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Complete nucleotide sequence of mouse immunoglobulin μ gene and comparison with other immunoglobulin heavy chain genes

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ABSTRACT

We have determined the complete nucleotide sequence (2168 bases) of the immunoglobulin μ gene cloned from newborn mouse DNA. The cloned 13kb fragment contained the entire constant region gene sequence that is interrupted by three intervening sequences at the junction of domains as previously shown in the γ1, γ2b and α genes. The amino acid sequence predicted by the nucleotide sequence agrees with that of the μ chain secreted by a myeloma MOPC104E except for 8 residues out of 448 residues. The homologous domains of the μ, γ1 and γ2b genes are more similar to each other than the different domains of the μ genes are. The result implicates that the class of the immunoglobulin heavy chain genes diverged after the heavy chain genes established the multi-domain structure. The short intervening sequences of the μ and γ genes are more conserved than the coding sequences except for the COOH-terminal domains. The results implicate that the nucleotide sequence of the intervening sequence is under selective pressure, possibly to maintain a secondary structure of the nuclear RNA to be spliced.

INTRODUCTION

Immunoglobulins comprise two polypeptides, light(L) and heavy(H) chains, each having a variable(V) region which determines antigen-binding specificity and a constant(C) region which mediates biological effector functions. Immunoglobulins can be divided into five major classes, IgM, IgG, IgA, IgD and IgE, which are defined by their heavy chain constituents, μ, γ, α, δ and ε, respectively. The C portion of the H chain contains three or four homology units or domains of similar size (about 110 amino acid residues). The μ chain has four domains (CH_1, CH_2, CH_3 and CH_4) in addition to a COOH-terminal segment composed of 19 residues (1,2). The order of mouse H chain genes is proposed to be as follows; V_H, spacer, μ, γ3, γ1, γ2b, γ2a and α (3).

The μ gene is of particular interest because precursors of all antibody-forming cells express the μ chain prior to the expression of the immunoglobulins of other classes. During a course of differentiation a given lymphocyte associates a single V region successively with two or more different classes of H chain C regions, which is called H chain class switch.

Based on the comparative studies on the germline and rearranged γ subclass genes we have proposed that the complete γ gene is formed by two or more recombination events (4,5). The first type of recombination completes the V gene which is expressed as a part of the μ chain. The second type of recombination switches the C part from μ to γ or to α without affecting the V region sequence. Both types of recombination result in the deletion of the inbetween DNA segment from the chromosome (6-11). Similar results were also reported by other investigators (12,33).

We have already determined the complete nucleotide sequences of the γ1 and γ2b genes (13-14). Such sequences have unequivocally shown that immunoglobulin genes like most other eukaryotic genes are split by intervening sequences (IVS). IVSs not only separate the genes encoding V and C regions but also divide the C region genes of γ1, γ2b and α heavy chains into segments encoding each domain and the hinge region (13-18). These observations suggest that the domains of other H chain genes may be also separated by IVS. We have cloned the μ gene from newborn mouse DNA and shown by determination of a partial nucleotide sequence that IVSs separate the domains of the μ gene (4). Other investigators (19,20) also reported that each domain of the μ gene is separated by IVSs based on studies using the restriction site mapping, R loop formation and partial nucleotide sequencing. These studies, however, did not answer the question whether or not a small IVS is present anywhere else in the coding region.

Comparison of the nucleotide sequences of the γ1 and γ2b genes has revealed that the two genes share a significant homology at a specific contiguous segment of the γ gene, namely the CH_1 domain and the adjacent IVS (21). The results seem to implicate that two unequal crossing-over events might have occurred between ancestral γ1 and γ2b genes somewhere in the IVSs proximal to the CH_1 domain. Although mutual homology of H chain genes was assessed by mRNA-cDNA hybridization study (22), direct comparison of the nucleotide sequences of the γ genes with those of other H chain genes would give more information concerning mutual evolutional relationship among the immunoglobulin H chain genes.

We will show in this paper the complete nucleotide sequence of the μ gene. The sequence shows that the μ gene is divided by IVSs into four coding segments, each corresponding to one of the four domains; there is no IVS between the DNA segments encoding the CH_4 domain and the COOH-terminal segment. Comparison of the nucleotide sequences of the μ and γ genes supports the idea that the multi-domain structure of the immunoglobulin gene was

established before divergence of the heavy chain class (23). The sequence also provides a basis for future comparison to investigate evolutionary aspects of the immunoglobulin genes.

