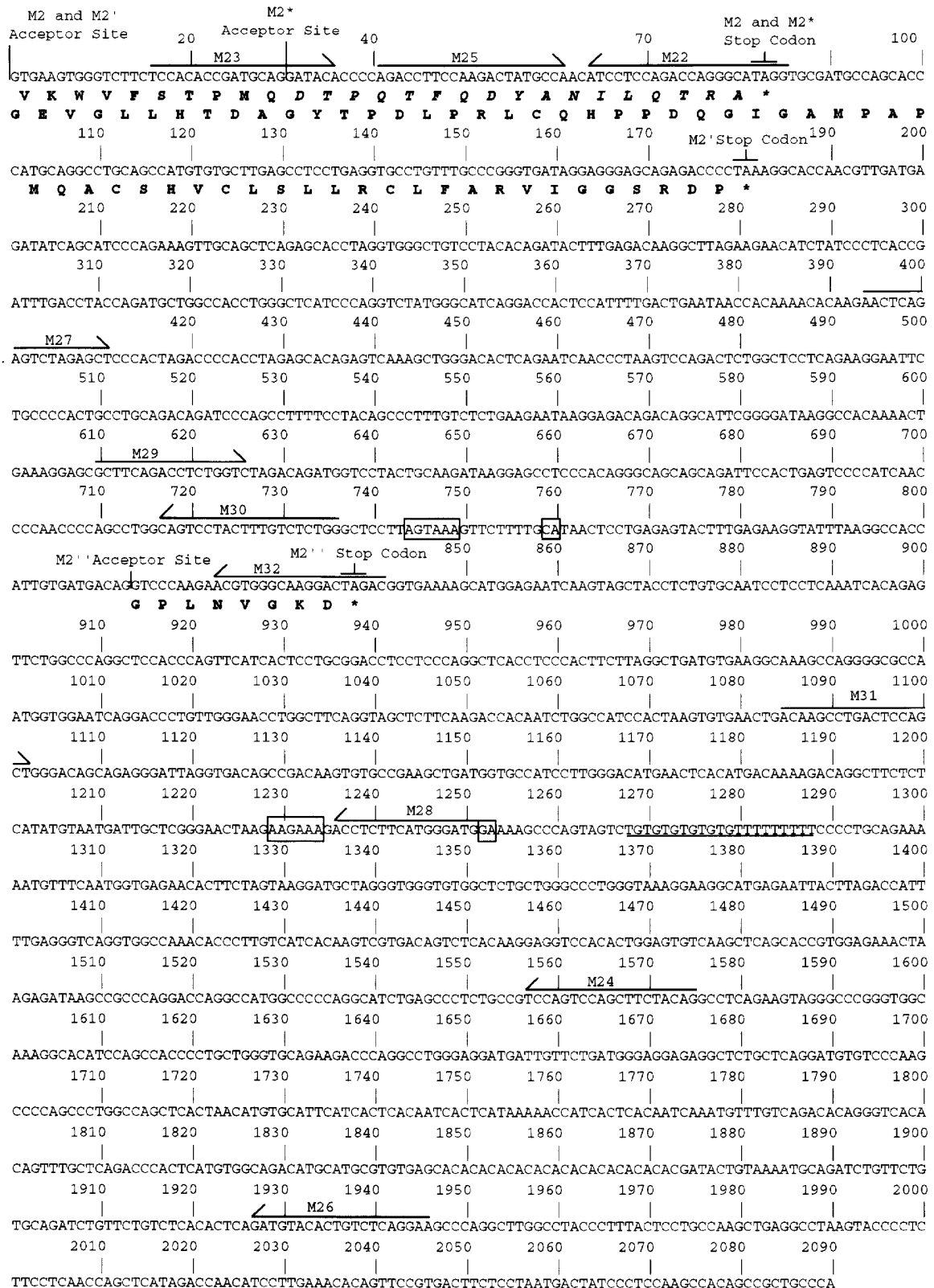


Fig. 2. Genomic sequence of the mouse M2 exon and its 3' flanking region. The acceptor splice sites of exons M2 and M2' (nt 1), M2* (nt 31) and M2' (nt 814), and their stop codons are indicated. The amino acid sequence corresponding to each open reading frame is shown in one-letter code below the nucleotide sequence. The overlapping amino acid sequence of the M2 and M2* exons is shown in italics. The two hexamer poly(A) signals and the sites of cleavage and poly(A) addition are boxed. The (G + T) rich element is underlined. Oligonucleotides used for PCR and sequencing are indicated by half arrows above the nucleotide sequence.

