

STRUCTURE OF THE GENE FOR THE α -CHAIN OF THE MOUSE HIGH AFFINITY RECEPTOR FOR IgE (Fc ϵ RI)

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Full-length genomic clones for the α -chain of mouse Fc ϵ RI were isolated and the exon/intron structure of the gene determined. The gene consisted of 5 exons, of which the first and second comprised the 5' untranslated region and the leader sequence; the third and fourth, the extracellular domain; and the fifth, the transmembrane and cytosolic domains, plus the 3' untranslated region. The upstream region was highly homologous to that of the rat counterpart. Primer extension and RNase protection analyses revealed multiple transcription initiation sites, between 30 and 120 nucleotides 3' of the putative TATA box. Comparison with other Fc receptor genes (rat Fc ϵ RI, mouse Fc γ RIII α , human Fc γ RIIIB and human Fc γ RIIIA α) revealed a high degree of gene organization conservation.

Ag recognition and the cellular activation that occurs as a result of such recognition are mediated by multisubunit receptors such as mIg² and TCR. Recently, it has been recognized that antibody-mediated cellular recognition events, based on the binding of the antibodies to specific FcR, may result in similar cellular activation (1-3). Two of the FcR, Fc ϵ RI and Fc γ RIII, share a common chain (γ) although they have distinct ligand binding chains (4). Indeed, the conserved sequence motif (5) shared by the FcR γ chain, the TCR complex ζ and η chains and the mIg-associated proteins IgM α and Ig β (5, 6), and the fact that the ligand binding chains of these receptors (α for the FcRs, α and β for the TCRs, H, L chains for mIg) are Ig supergene family members, suggests that mIg, the TCR, and the FcR should be regarded as highly homologous types of recognition structures. Recent evidence indicates that engagement of the TCR causes exocytosis of granules from CD8⁺ T cells (7), a function comparable to the major function of Fc ϵ RI, the exocytosis of granules from mast cells and basophils (8), while cross-linkage of Fc ϵ RI and of Fc γ RIII leads to production of a set of cytokines (9-12), a function long known to result from the engagement of the TCR. The functional and structural homology of these receptors reiterates the relatedness of these "multichain immune

recognition receptors."

Complementary DNA clones for all of the known chains of Fc ϵ RI (α , β , γ) have been obtained (6, 13-19) and genomic clones for the α -chain of rat Fc ϵ RI have been reported (20). In view of the physiologic importance of Fc ϵ RI, both for its essential role in mediating acute allergic inflammatory responses through the release of histamine and related products and for its stimulation of production of a set of cytokines by mast cells and basophils, an understanding of the regulation of expression of the ligand binding chain (α) is of particular importance. Such information should also aid in understanding of the distinctive range of cellular expression of Fc ϵ RI and Fc γ RIII, the former being limited to mast cells and basophils while the latter are also expressed on NK cells and macrophages (3). Furthermore, efforts to create mice that overexpress the α -chain, in which the α -chain has been inactivated by gene targeting or in which cells that are α -chain-positive have been deleted by promoter-determined toxin expression will depend upon an understanding of the murine α -chain promoter region. We report the isolation and characterization of genomic clones spanning the entire gene for the α -chain of murine Fc ϵ RI including 4.4 kb upstream of the transcription initiation site.

MATERIALS AND METHODS

Genomic library screening. A genomic library constructed from adult BALB/c mouse liver DNA in bacteriophage vector EMBL-3 (Clontech catalog No. ML1030, Clontech Labs., Palo Alto, CA) was screened (21) with a full-length murine Fc ϵ RI α -chain cDNA (18), labeled with α -³²P-dCTP (Amersham Corp., Arlington Heights, IL) by random hexamer method. Six positive λ phages were isolated by screening approximately 3×10^6 plaques. Large quantities of bacteriophages from the six positive clones were prepared in liquid culture. Phage DNA was purified and digested with XhoI to excise the genomic DNA inserts. Digested phage DNA was electrophoresed on a 0.8% agarose gel and transferred to a Nytran membrane (Schleicher & Schuell, Keene, NH). ³²P-labeled DNA fragments corresponding to either 5' or 3' portion of the cDNA were used to probe the phage DNA blot. Two phages (λ A1 and λ A4) hybridized to both 5'- and 3'-specific probes and presumably contain the complete α -chain gene. These two phages were selected for further analysis.