METHOD

Cloning and Subcloning of the μ Gene: The 13 kilobase(kb) EcoRI fragment containing the structural sequence of the μ gene was previously cloned from newborn BALB/c mouse DNA using λgtWES·λB as an EK2 vector (4). The recombinant phage and the insert were referred to as λgtWES·IgH701 and IgH701, respectively. To obtain a large amount of DNA, the HindIII-digested fragments of the 13kb insert DNA were subcloned into the HindIII site of pBR322 (see restriction map of Fig. 1). The two subclones, pIgH701-HindIII C and pIgH701-HindIII D which contain the 5' and 3' halves of the coding region of the μ gene, respectively, were used for sequencing. Cloning experiments were done under P2-EK2 or P3-EK1 conditions.

Restriction Mapping of the μ Gene: A restriction map of IgH701 was constructed by digestion with combinations of restriction endonucleases. For this purpose, the fragment to be mapped was 5'-labeled with [^{32}P-γ] ATP (New England Nuclear) and polynucleotide kinase, then cut with a restriction enzyme and resolved on a 4-5% polyacrylamide gel. Comparison between the original fragment and those generated by digestion permitted determination of the restriction sites.

DNA Sequence Analysis: The DNA fragment used for nucleotide sequencing were obtained from the clone λgtWES·IgH701 and the subclones; pIgH701-HindIII C and pIgH701-HindIII D. The fragments to be sequenced were isolated on a 3-5% polyacrylamide gel and either labeled at the 5' ends with polynucleotide kinase and [^{32}P-γ] ATP (specific activity, 7000Ci/mmol) after treatment with bacterial alkaline phosphatase. Alternatively the fragments were labeled at the 3' ends with [^{32}P-α] dCTP (specific activity, 2000Ci/mmol, New England Nuclear) and reverse transcriptase from avian myeloblastosis virus. The restriction fragments labeled at one end only were obtained by the second restriction cleavage or strand separation. Sequencing was performed using partial chemical degradation method of Maxam and Gilbert (24). Four base-specific reactions (G, A>C, C+T, C) were used and samples were separated by a thin (0.5 mm) polyacrylamide gel as described (13,25).

Alignment of the Nucleotide Sequence: Sequences were aligned to maximize homology by a computer using a modified program of Needleman and Wunsch (6, 27). Similarity index was computed as 100-(% gap + % transition + % trans-

version). The percentage was calculated relative to corrected length which is obtained by adding the number of base pairs and the number of gaps of the aligned sequences.

RESULTS AND DISCUSSION

Restriction Mapping and Sequencing Strategy: Figure 1a shows the cleavage sites of the restriction endonucleases; BamHI, BglII , HindIII, KpnI, SmaI, SacI, XbaI, XhoI and HhaI in the 13kb EcoRI fragment containing the μ gene (IgH701). SalI did not cleave the IgH701 DNA. A detailed restriction map of the structural region is shown in Fig. 1b.