ent probes corresponding to distinct parts of the $C\epsilon$ gene. The m ϵ C probe contained CH3 and CH4 exon sequences and was used to detect all ϵ mRNAs. As shown in Fig. 3, this probe hybridized to three mRNA species. The smallest, most intensely hybridizing mRNA was ~ 1.9 kb long and corresponded in size to the secretory type of ϵ mRNA. The two larger ϵ mRNA species had molecular sizes of 2.7 and 3.2 kb and both also hybridized to the probe containing the complete M1 exon. The difference in size between these two mRNAs was consistent with the distance between the two polyadenylation sites, indicating that they corresponded to differentially polyadenylated membrane ϵ mRNAs. This was confirmed with a probe located in the region between the two poly(A) sites, which hybridized only to the 3.2 kb transcript. Interestingly, the 3.2 kb transcript appeared to be more abundant than the 2.7 kb species (as judged by the hybridization signals obtained with the m ϵ C and mM1 probes), suggesting that the second poly(A) signal is more active than the first one.

Alternatively spliced membrane exon transcripts are produced by I29 ϵ^+ cells and mouse splenocytes

In recent studies we and others have shown that the human $C\epsilon$ gene produces a number of alternatively spliced ϵ mRNA molecules (Batista *et al.*, 1995a,b; Diaz-Sanchez *et al.*, 1994; Efremov *et al.*, 1993; Hellman, 1993; Peng *et al.*, 1992; Zhang *et al.*, 1992, 1994). To investigate if similar events occur in the mouse $C\epsilon$ locus, RT-PCR analysis was done on RNA extracted from the murine IgE secreting lymphoma (I29 ϵ^+) and from freshly isolated

mouse splenocytes. The cDNAs were amplified with primers located in the CH4 exon (E41) and at the end of the M2 3' untranslated region (M28) (Fig. 4(a)). Three bands of approximately 1.4 kb, 1.3 kb and 0.5 kb were obtained from both cell types, all of which hybridized with an M2 exon PCR fragment spanning the region between oligonucleotides M29 and M26 (Fig. 4(b)). Cloning and sequencing of the PCR fragments showed that they represent four different ϵ mRNA species (Figs 4 and 5). The first one corresponded to the classical membrane species whose structure had been predicted based on splice sites consensus sequences. This species (m ϵ CH4-M1-M2) is spliced from the CH4 exon donor site, located 23 nt upstream of the CH4 stop codon, to the acceptor site of the M1 exon, and subsequently from the M1 to the M2 exon. One of the novel mRNAs designated m ϵ CH4-M1-M2* was obtained from the same band as the m ϵ CH4-M1-M2 splice product. This RNA spliced to a new acceptor splice site in the M2 exon located 30 nt downstream to the splice site of the m ϵ CH4-M1-M2 form. Since there is no shift in the reading frame, this second membrane isoform should end at the same stop codon as the classical membrane isoform, but should be shorter for 10 aa in the cytoplasmic domain (Fig. 2).

The m ϵ CH4-M2' mRNA species was spliced directly from the donor splice site of the CH4 exon to the acceptor site of the M2 exon. This resulted in a mRNA which lacked the entire M1 exon. The absence of the M1 exon in this form resulted in a shift of the M2 exon reading frame. The new reading frame of this exon (M2') encodes 59 aa in contrast to the 27 residues of the M2 exon in the m ϵ CH4-M1-M2 isoform (Fig. 2).