Restriction enzyme mapping. The genomic DNA inserts from bacteriophages λ A1 and λ A4 were excised with XhoI and subcloned into the XhoI site of the plasmid vector pGEM7. An internal XhoI site exists in both clones (Fig. 1); therefore, two DNA fragments were generated from each phage and subcloned individually. Restriction maps of the inserts were initially obtained by partial enzyme digestion method (22). pGEM7 plasmids harboring genomic DNA were digested with PvuII, which cuts the vector but does not cleave DNA inserts. The PvuII-cleaved plasmids were then partially digested with appropriate restriction enzymes and electrophoresed on 0.6% agarose gel at low voltage (2 V/cm). The gel was denatured and neutralized after electrophoresis and DNA transferred to two sheets of Nytran membrane using the "sandwich blotting" method (21). Two plasmid DNA fragments (XhoI-PvuII fragments) from pGEM7 position 37-1762 and 2890-37 were isolated as probes. These fragments were labeled with [³²P] dCTP by random hexamer method and hy-

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² Abbreviations used in this paper: mIg, membrane Ig; Fc ϵ RI, high affinity receptor for IgE.

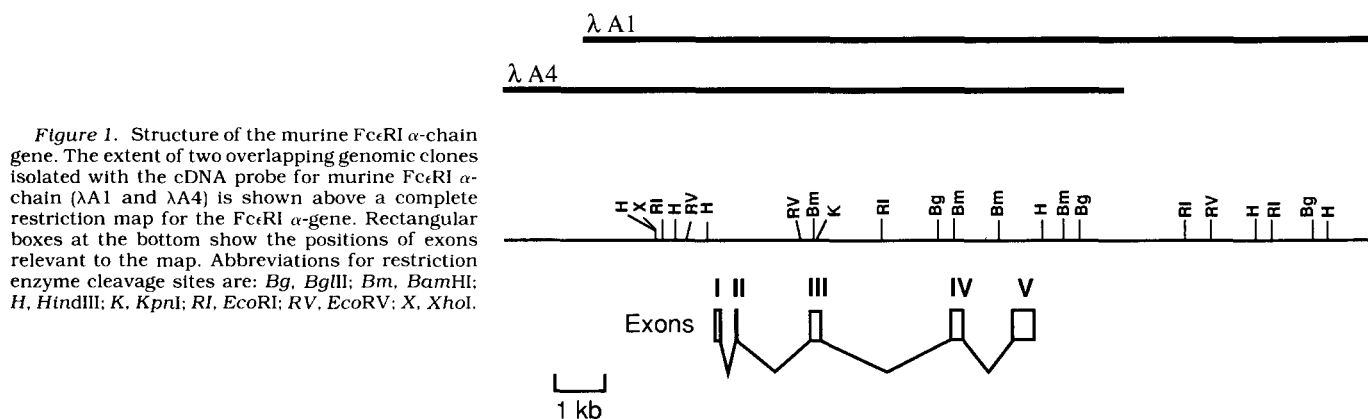


Figure 1. Structure of the murine Fc ϵ RI α -chain gene. The extent of two overlapping genomic clones isolated with the cDNA probe for murine Fc ϵ RI α -chain (λ A1 and λ A4) is shown above a complete restriction map for the Fc ϵ RI α -gene. Rectangular boxes at the bottom show the positions of exons relevant to the map. Abbreviations for restriction enzyme cleavage sites are: Bg, BglII; Bm, BamHI; H, HindIII; K, KpnI; RI, EcoRI; RV, EcoRV; X, XhoI.

bridized to the two partially digested DNA blots. The cleavage site for restriction enzymes were determined by the lengths of ^{32} P-hybridized DNA fragments on the blots. Based on the initial map, the genomic DNA insert was cleaved and subcloned. A refined map was obtained (Fig. 1) by verifying the enzyme digestion sites within the subfragments and by sequencing ambiguous regions.

Nucleotide sequence analysis. Chain-termination sequencing reactions were performed on double-stranded DNA using a Sequenase kit purchased from United States Biochemicals (Cleveland, OH). The first set of primers was synthesized based on information from the cDNA sequence. When a nucleotide sequence was determined, a new primer was made to continue the sequencing. Locations of exons were determined by diagnostic restriction sites encountered within or near individual exons.