To sequence the μ gene, we isolated DNA fragments containing the

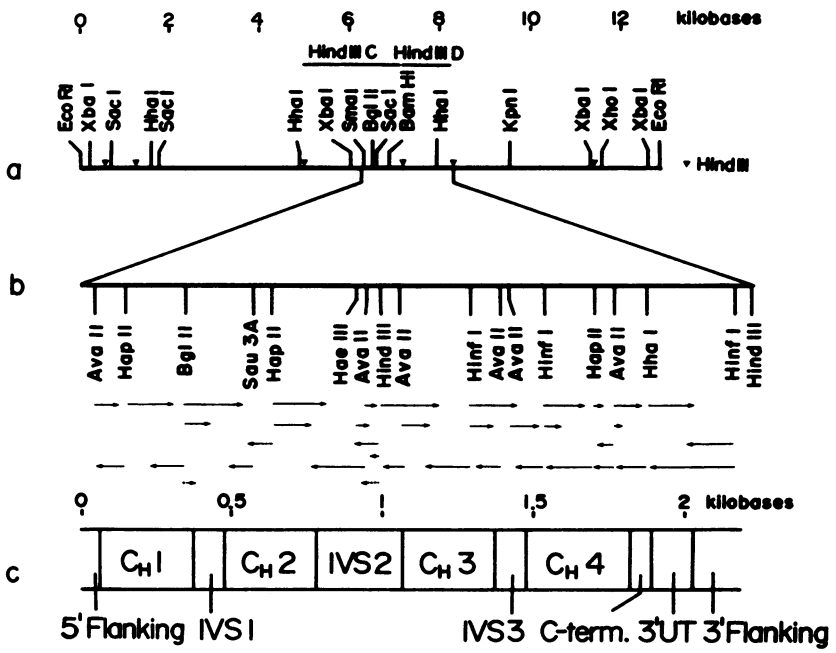


Figure 1. Diagram of restriction endonuclease cleavage site in the μ gene and strategy for sequencing.

a, the restriction cleavage sites of IgH701. The Hind III D fragments, which contained the structural sequences and were subcloned into pBR322, were indicated at the top of the figure. b, the detailed restriction map of the structural region. Only the restriction sites used for sequencing are shown. The horizontal arrows indicate the direction and range of the sequence obtained from these sites. The dotted arrows show the sequences determined by the 3' end labeling. c, the structure of the mouse μ genes. IVS, intervening sequence; UT, untranslated sequence; C-term, C-terminal segment.

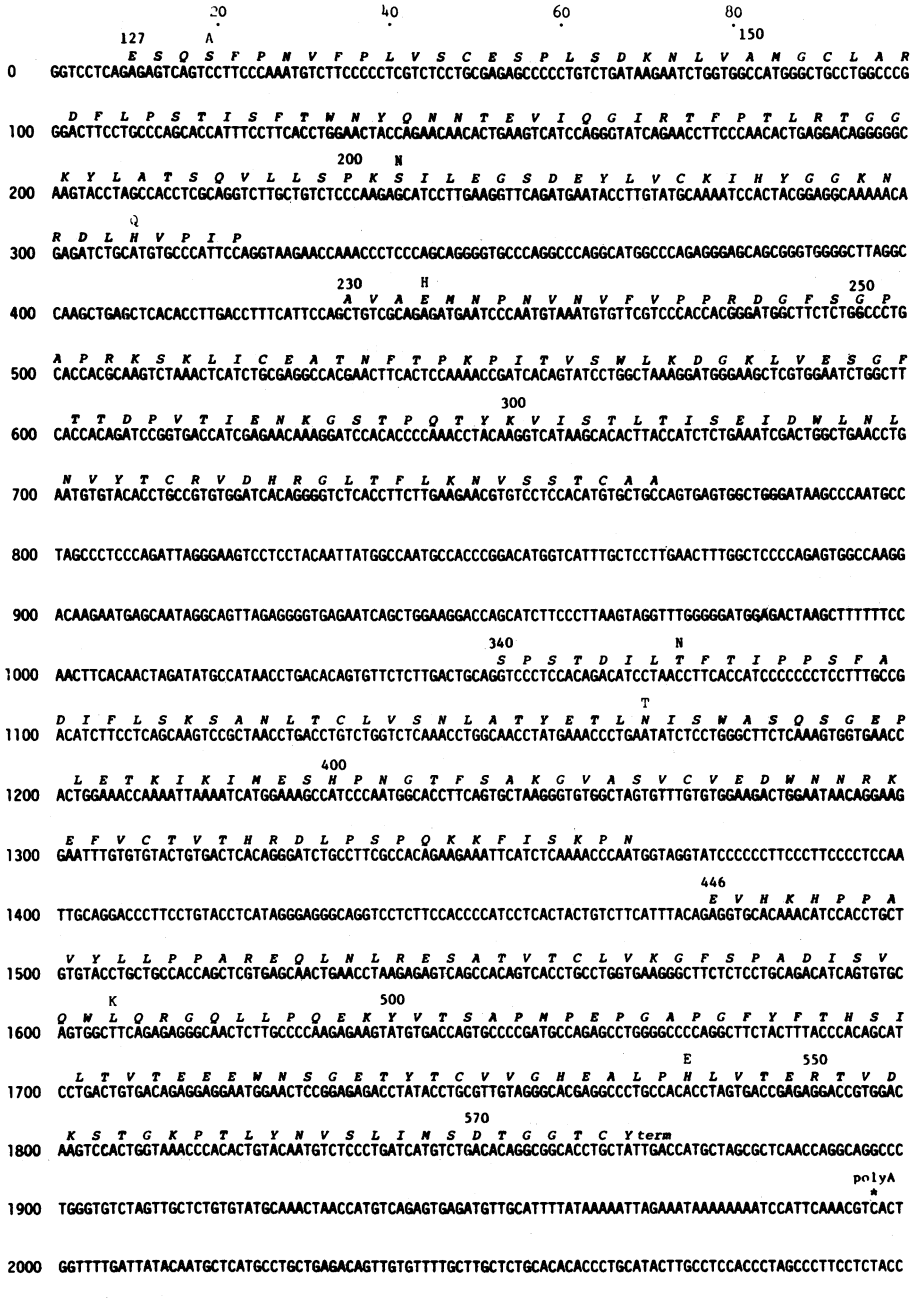
structural gene from the original clone (λgtWES·IgH701) and from two subclones (pIgH701-HindIII C and pIgH701-HindIII D). λgtWES·IgH701 DNA was digested with HhaI and then the HhaI fragments (3kb) containing the μ gene were separated from phage DNA fragments on a 3% polyacrylamide gel, since the outer fragments of λgtWES were digested with HhaI into many fragments smaller than 1kb. The subclones were digested with HindIII and the inserts were isolated. The fragments thus obtained were cut with the restriction endonucleases indicated in Figure 1b and labeled at their termini as described in Methods. DNA fragments labeled only at one end were used as substrates for partial chemical degradation (24). Regions were sequenced in both 5'→3' and 3'→5' directions wherever feasible. Restriction sites used for sequencing and the direction and range for sequencing are shown at the bottom of Figure 1b.