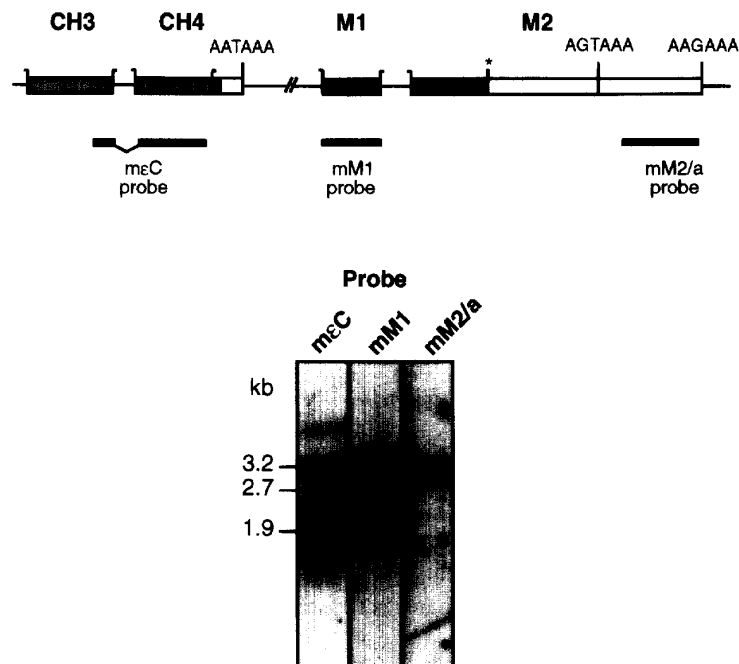


Fig. 3. Northern blot analysis of murine ϵ mRNAs. Upper panel shows the schematic representation of the 3' end of the murine ϵ gene and the position of the probes used for hybridization. Bottom panel shows an autoradiogram with the predominant ϵ mRNA species expressed by the IgE producing lymphoma I29 ϵ^+ .

