Further evidence that BALB/c and C57BL/6 γ2a genes originate from two distinct isotypes

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Gene conversion by the corresponding γ2b gene has been proposed to explain the multiple differences between the nucleic acid sequences of BALB/c (Igh-1ᵃ) and C57BL/6 (Igh-1ᵇ) γ2a immunoglobulin allelic genes. However, genetic analysis indicates that duplicated forms of γ2a genes are not only present in Eastern Asia, but also in European wild mouse populations which suggests a widespread phenomenon. In order to verify whether the γ2a-related isotypic genes, namely γ2c and γ2a, could correspond to those present as alleles in domestic mice (Igh-1ᵇ and Igh-1ᵃ), a genomic library from *Mus m.musculus* strain (MAI) was constructed. Extensive mapping of the recombinant phages and Southern blot analysis with several restriction enzymes gave the complete organization of these loci: γ2b (18 kb) γ2c (17 kb) γ2a (14 kb) ε. The homology in flanking, coding and intervening region sequences indicates that MAI γ2c and γ2a related genes correspond to C57BL/6 and BALB/c Igh-1 alleles respectively. Also, Southern blot analysis using several probes derived from exonic and intronic regions between γ2b and γ2a genes shows a 2.0- to 3.0-kb difference in the distance between γ2b and γ2a genes of BALB/c strain as compared to C57BL/6. Taken together, these results indicate that BALB/c and C57BL/6 γ2a genes could originate from different isotypes.

Key words: allotypes/evolution/gene organization/immuno-globulins/isotypes

Introduction

Serological (Herzenberg *et al.*, 1968) and molecular cloning experiments (Shimizu *et al.*, 1981, 1982a) have shown that mouse immunoglobulin heavy chain constant region(s) (Igh-C) are encoded by eight tightly linked genes which are inherited as Igh haplotypes. With the exception of γ3, all of these isotypic genes present multiple allelic forms (Lieberman, 1978). Among these, γ2a isotype seems to be the most polymorphic, with a large number of antigenic specificities defining 12 allelic forms (Igh-1 series) in domestic mice (Lieberman, 1978; Green, 1979; Parsons *et al.*, 1986). However, all the γ2a allotypes, with the exception of Igh-1ᵇ (Huang *et al.*, 1983) are related to Igh-1ᵃ with no intermediary form between them. Furthermore, sequence analysis of Igh-1ᵃ (BALB/c) and Igh-1ᵇ (C57BL/6) alleles showed 10% differences at the nucleotide level and ~15%

of the deduced protein level (Schreier *et al.*, 1981). These differences are mainly located in the 5' and 3' flanking, hinge, CH3 and 3' untranslated regions (Schreier *et al.*, 1981; Ollo and Rougeon, 1983).

Such differences give rise to questions concerning the molecular mechanisms that lead to a high allelic divergence in Igh-1 (γ2a) locus while the corresponding Igh-6 (μ) and the neighbouring Igh-3 (γ2b) and Igh-5 (ε) alleles differ in only a few positions (Ollo and Rougeon, 1983; Schreier *et al.*, 1986; Shinkai *et al.*, 1988). It has then been suggested that non-reciprocal gene conversion of γ2a locus by γ2b gene could introduce sequence homogeneity between these isotypic genes and, consequently, generate extensive divergence and polymorphism in Igh-1 alleles (Ollo and Rougeon, 1983).

However, a few years ago, duplicated forms of γ2a locus were found in Japanese mice *Mus musculus molossinus* (Shimizu *et al.*, 1982b) and *Mus musculus* subspecies of Chinese wild mice (Fukui *et al.*, 1984). These observations were further extended to European *M.m.musculus* strains originating from different geographical areas (Jouvin-Marche *et al.*, 1989), indicating that γ2a duplication is common not only in Eastern Asia but also among European wild mice.

In a previous paper we suggested that the divergent γ2a alleles of BALB/c and C57BL/6 (Igh-1ᵃ and Igh-1ᵇ respectively) could derive from two distinct isotypes related to γ2a, namely γ2a and γ2c, frequently observed in wild mouse populations (Jouvin-Marche *et al.*, 1989). In order to confirm this hypothesis, the two γ2a related genes from an inbred *M.m.musculus* strain (MAI) were cloned and sequenced. Comparison of nucleotide sequences in the flanking, coding and intervening regions revealed a strong homology between MAI γ2a and BALB/c Igh-1ᵃ, and between MAI γ2c and C57BL/6 Igh-1ᵇ. The physical linking of γ2a and γ2c as distinct isotypes is confirmed. Moreover, we have strong evidence that BALB/c and C57BL/6 γ2a genes could originate from distinct isotypes.