The μ Gene Contains Three Intervening Sequences which Separate Domains: The complete nucleotide sequence of the μ chain C region gene is shown in Figure 2. The total number of the nucleotides determined is 2168, which includes the coding sequence (1308 base pairs), IVS(496 base pairs), the 3' untranslated sequence (125 base pairs), and the 5' and 3' flanking sequences (239 base pairs).

The sequence unequivacally demonstrates that IVS divides the μ gene into four segments, each coding for a functional unit of the μ chain protein, namely a domain. The general structure of the μ gene is shown in Figure 1c. The DNA segments coding for the CH_1, CH_2, CH_3 and CH_4 domains are 315, 339, 318 and 336 base pairs long, respectively. IVS 1, IVS 2 and IVS 3 are 110, 279 and 107 base pairs long, respectively. The results are in agreement with previous reports that each domain of the μ gene is separated by IVS, based on studies using the restriction site mapping, R loop formation and partial nucleotide sequencing (4,19,20). In addition, the nucleotide sequence has shown that the CH_4 domain and COOH-terminal segment are not separated by an IVS but encoded by a cotiguous segment of DNA.

The present study as well as recent studies on immunoglobulin H chain gene (13-20) indicate that IVSs split H chain genes of various classes, γ1, γ2b, α and μ into the homologous sets of segments, namely, the domains and the hinge region. The most probable conclusion to be drawn from these results is that the IVS was introduced into the H chain gene before divergence of the H chain classes.

The Coding Sequence: The nucleotide sequence was correlated with the amino acid sequence already determined for the μ chain protein secreted by a myeloma MOPC104E (2). Our sequence disagreed at 8 of 443 positions (positions



130, 202, 225, 233, 347, 379, 489 and 545) as shown in Figure 2. Since five of them (positions 130, 202, 225, 347 and 379) are explained by substitution of one nucleotide, these differences might be due to the mutations in the myeloma cells. Our sequence disagreed with the partial nucleotide sequence of the μ chain cDNA clone reported by Calame et al. (20) at one base, TCT(S) instead of ACT at position 308.

During differentiation of B-lymphocytes two forms of the μ chain are synthesized. The membrane-bound μ chain is reported to be different from the μ chain secreted into the blood (2,28,29). The μ gene in the clone IgH701 encodes a secreted form of the μ chain. Other investigators have shown that this clone contains at its 3' side an additional segment which codes for the COOH-terminal segment of the membrane-bound μ chain (30). Such a segment seems to replace the COOH-terminal segment of the secreted μ chain by splicing mechanism.