Primer extension analysis. A synthetic oligonucleotide primer (dGGCAGACCTTCCAGTGACCA; complementary to position 41–60 of the cDNA sequence) was labeled with T4 polynucleotide kinase (New England Biolabs, Beverly, MA) and γ - ^{32} P ATP (ICN, 7000 Ci/mmol, ICN Biochemicals, Cleveland, OH) to a specific radioactivity of $\sim 10^9$ cpm/ μ g. Fifteen μ g of poly(A)+RNA was mixed with the ^{32} P-labeled primer (10^6 cpm) in a final volume of 20 μ l containing 100 mM Tris (pH 8.0), 100 mM KCl, 10 mM MgCl₂, and 10 mM DTT. The mixture was heated at 90°C for 2 min and annealed at 42°C for 1 hr. At the end of incubation, RNasin (60 U), dATP, dCTP, dGTP, dTTP (each to a final concentration of 0.25 mM), actinomycin-D (Boehringer Mannheim Biochemicals, Indianapolis, IN) (to a final concentration of 50 μ g/ml) and AMV reverse transcriptase (40 U) were added. The reaction volume was adjusted to 40 μ l with H₂O. The reverse transcription reaction was carried at 42°C for 1 hr. The mixture was extracted with phenol/chloroform and precipitated with ethanol. The dried pellet was dissolved into sequencing gel-loading buffer, boiled for 3 min, and electrophoresed on a 6% sequencing gel. A genomic DNA clone was sequenced using the same oligonucleotide primer and loaded on the same gel as molecular weight standards.

RNAse protection assay. A ^{32} P-labeled single-strand RNA was prepared using an SP6 riboprobe kit (Promega, Madison, WI). The DNA template was a HindIII-NcoI fragment of the Fc ϵ RI α gene subcloned into pGEM7 plasmid. This DNA fragment contains part of the first intron, first exon, and ~ 370 bp of the 5'-flanking sequence of the gene. RNAse protection was done according to a standard protocol (22). Briefly, 20- μ g samples of total RNA were precipitated with ethanol, and the pellets were air-dried. Thirty μ l of hybridization buffer (40 mM PIPES, pH 6.4, 0.4 M NaCl, 1 mM EDTA, 80% formamide, 10^6 cpm of single-strand RNA probe) was added to each RNA pellet and incubated at room temperature for 10 min. The mixtures were pipetted 30 times to facilitate solubilization. The samples were heated to 90°C for 5 min and annealed at 42°C overnight. Three-hundred and fifty μ l of digestion buffer (10 mM Tris, pH 7.5, 300 mM NaCl, 5 mM EDTA) containing RNase A (40 μ g/ml) and RNase T1 (2 μ g/ml) was added to each sample and incubated for 60 min at 30°C. Ten μ l of 20% SDS and 5 μ l of 10 mg/ml proteinase K were added and further incubated at 37°C for 15 min. The samples were extracted with phenol-chloroform and precipitated with ethanol. Pellets were dissolved into 4 μ l of sequencing-loading buffer and electrophoresed on a 6% sequencing gel.

RESULTS AND DISCUSSION

Structure of the murine gene encoding Fc ϵ RI α -chain.

Using the murine Fc ϵ RI α -chain cDNA as probe, six positive bacteriophage clones were isolated that consistently

hybridized after four rounds of plaque purification. To identify the clones that presumably contain the complete sequence of the Fc ϵ RI α -chain gene, we isolated two cDNA fragments corresponding to the 5' and 3' portions of the cDNA. These fragments were used to probe duplicate blots of the bacteriophage clones. Genomic inserts from two phages (λ A1 and λ A4) hybridized strongly to both cDNA fragments (data not shown). These two clones were used to construct a restriction map for the murine Fc ϵ RI α -chain gene (Fig. 1).