Results

Organization of γ2 loci in M.m.musculus

A genomic library from liver DNA of an inbred *M.m. musculus* strain (MAI) was screened with a 380-bp *XhoI/PstI* fragment from pBG2a-4 (Ollo *et al.*, 1981) covering the CH1-IVS1 region of γ2aᵃ gene. Out of 1.5 × 10⁶ recombinants analysed, six different clones were isolated and extensively mapped (Figure 1). Overlaps between phage clones were established on the basis of common restriction fragments (RF) and by hybridization with several probes derived from different clones as specified in Figure 1. Size and organization of the RF described in Figure 1 were confirmed by Southern blot analysis of genomic DNA from MAI, BALB/c and C57BL/6 strains. Overlapping phages indicate that γ2b—γ2c loci lie on the same 20-kb *XhoI* or
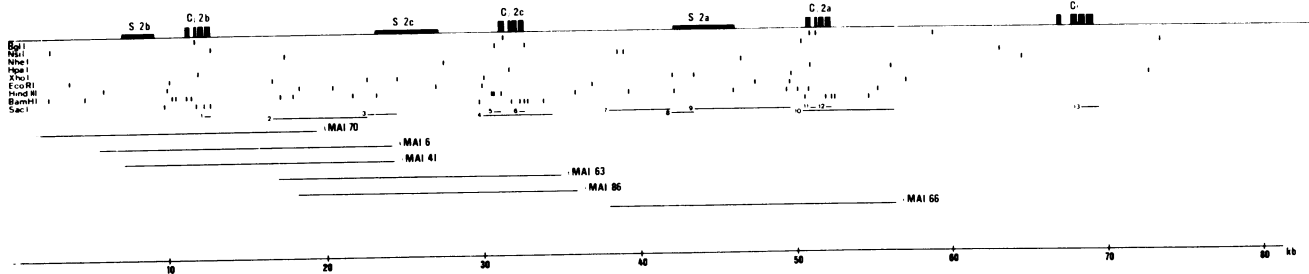
Fig. 1. Organization and restriction map of γ2 locus of *M.m.musculus* MAI strain. Structural genes are shown by open boxes at the top line. Switch region of each γ2 gene is indicated. Overlapping phage clones were mapped with the restriction enzymes indicated on the left. *Sac*I mapping covers only the flanking, coding and intervening regions of γ2b, γ2c and γ2a loci. Horizontal bars indicate fragments used as probes in this study. The ε probe (fragment 13) is described in Materials and methods. DNA regions covered by isolated λ clones are shown in horizontal bars with abbreviated names.

*Bgl*I fragment. Using probes 12 and 6 we verified that MAI γ2a and γ2c genes are located on a 23-kb and a 12-kb *Eco*RI fragment respectively (Figure 2). Furthermore the 23-kb fragment was also hybridized with probe (probe 13) confirming that γ2a and ε genes lie on the same *Eco*RI fragment as observed in BALB/c and C57BL/6 mice strains (Shimizu *et al.*, 1981).

Finally, the linkage between γ2c and γ2a was established by (i) hybridization of the 12-kb genomic *Eco*RI fragment containing γ2c with probe 7 derived from the 5' extremity of the MAI 66 clone, and (ii) hybridization with probes 6 and 9 from the 19.4-kb *Bgl*I or *Xho*I fragment which links MAI γ2c CH1-IVS1 regions to MAI γ2a CH1 domain (Figures 1 and 3). These results confirm that γ2c and γ2a are organized in tandem on the same chromosome and should be considered as two distinct isotypes and establish the gene order γ2b-(18 kb)-γ2c-(17 kb)-γ2a-(14 kb)-ε in MAI genome.