The boundaries of the domain determined by the nucleotide sequence are different from those assigned by the amino acid sequence. The nucleotide sequence shows that the CH_1, CH_2 and CH_3 domains end at residues 229, 339 and 445, respectively. On the other hand Kehry et al. (2) assigned that the CH_1, CH_2 and CH_3 domains terminate at residues 232, 339 and 446, respectively.

Comparison between Domains: We have compared the nucleotide sequences of the domains of the μ gene with each other in every combination. Sequences were allinged to maximize homology by a computer using a modified program of Needleman and Wunsch (26). As shown in Table 1 the similarity indices between two domains are almost constant (39-42%) except that the similarity index between the CH_2 and CH_4 domains is significantly higher (46%) than those between the other combination. The similarity indices between the domains of the μ gene are similar to those between the domains of the γ1 gene which range 40-43% (13). The indices between the domains of each immunoglobulin gene are

Figure 2. Nucleotide sequence of the mouse μ gene.
The nucleotide sequence of the strand corresponding to the mRNA is displayed 5' to 3' at the bottom line. The amino acid predicted by the nucleotide sequence is shown above the coding sequence in italic letters. The numbers show the amino acid residues corresponding to those of MOPC104E μ chain protein in which the position 270 is deleted. The amino acid sequence of MOPC104E is presented on the top line. Only the amino acids which disagree with the predicted residue are shown. Amino acids are expressed by a one letter code as follows: (A) alanine; (C) cysteine; (D) aspartic acid; (E) glutamin acid; (F) phenyl alamine; (G) glycine; (H) histidine; (I) isoleucine; (K) lysine; (L) leucine; (M) methionine; (N) asparagine; (P) proline; (Q) glutamine; (R) argininine; (S) serine; (T) threonine; (V) valine; (W) tryptophan; (Y) tyrosine.

Table 1: Homology between Domains of the μ Gene

Domains compared	Nucleotides compared	Matched bases	Gap (bases)	Corrected length (bases)	Similarity Index (%)
CH_1:CH_2	315:339	156	132	393	40
CH_1:CH_3	315:318	150	129	381	39
CH_1:CH_4	315:336	149	108	387	39
CH_2:CH_3	339:318	165	129	393	42
CH_2:CH_4	339:336	174	81	378	46
CH_3:CH_4	318:336	152	90	372	41

significantly higher than the values (36-38%) obtained by the comparison between unrelated DNA segments (13). These results seem to implicate that the domains of the μ and γ genes evolved at about the same time. Therefore, the classes of the immunoglobulin H chain gene seem to have evolved after the immunoglobulin gene completed the three or four domain-structure as suggested from amino acid sequence studies (23).

Comparison between μ and γ Genes: We have compared the nucleotide sequences of the homologous domains of the μ, γ1 and γ2b gene. As shown in Table 2 the CH_2 domains of the γ genes are more homologous to the CH_3 domain of the μ gene than to the CH_2 domain of the μ gene. Similarly, the CH_3 domains of the γ genes are more homologous to the CH_4 domain of the μ gene than to the CH_3 domain of the μ gene. If one follows this order the CH_2 domain of the μ gene may correspond to the hinge regions of the γ genes as suggested by Putnam et al. (1). The COOH-terminal domains of the μ and γ genes are most conserved among domains, suggesting that a stronger selection pressure may have operated on the COOH-terminal domains of the heavy chain gene. The results are consistent with the report that the CH_4 domain is the most conserved domain between human and mouse μ chains (2).