Both phage clones contain the complete sequence of the α -chain gene. The gene consists of five exons, which are located within a 6.7 kb-HindIII fragment. The nucleotide sequences of exons were determined and shown to match the cDNA sequence (18). The lengths of exons and introns and the exon-intron junctions are shown in Table I. The 5' splice donor sequences and 3' splice acceptor sequences of the introns show good homology to the relevant mammalian consensus sequences (23). Exon I encodes the 5'-untranslated region and the bulk of the leader sequence. Exon II is only 21 nucleotides long and encodes the remainder of the leader sequence. Exons III and IV encode the extracellular domain. The transmembrane domain and cytoplasmic tail are encoded by exon V. Exon V also contains a 3'-untranslated region of 206 nucleotides.

Analysis of the 5'-flanking region and the transcription initiation sites. The sequence of the upstream flanking region of the Fc ϵ RI α -chain is shown in Figure 2. A putative TATA box and a CAAT box were found. A reverse complement of the μ E3 box, which is present in Ig H chain enhancer, was found in more upstream location (Fig. 2).

Primer extension assay was carried out to map the 5' border(s) of the mRNA of the Fc ϵ RI α -chain gene. Poly(A)+ RNA was prepared from a murine mast cell line CFTL-12, which expresses high levels of Fc ϵ RI, and several Fc ϵ RI-negative cell lines, including a myeloid line (FDC-

TABLE I
Organization of the murine Fc ϵ RI α -chain gene^a

Exon No. and Size (bp)	5' Splicing Donor	Intron No. and Size (kb)	3' Splicing Acceptor
I. 102	CATGTgtaagtacag	1. 0.4	gattttgcagCTCCT
II. 21	GACAGgtgagttagt	2. 1.4	cttctttagCCAAT
III. 258	GCAAGgtacgttcca	3. 2.6	tccattctagCTTAC
IV. 255	AAAAGgttaagttgat	4. 0.9	tgtttttcagATTGG
V. 376	CACGT		

^a Nucleotides in exons are capitalized; nucleotides in introns are shown in lower case letters.

TGATAACAATAATTTATACATTTTAAAGCATAAATCATTCTAATATATATTATATAAACAT -534
 TTTAAGTCTAAATCATTTTAAATAACTATAAATTTGCAAGTTTAACTAATTTTTTTTAT -474
 TTTTATTTTTTATGATATTCCTGAGCATATTTTATTAAATATGTCGTAATAATCTGAT -414
 GGTAAGCAATACTATCTGGCAATAAAAAAATCTGACAACAACAAAGCTTTATTATTGAA -354
 ACAACAGTATTTGATTGCTTCTAGCACAAACATAAAATGGACACTAATGTTTTCTGTTGA -294
 AAACATTCATGTTTTCTCTATACTCTGTGTTTAAAGTCTCAAATCAAATCTTGTGCAT -234
 TGAATGTCACCTTAAGGAGGAATCCACATGACTCTGCTTAGACCCTTGGTCCCTGCCTAT -174
 CAGATTCTAGGTGTCTAGCTGAAGGTACAATAGGAGAAAATGGGTGACTATAAAATTGCC -114
 AATTTGGCACAACCCTATTATTATCTCTGGAGAAAGCCAAGCCCAGGCATAGCTGATGAG -54
 TTAACCAGATACAACACAGAAGACATTTCTCTCTCTTGTGGTTTCAAGCCTATTTTCG +7
 AAGCCATAGCTCTCTGGTGCAGTTAGCACCTGAAGGTGCAGGGGCGATGGTCACTGGAAG +67

The putative TATA box, CAAT box, and reverse complement of μ E3 site are underlined. The sequence shown was confirmed by sequencing both strands. Arrows indicate the principal potential initiation sites of mRNA deduced from primer extension assays (data shown in Fig. 3). Numbering is based on designating the 3' initiation sites as +1 and +2.

Figure 2. Nucleotide sequence of the 5' upstream region of the murine Fc ϵ RI α -chain gene. The putative TATA box, CAAT box, and reverse complement of μ E3 site are underlined. The sequence shown was confirmed by sequencing both strands. Arrows indicate the principal potential initiation sites of mRNA deduced from primer extension assays (data shown in Fig. 3). Numbering is based on designating the 3' initiation sites as +1 and +2.