γ2b – γ2a distance is not the same in BALB/c and C57BL/6 strains

While this work was in progress an unexpected RF polymorphism in the region covering the γ2b–γ2a gene was observed between BALB/c and C57BL/6, used as control mice containing divergent γ2a alleles (Schreier *et al.*, 1981; Ollo and Rougeon, 1983). A more detailed Southern blot analysis was undertaken using several probes derived from MAI recombinant phages. Indeed, using probes 7, 8 and 9 respectively, we confirmed the presence of the three 4.0-, 1.8- and 5.0-kb *Eco*RI intervening fragments between γ2b and γ2a in BALB/c strain as described elsewhere (Shimizu *et al.*, 1981). However, these *Eco*RI fragments measured at least 5.4, 1.8 and 5.6 kb in C57BL/6 strain (Figure 4). The BALB/c pattern was also observed in C58, 129, DBA/2, RF, AKR, NZB, C3H and PL/J domestic strains corresponding to a, c, d, e and j Igh-1 alleles respectively. The C57BL/6 pattern was found in SJL and SM/J strains carrying the Igh-1[b] allotype. Furthermore, *Bgl*I or *Xho*I RF hybridized with probes 1 and 5 showing that γ2b and γ2a lie on the same 18-kb fragment in BALB/c, whereas these two isotypes lie on the same 20-kb fragment in C57BL/6 strain (Figure 4b). These results indicate a 2.0- to 3.0-kb difference in the γ2b and γ2a gene distance between BALB/c and C57BL/6 strains.

BALB/c and C57BL/6 γ2a genes could originate from different isotypes linked in wild mice

The *Eco*RI fragments containing γ2a (fragment 10) and γ2c (fragment 4) constant region genes were isolated from MAI

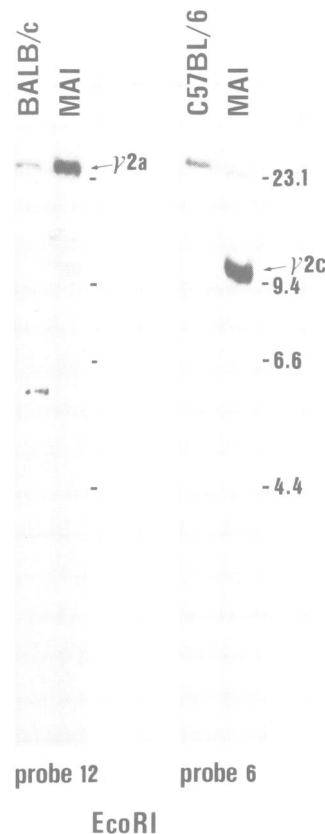**Fig. 2.** Southern blot hybridization of BALB/c, C57BL/6 and MAI DNA with specific γ2a and γ2c probes, corresponding to the *Sac*I fragments containing the CH3 region exon. Fragment sizes were estimated by comparison to λ phage DNA digested with *Hin*dIII (marker sizes in kb on the right). Arrows indicate specific hybridization of MAI γ2a and γ2c isotypes.

66 and MAI 63 phage clones respectively (Figure 1), subcloned into plasmid for detailed restriction analysis and the nucleotide sequences of the flanking, coding and intervening (IVS) regions were determined.

γ2a and γ2c MAI sequences were aligned with published BALB/c and C57BL/6 γ2a sequences (Ollo *et al.*, 1981; Schreier *et al.*, 1981; Yamawaki-Kataoka *et al.*, 1981; Ollo and Rougeon, 1983) in a way to maximize homology (Figure 5). Lengths of homologous regions are similar between the four genes except for IVSs and hinge regions between BALB/c and C57BL/6 alleles (Ollo and Rougeon, 1983) and between MAI γ2a and γ2c isotypes. Percentage identities in coding and non-coding regions between MAI, BALB/c and C57BL/6 γ2a related genes are summarized in Table
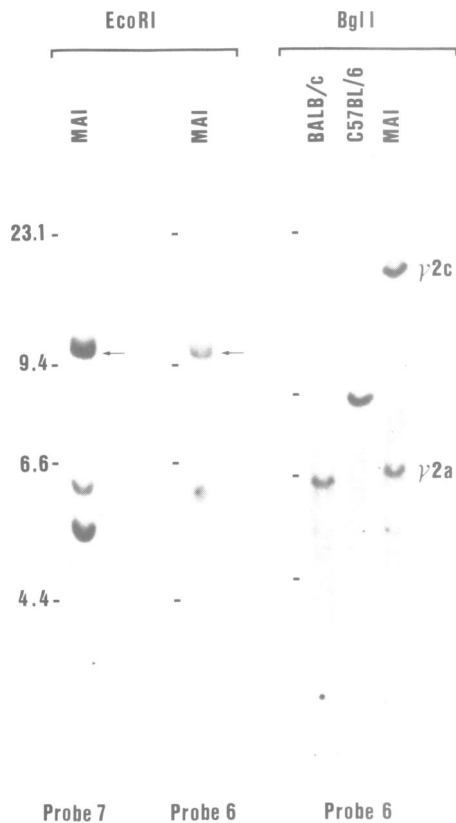
Fig. 3. Southern blot analysis showing the linkage between MAI γ2c and γ2a constant regions. Arrows indicate the hybridization of the same 12-kb EcoRI fragment with probe 7 covering the 5' extremity of clone λ MAI 66 and with probe 6 covering the CH3 region of MAI γ2c. Hybridization of a 19.4-kb BglI restriction fragment spanning the MAI γ2c to MAI γ2a CH1 region. In BALB/c, C57BL/6 and MAI γ2a the hybridization corresponds to a BglI fragment spanning from the hinge region to the 3' flanking region. Size markers are indicated on the left of the figure.