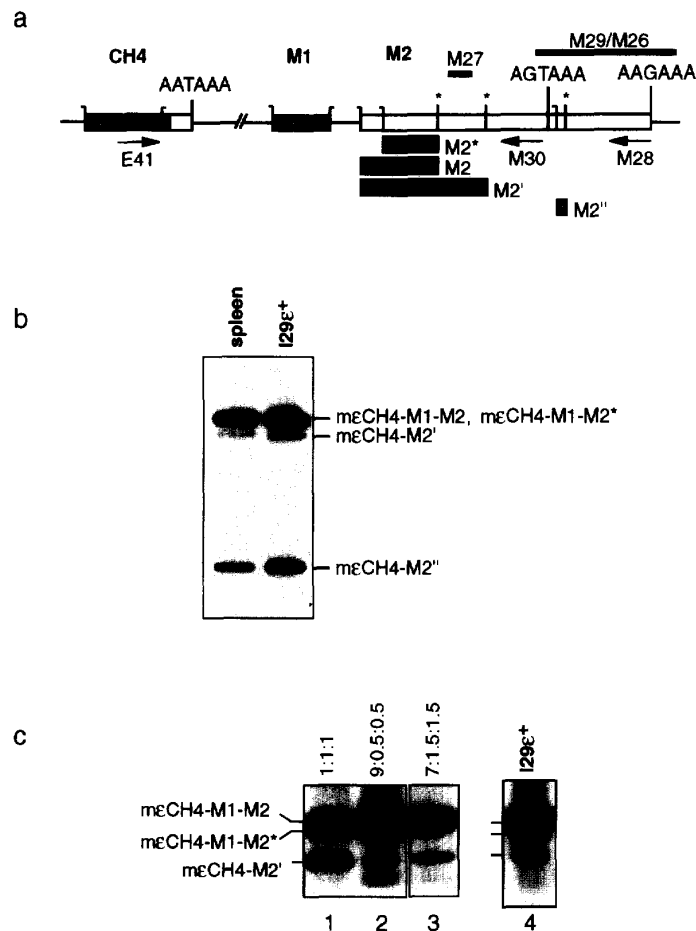


Fig. 4. Detection of alternatively spliced murine ϵ mRNAs in I29 ϵ^+ cells and normal mouse splenocytes. (a) Schematic representation of the 3' end of the $C\epsilon$ gene with the location of splice sites, stop codons and primers used for PCR amplification (E41, M30 and M28). The four different coding regions derived from the M2 exon are indicated below the exon. (b) RT-PCR products obtained by amplification with oligonucleotides E41 and M28. The M29/M26 probe was used for the Southern blot hybridization. The identity of each band in the autoradiogram is indicated. (c) Quantification of the relative amounts of m ϵ CH4-M1-M2, m ϵ CH4-M1-M2* and m ϵ CH4-M2' transcripts in I29 ϵ^+ cells (lane 4). The ϵ cDNA was amplified with the E41/M30 primer pair and hybridized with the M27 oligonucleotide. Standards containing known quantities of cloned fragments corresponding to the m ϵ CH4-M1-M2, m ϵ CH4-M1-M2* and m ϵ CH4-M2' variants were amplified under the same conditions (lanes 1–3). The relative amount of plasmid DNA in each standard (m ϵ CH4-M1-M2: m ϵ CH4-M1-M2*: m ϵ CH4-M2') is indicated above each lane.

The fourth ϵ mRNA species (m ϵ CH4-M2'') revealed another novel splice site located within the M2 exon. This acceptor splice site is located in the 3' untranslated region of the M2 exon, 728 nt downstream of the stop codon from the M2 reading frame and 631 nt downstream from the stop codon of the M2' reading frame. Thus, this species lacks completely the amino-acid sequences encoded by the M1 and M2 or M2' exons, but contains eight other amino-acids encoded by a small exon designated M2'' (Fig. 2).

Since the PCR strategy used in the above assay would not have detected ϵ transcripts that were polyadenylated at the first poly(A) site, we did an additional RT-PCR experiment in which a primer upstream of the first poly(A) site (M30) was used, together with the CH4 exon

primer (Fig. 4(a)). Three bands corresponding in size to the m ϵ CH4-M1-M2, m ϵ CH4-M1-M2* and m ϵ CH4-M2' species were obtained, indicating that these alternatively spliced transcripts can be polyadenylated at either site (Fig. 4(c), lane 4). The m ϵ CH4-M2'' species was not detected in this experiment because the M2'' exon is located downstream to the first poly(A) site. The band corresponding to the m ϵ CH4-M1-M2 isoform was of the strongest intensity, suggesting that this is the most abundant membrane ϵ transcript. To investigate if the PCR strategy gives a true estimate of the relative amounts of the alternatively spliced ϵ transcripts, we performed experiments with standards containing different relative amounts of cloned DNA fragments corresponding to the m ϵ CH4-M1-M2, m ϵ CH4-M1-M2* and m ϵ CH4-M2'

species (analysis of three representative standards is shown in Fig. 4(c), lanes 1–3). These experiments showed that the three ϵ species are amplified with equal efficiency. Analysis of I29 ϵ^+ mRNA gave a similar value as the standard containing m ϵ CH4–M1–M2, m ϵ CH4–M1–M2* and m ϵ CH4–M2' plasmids at a 9:0.5:0.5 ratio, respectively, indicating that the m ϵ CH4–M1–M2 species is approximately 10 times more abundant than the other two species.

DISCUSSION

The present study describes the characterization of the two poly(A) signals of the murine membrane ϵ transcripts. In addition, it provides the complete sequence of the 3' untranslated region of these transcripts and 836 nt of the 3' flanking region sequence. The first poly(A) signal used by the mouse membrane ϵ transcripts is an AGTAAA hexamer located 743 nt downstream from the beginning of the M2 exon. The site of cleavage and polyadenylation is a CA dinucleotide located 9 nt further downstream. Though the AGTAAA hexamer is not identical to the consensus AATAAA and has only 30% of its activity (Sheets *et al.*, 1990), it has been found in a number of eucaryotic genes. Most interestingly, this hexamer constitutes the polyadenylation signal sequence of the human membrane ϵ transcripts (Batista *et al.*, 1995b). However, in contrast to humans, the first mouse poly(A) site does not contain the conserved (G+T) rich element. The absence of this element could further weaken this poly(A) site, since even changes in the position of the (G+T) rich regions have been shown to result in dramatic reduction of 3' end processing activity (Chen *et al.*, 1995). Unlike the human C ϵ gene, the mouse M2 exon contains another poly(A) signal sequence located 486 nt downstream to the first hexamer. This AAGAAA signal sequence is found even less frequently in eucaryotic genes, and has been attributed only 6% of the polyadenylation activity of the consensus AATAAA (Sheets *et al.*, 1990). However, this distal poly(A) site contains