We have also compared the nucleotide sequences of IVSs of the heavy chain genes. We have chosen short IVSs of similar length (98-120 base pairs) because an excessive difference in length makes the direct comparison of two sequences meaningless. As shown in Table 3 similarity indices of short IVS of the μ and γ genes ranged from 47 to 58% whereas those of the coding domains fell between 40 to 46% except for the COOH-terminal domains. The results indicate that

Table 2: Comparison of the Homologous Domains of the μ and γ Genes

Domains compared	Nucleotides compared	Matched bases	Gap (bases)	Corrected length (bases)	Similarity Index (%)
μCH_1:$\gamma 1 CH_1$	315/291	150	72	339	44
μCH_2:$\gamma 1 CH_2$	339/321	163	150	405	40
μCH_3:$\gamma 1 CH_2$	318/321	163	75	357	46
μCH_3:$\gamma 1 CH_3$	318/321	159	111	375	42
μCH_4:$\gamma 1 CH_3$	336/321	188	51	354	53
μCH_1:$\gamma 2b CH_1$	315/291	145	90	348	42
μCH_2:$\gamma 2b CH_2$	339/330	163	111	390	42
μCH_3:$\gamma 2b CH_2$	318/330	163	72	360	45
μCH_3:$\gamma 2b CH_3$	318/321	152	93	366	41
μCH_4:$\gamma 2b CH_3$	336/321	191	54	357	54

Table 3: Comparison of Intervening Sequences of the μ and γ Genes

IVS compared	Nucleotides compared	Matched bases	Gap (bases)	Corrected length (bases)	Similarity Index (%)
μIVS1: μIVS3	110/107	71	27	122	58
μIVS1: γ1IVS2	110/98	69	48	128	54
μIVS1: γ1IVS3	110/120	71	47	140	51
μIVS1:γ2bIVS2	110/107	71	47	132	54
μIVS1:γ2bIVS3	110/112	69	42	132	52
μIVS3: γ1IVS2	107/98	68	37	121	56
μIVS3: γ1IVS3	107/120	68	62	145	47
μIVS3:γ2bIVS2	107/107	68	54	134	51
μIVS3:γ2bIVS3	107/112	69	43	131	53

short IVSs of the μ and γ genes are more conserved than the coding sequences except for the COOH-terminal domains. The obvious implication is that the nucleotide sequence of the IVS is under selective pressure, probably to maintain a secondary structure of the nuclear RNA to be spliced. Such conclusion is also supported by the previous observation that the homologous IVSs of the γ1 and γ2b genes are more conserved than the third bases of codons in the homologous coding sequences (21).

It is known that the nucleotide sequences adjacent to the splicing site are relatively conserved among many IVSs. However, the homologous base-pairs of the μ and γ gene IVSs are not confined to the boundary regions of the IVSs when they are aligned so as to have maximal matching bases. Comparison of mouse and rabbit β globin genes has shown that the IVSs diverged much faster than coding sequences (31). Similar results were obtained by comparison of mouse γ1 and γ2b genes (21). Discrepancy between the present and previous results is probably due to difference in the selection pressure operating on the coding sequences. It is likely that the coding sequences of the heavy chain genes of the different classes the μ and γ chains were allowed to diverge from each other faster than those of the two genes in the γ subclass the γ1 and γ2b genes as well as those of the same globin genes in different species. On the other hand, small IVSs in various genes appear to be conserved by a weak but constant selection pressure.

Codon Usage of the μ *Gene:* Codon choices of the μ gene are summarized in Table 4. The codon usage is biased like other eukaryotic genes. All codons are used at least once with the exceptions of UUA(Leu), GCG(Ala) and CGA(Arg). Other codons which are rarely used are CCG(Pro), CGX(Arg), AUA(Ile), ACG(Thr) and GUU(Val). Certain codons are strongly preferred for some amino acids; for example, UUC(Phe), CUG(Leu), AUC(Ile), ACC(Thr) and GUG(Val). These biases in codon usage are shared more or less with the other immunoglobulin genes so far sequenced, γ1 and γ2b genes (13,14,15). As noticed previouly (32) codons with the doublet CG are rarely used. G or C is preferred at the third position. Out of 456 codons 175 (38%) codons terminate in C, 113 (25%) in G, 81 (18%) in A and 87 (19%) in T. These numbers are essentially similar to those observed in the γ1 and γ2b genes.

3' Untranslated Region: Comparison of the nucleotide sequences of the μ gene and the μ chain cDNA (30) indicate that the 3' untranslated sequence is 125 nucleotides long, starting at CCA and ending at GTC. The characteristic hexanucleotide AATAAA is found 20-25 bases before the poly(A) addition site. The 3' untranslated regions of the μ, γ1 and γ2b genes were compared with each

Table 4: Codon usage in C regions of immunoglobulin μ and γ2b chains

The number of times of each amino acid codon used in immunoglobulin gene is calculated. The γ2b gene sequence data are take from ref. 14.