I and clearly indicate that MAI γ2a corresponds to BALB/c Igh-1[a], whereas MAI γ2c is equivalent to C57BL/6 Igh-1[b]. Comparisons between MAI γ2a and BALB/c Igh-1[a] sequences indicate a total conservation of CH1, hinge and CH3 domains. In the CH2 domain, only three transitions in positions 947, 953 and 1013 and two transversions in positions 1033 and 1159 are observed. Again the CH1 domain is totally conserved between the corresponding MAI γ2c and C57BL/6 Igh-1[b] genes. With the exception of the hinge and IVS2 sequences all other regions present >96% nucleotide homology. From the 15 modifications observed in the hinge region, seven correspond to transitions and eight to transversions. A predominance of amino acid changes over silent modifications is also observed in this region (Table II). The 5' flanking regions of BALB/c and MAI γ2a genes as well as those of C57BL/6 Igh-1[b] and MAI γ2c genes were also highly conserved. From the 122-bp sequence compared only one T → C transition in position 42 in the case of γ2a and three T → C transition in positions 73, 74 and 75 and one T → A transversion in position 32 in the case of γ2c were found. Similar results are also observed when 3' untranslated and 3' flanking regions are compared among these strains. The region surrounding the poly(A) addition site is strongly conserved among the four genes compared (Figure 5). However, the homology falls off sharply when MAI γ2a and γ2c or BALB/c and C57BL/6

γ2a genes are compared either in the coding or in intervening and flanking regions (Table I). Finally, divergence between MAI γ2a and γ2c isotypes appears equivalent to that observed between BALB/c and C57BL/6 γ2a genes, confirming their origin from distinct isotypes.

Discussion

In this paper we present the complete organization of the immunoglobulin heavy chain γ2 loci in an inbred M.m. musculus strain. The duplicated γ2a genes, namely γ2c and γ2a, lie in tandem on the same chromosome between γ2b and ε constant region genes and should be considered as distinct isotypes. Analysis of homology at nucleotide and amino acid levels shows that MAI γ2a strictly corresponds to the BALB/c Igh-1[a] allele and a high homology is observed between MAI γ2c and C57BL/6 Igh-1[b] allele. Furthermore, Southern blot analysis with different probes covering the region between γ2b and γ2a shows that this region is at least 2.0−3.0 kb longer in C57BL/6 than in BALB/c mice. Surprisingly, the analysis of the homology levels in the flanking regions from BALB/c and C57BL/6 γ2a showed the same level of divergence as those observed between MAI γ2a and γ2c isotypes. Taken together, these results strongly suggest that BALB/c and C57BL/6 γ2a genes are not derived from a common ancestral gene and could have different evolutive stories.