a 21 nt long (G+T) rich region which is located 14 nt downstream to the cleavage site. The presence of this element could strengthen the 3' end processing activity of the distal poly(A) site and could account for the Northern blot data which showed that this site is preferentially utilized by the membrane ϵ transcripts.

Differential polyadenylation of membrane transcripts is also a feature of other mouse Ig H-chain genes. The mouse C δ gene contains two distal poly(A) sites that give rise to two types of membrane δ transcripts, whereas three poly(A) sites are present in the second membrane exon of the mouse C α gene (Cheng *et al.*, 1982; Word *et al.*, 1983). The striking feature of the murine membrane ϵ poly(A) signals is that both of them (AGTAAA and AAGAAA) are different from the consensus AATAAA and ATTAAA sequences and have a much lower polyadenylation activity, whereas all other mouse and human Ig H chain genes, with the exception of human C ϵ , contain the consensus sequence. Presence of inefficient poly(A) signals in the membrane C ϵ locus could constitute an additional aspect in the regulation of IgE production, preventing high level expression of membrane ϵ transcripts.

We also investigated the type of ϵ mRNA expressed by a mouse IgE-producing lymphoma cell line and by normal mouse splenocytes. This allowed us to define the structure of the membrane exons and the positions of splice sites within them. We show that the predicted splice sites in the CH4, M1 and M2 exons are active and that two novel acceptor splice sites are present within the M2 exon. As in humans, utilization of these splice sites generates a number of ϵ mRNA variants which contain membrane-exon sequences (Fig. 5). Estimation of their relative amounts with a quantitative RT-PCR assay indicated that these alternatively spliced transcripts are expressed at substantially lower levels than the classical membrane IgE isoform (m ϵ CH4–M1–M2).

The pattern of splicing that produces the newly described murine variants is rather similar to the one of the human ϵ locus, although none of the variants bears

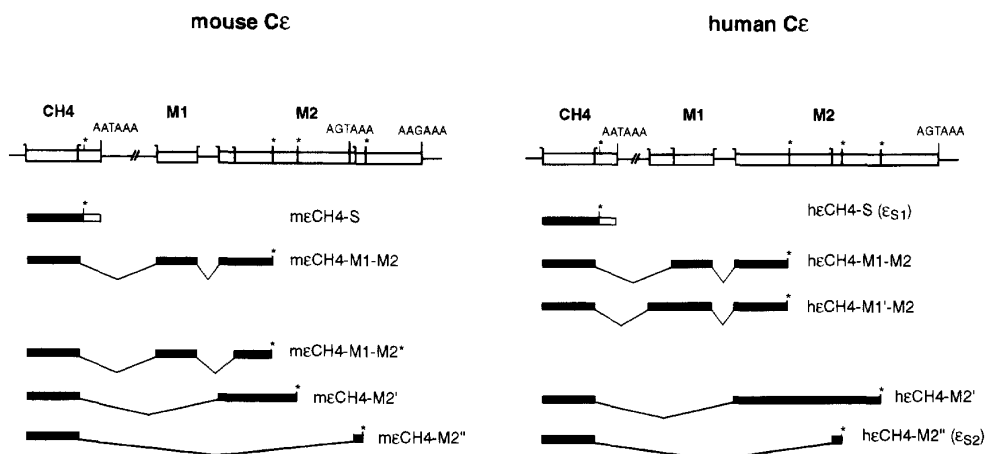


Fig. 5. Comparison of mouse and human ϵ transcripts that are generated by alternative splicing between the CH4, M1 and M2 exons.