1), a T-lymphoma line (EL-4), and a pre-B cell line (18-81A20). Using an oligonucleotide primer complementary to the leader sequence of the gene, several cDNA species with heterologous 5'-borders were identified by primer extension (Fig. 3A). The longest mast cell-specific transcripts terminate ~30 bp downstream of the putative TATA box, consistent with their representing transcription initiation sites. However, they represent relatively minor transcripts with more abundant ones terminating about 120 nucleotides downstream from the TATA homology. These results were obtained with two independently isolated RNA preparations from CFTL-12 cells. RNase protection analysis (Fig. 3B) reveals fragments protected by mast cell RNA that are similar in length to the largest fragments obtained by primer extension. However, shorter fragments are also protected, suggesting that the more 3' sites identified by primer extension may constitute true start sites rather than simply reflecting failure of the reverse transcriptase to propagate. RNA from a T lymphoma (EL-4), an Abelson murine leukemia virus transformed pre-B cell (18-81 A20) and a factor-dependent myeloid cell line (FDC-1) did not give rise to reverse transcripts nor did they protect the labeled RNA probe. Inasmuch as the 3' transcript initiation sites represent a dominant population of the mRNA, we designated these sites as +1/+2. The doublet bands ~30 bp 3' of the putative TATA box lie at positions -93/-94 (Fig. 2).

Comparison with other FcR genes. The organization of the mouse Fc ϵ RI α -chain appears closely related to that previously reported for rat Fc ϵ RI α , mouse Fc γ RIII α , human Fc γ RIIIB, and human Fc γ RIIIA α (24). Each has five exons of essentially the same size. In each case, the first and second exon comprise the leader. The third and fourth exons encode extracellular domains and the fifth codes for the transmembrane, cytosolic, and 3' untranslated regions.

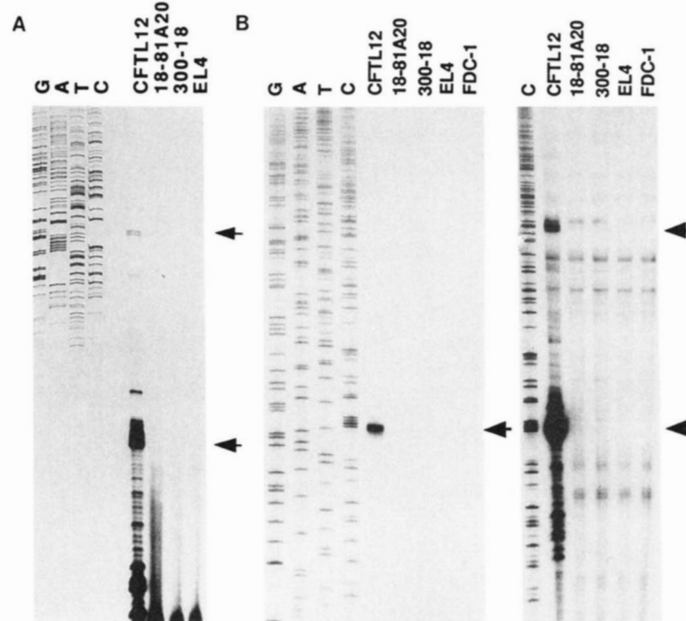


Figure 3. Mapping the transcription initiation sites of the Fc ϵ RI α gene. A, Primer extension analysis of Fc ϵ RI α -chain mRNA. Poly(A)⁺ RNA from various cell lines were isolated, subjected to primer extension, and analyzed on a 6% sequencing gel. The DNA sequencing ladder was generated with the same primer on cloned Fc ϵ RI α gene fragment and is shown on the left as a marker. G, A, T, and C refer to individual sequencing reactions. The lower arrow indicates the major (3') initiation sites (position +1/+2 in Fig. 2) and the upper arrow to the minor (5') initiation site (positions -93/-94 in Fig. 2). B, RNase protection analysis of Fc ϵ RI α -chain mRNA. Total RNA (20 μ g) isolated from various cell lines were subject to RNase protection analysis with a ³²P-labeled riboprobe spanning ~370 bp of 5'-flanking region, first exon, and part of the first intron. DNA sequencing ladders of pGEM3 plasmid with m13/PUC reverse primer are shown. The results from RNA of different cells with a short exposure of the gel to a X-ray film are shown on the left; the arrow indicates the major (3') initiation site. On the right, the same set of the samples with 8 times longer exposure times are shown in order to reveal the minor (5') initiation site indicated by the upper arrow.