Amino acid	Codon	γ2b	μ	Amino acid	Codon	γ2b	μ	Amino acid	Codon	γ2b	μ	Amino acid	Codon	γ2b	μ
Phe	UUU	1	3	Ser	UCU	5	9	Tyr	UAU	2	5	Cys	UGU	4	4
	UUU	8	16		UCC	10	14		UAC	8	8		UGC	7	8
Leu	UUA	0	0		UCA	6	4	Stp	UAA	0	0	Stp	UGA	1	1
	UUG	2	4		UCG	0	2		UAG	0	0	Trp	UGG	6	7
Leu	CUU	1	4	Pro	CCU	6	5	His	CAU	2	3	Arg	CGU	0	2
	CUC	8	6		CCC	8	14		CAC	6	7		CGC	0	1
	CUA	2	5		CCA	15	15	Gln	CAA	2	5		CGA	0	0
	CUG	0	22		CCG	2	3		CAG	7	7		CGG	2	2
Ile	AUU	2	3	Thr	ACU	9	6	Asn	AAU	4	11	Ser	AGU	6	6
	AUC	12	18		ACC	10	25		AAC	10	13		AGC	13	7
	AUA	1	1		ACA	8	12	Lys	AAA	12	11	Arg	AGA	3	4
Met	AUG	4	5		ACG	2	1		AAG	8	15		AGG	2	5
Val	GUU	1	2	Ala	GCU	5	8	Asp	GAU	7	9	Gly	GGU	4	7
	GUC	13	10		GCC	3	10		GAC	10	7		GGC	3	11
	GUA	3	4		GCA	2	4	Glu	GAA	1	12		GGA	6	3
	GUG	16	20		GCG	0	0		GAG	14	15		GGG	4	5

other. A significant homology was found among three sequences as shown in Figure 3. The 3' untranslated regions of these genes seem to be derived from a common ancestral sequence but that of the μ gene has insertions at several places.

Deletion of Segment from the μ Gene Fragment: During cloning of the μ gene fragment (IgH701) we also obtained a shorter clone containing a 10.5kb insert (IgH714). When characterized by restriction enzyme cleavage analysis and by Southern blot hybridization technique IgH714 was shown to be a variant of IgH701 which was produced during cloning. IgH714 has deleted a 2.5kb segment which is located about 2-5kb away from the 5' end of IgH701. When

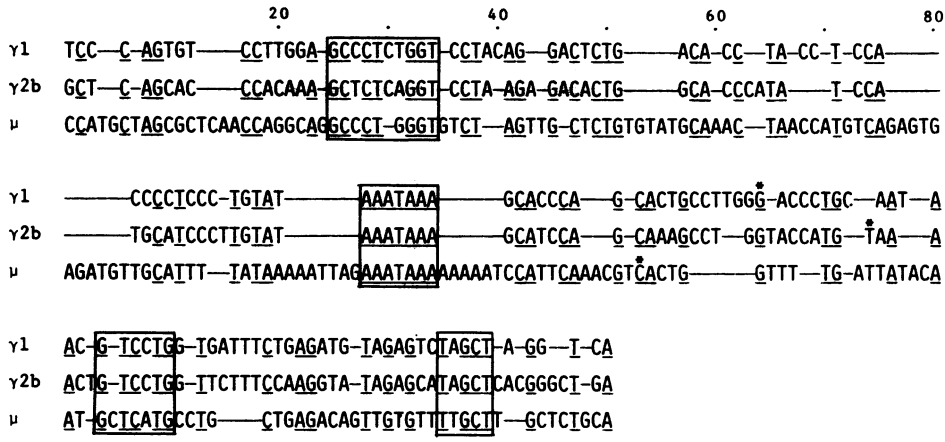


Figure 3. Comparison of the 3' terminal portion of mouse γ1, γ2b and μ genes.

Three sequences are aligned to maximize homology. The common sequences are underlined and long homology regions are boxed. Dashes indicate artificial deletions required for maximal homology. (*), the poly (A) addition sites.

IgH701 was recloned into pBR322 such deletion was rather frequent. This is probably because this region contains extremely repetitive sequences (5, unpubilished data).

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