significant amino acid homology to their human counterparts (Fig. 5). One of the new ϵ mRNAs is produced by splicing of the CH4 exon to a novel acceptor splice site inside the M2 exon. This splicing generates an alternative membrane variant of murine IgE (m ϵ CH4-M1-M2*) that is 10 aa shorter in the cytoplasmic domain than the classical m ϵ CH4-M1-M2 form. This is opposite to what has been observed in humans, where the two membrane variants differ in the length of the extracellular membrane-proximal domain (Batista *et al.*, 1995b, 1996a; Peng *et al.*, 1992). The other two alternatively spliced ϵ mRNAs exclude the M1 exon and splice directly into the M2 exon. One of them (m ϵ CH4-M2') splices to the first acceptor site of the M2 exon and generates an mRNA that encodes an IgE H-chain, 52 aa longer than the classical secreted protein (m ϵ CH4-S). This form is analogous to the M2' form in humans, which contains an additional 134 aa tailpiece that prevents its assembly and secretion (Batista *et al.*, 1995a). The second form that excludes the M1 exon is produced by splicing of CH4 to a previously undescribed acceptor splice site inside the M2 exon, between the two poly(A) sites. This isoform (m ϵ CH4-M2'') is of considerable interest since it is analogous to the human ϵ CH4-M2'' transcript which encodes the secreted ϵ_{S2} isoform (Batista *et al.*, 1996b). Both human and mouse ϵ CH4-M2'' transcripts are generated by splicing to a small exon which encodes only eight amino acids. However, the human and mouse M2'' exons do not share any sequence homology, although there is a similar net charge of the carboxyl terminal tails encoded by these two exons (Fig. 6). Furthermore, whereas the human ϵ CH4-M2'' transcript is readily detectable by Northern blot analysis, the same is not true for the mouse ϵ CH4-M2'' variant, which implies that the mouse M2'' form is significantly less represented.

In conclusion, we have shown that alternative splicing of membrane exon sequences also occurs in the mouse C ϵ locus. This finding is different from that reported for the rat C ϵ gene, in which only one type of membrane transcript was observed (Aveskogh and Hellman, 1995). This finding, as well as the fact that the mouse ϵ variants do not share any amino acid homology with the expressed human IgE isoforms, indicate that there is considerable

variability in the type of IgE produced between different species.

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REFERENCES

- Aveskogh M. and Hellman L. (1995) A single major transcript encodes the membrane bound form of rat IgE. *Scand. J. Immun.* **42**, 535–539.
- Batista F. D., Anand S., Presani G., Efremov D. G. and Burrone O. R. (1996a) The two membrane isoforms of human IgE assemble into functionally distinct B-cell antigen receptors. *J. Exp. Med.* **184**, 2197–2205.
- Batista F. D., Efremov D. G. and Burrone O. R. (1995a) Characterisation and expression of alternatively spliced IgE heavy chains transcripts produced by peripheral blood lymphocytes. *J. Immun.* **154**, 209–218.
- Batista F. D., Efremov D. G. and Burrone O. R. (1996b) Characterisation of a second secreted IgE isoform and identification of an asymmetric pathway of IgE assembly. *Proc. natn. Acad. Sci. U.S.A.* **93**, 3399–3404.
- Batista F. D., Efremov D. G., Tkach T. and Burrone O. R. (1995b) Characterisation of the human immunoglobulin epsilon mRNAs and their polyadenylation sites. *Nucl. Acids Res.* **23**, 4805–4811.
- Bensmana M. and Lefranc M. P. (1990) Gene segments encoding membrane domains of the human immunoglobulin gamma 3 and alpha chains. *Immunogenetics* **32**, 321–330.
- Chen F., MacDonald C. C. and Wilusz J. (1995) Cleavage size determinants in the mammalian polyadenylation signal. *Nucl. Acids Res.* **23**, 2614–2620.
- Cheng H. L., Blattner F. R., Fitzmaurice L., Mushinski J. F. and Tucker P. W. (1982) Structure of genes for membrane and secreted murine IgD heavy chains. *Nature* **296**, 410–415.
- Chomczynski P. and Sacchi N. (1987) Single-step method of RNA isolation by acid guanidinium thiocyanate–phenol–chloroform extraction. *Anal. Biochem.* **162**, 156–159.
- Diaz-Sanchez D., Dotson A. R., Takenaka H. and Saxon A. (1994) Diesel exhaust particles induce local IgE production *in vivo* and alter the pattern of IgE messenger RNA isoforms. *J. Clin. Invest.* **94**, 1417–1425.
- Dorai H. and Gillies S. D. (1989) The complete nucleotide sequence of a human immunoglobulin genomic C mu gene. *Nucl. Acids Res.* **17**, 6412–6412.
- Efremov D. G., Batista F. D. and Burrone O. R. (1993) Molecular analysis of IgE H-chain transcripts expressed *in vivo* by peripheral blood lymphocytes from normal and atopic individuals. *J. Immun.* **151**, 2195–2207.
- Frohman M. A., Dush M. K. and Martin G. R. (1988) Rapid production of full length cDNAs from rare transcripts: amplification using a single gene specific oligonucleotide primer. *Proc. natn. Acad. Sci. U.S.A.* **85**, 8998–9002.
- Hellman L. (1993) Characterisation of four novel ϵ chain mRNA and a comparative analysis of genes for immunoglobulin E in rodents and man. *Eur. J. Immun.* **23**, 159–167.
- Ishida N., Ueda S., Hayashida H., Miyata T. and Honjo T. (1982) The nucleotide sequence of the mouse immunoglobulin epsilon gene: comparison with the human epsilon gene sequence. *EMBO J.* **1**, 1117–1123.
- Max E., Battey J., Ney R., Kirsch I. and Leder P. (1982)

mouse		
.....EGTISTSLG NTSLRPS	ϵ CH4-S	412
..... PKNVGKD	ϵ CH4-M2''	
human		
.....QRAVS VNPGK	ϵ CH4-S (ϵ_{S1})	423
..... ESSRRGGC	ϵ CH4-M2'' (ϵ_{S2})	

Fig. 6. Comparison of the carboxyl-terminal sequences of the classical secretory mouse ϵ heavy chain (m ϵ CH4-S) and the novel murine m ϵ CH4-M2'' variant with the human secretory isoforms ϵ_{S1} and ϵ_{S2} . Dots indicate amino acid identity. The carboxyl-terminal tail unique to each isoform is shown in bold.

Numbers are relative to the first residue of C ϵ .

- Duplication and deletion in the human immunoglobulin ϵ genes. *Cell* **29**, 691–699.
- Peng C., Davis F. M., Sun L. K., Liou R. S., Kim Y. W. and Chang T. W. (1992) A new isoform of human membrane bound IgE. *J. Immun.* **148**, 129–136.
- Sambrook J., Fritsch E. F. and Maniatis T. (1989) *Molecular Cloning: A Laboratory Manual*, 2nd Edn. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.
- Sheets M. D., Ogg S. C. and Wickens M. P. (1990) Point mutations in AAUAAA and the poly(A) addition site: effects on accuracy and efficiency of cleavage and polyadenylation *in vitro*. *Nucl. Acids Res.* **18**, 5799–5805.
- Sitia R. (1985) Biosynthesis of membrane and secreted epsilon chains during lipopolysaccharide induced differentiation of an IgE⁺ murine B-lymphoma. *Mol. Immun.* **22**, 1289–1296.
- Struck F. and Collins J. (1994) Simple and rapid 5' and 3' extension techniques in RT-PCR. *Nucl. Acids Res.* **22**, 1923–1924.
- Word C. J., Mushinski J. F. and Tucker P. W. (1983) The murine immunoglobulin ϵ gene expresses multiple transcripts from a unique membrane exon. *EMBO J.* **2**, 887–898.
- Zhang K., Max E. E., Cheah H. K. and Saxon A. (1994) Complex alternative RNA splicing of ϵ -immunoglobulin transcripts produces mRNAs encoding four potential secreted protein isoforms. *J. Biol. Chem.* **269**, 456–462.
- Zhang K., Saxon A. and Max E. E. (1992) Two unusual forms of human immunoglobulin ϵ encoded by alternative splicing of ϵ heavy chain membrane exon. *J. Exp. Med.* **176**, 233–243.