Immunoglobulin γ2a duplication in Japanese and Chinese strains of mice has been assumed to result from an unequal crossing-over between two different haplotypes (Shimizu et al., 1982; Shinkai et al., 1988). However, this hypothesis explains neither (i) the high frequency of European M.m. musculus strains (Jouvin-Marche et al., 1989) in addition to Asian M.m.musculus (Shimizu et al., 1982; Fukui et al., 1984), European M.specilegus and M.spretoides strains (M.G.Morgado et al., in preparation) bearing the γ2a duplicated genes corresponding to BALB/c and C57BL/6 γ2a, nor (ii) the divergence in the amino acid and nucleotide sequences between Igh-1[a] and Igh-1[b] which appear very high for simple alleles. Although differential gene conversions of these γ2a alleles by the corresponding γ2b genes have been proposed to explain this divergence (Schreier et al., 1981; Ollo and Rougeon, 1983), it is difficult to understand how gene conversion could generate such a high degree of polymorphism only in the γ2a locus, whereas other Igh loci are just slightly polymorphic. Different models have been proposed to estimate the relative importance of independent point mutations versus gene correction mechanisms during gene evolution (Jaulin et al., 1985; Stephens, 1985). A preliminary analysis of divergent positions in MAI γ2a, γ2c and γ2b, in addition to BALB/c and C57BL/6 γ2a and γ2b genes according to both methods indicates that gene correction between γ2b- and γ2a-like genes does not seem to be the major mechanism to explain the divergence between γ2a-related genes. In most positions the differences observed could be attributed to point mutations. However, we cannot exclude the possibility that the clustered mutated positions in the CH3 and 3' untranslated regions could indicate a gene correction mechanism. Thus, we propose that BALB/c and C57BL/6 γ2a genes are derived from distinct isotypes widespread in natural populations of mice.
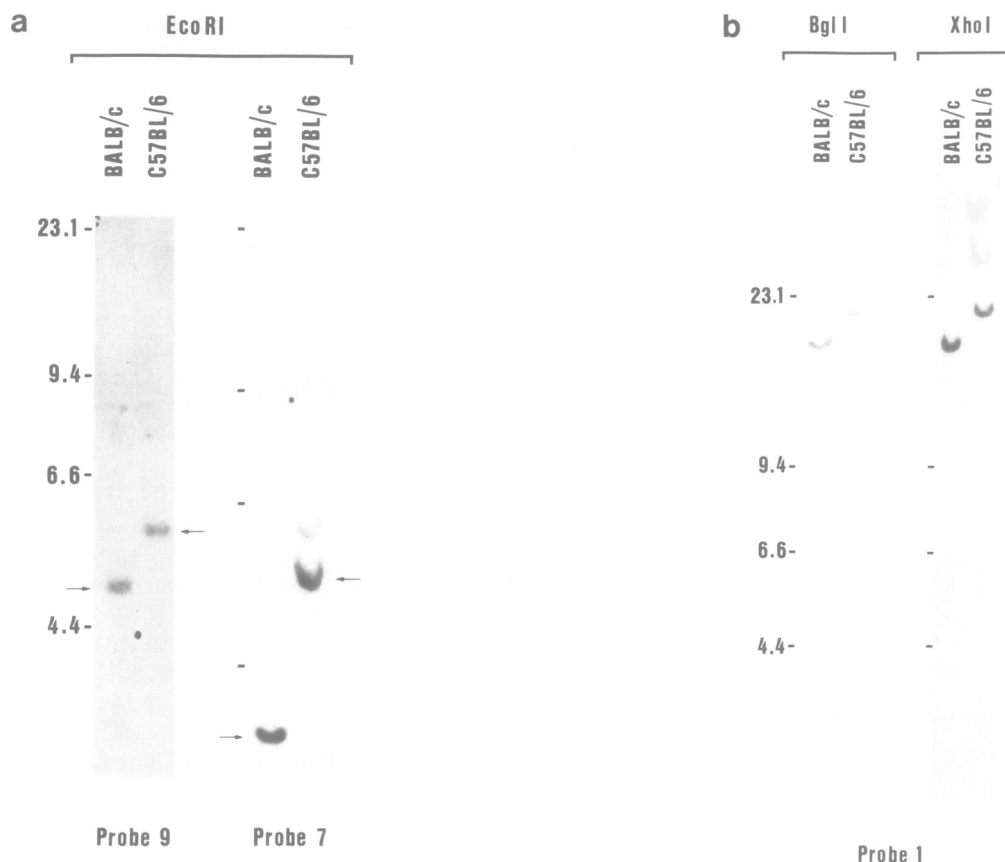
Based on the analysis of the frequencies of synonymous

Fig. 4. Southern blot analysis of BALB/c and C57BL/6 DNAs showing (**a**) the polymorphism in two out of three *Eco*RI fragments covering a large intronic region between γ2b and γ2a as indicated by the arrows, and (**b**) the difference in the genomic distance between γ2b and γ2a in BALB/c and C57BL/6 domestic mice. Size markers are indicated on the left of the figure.

(ks) and replacement (ka) substitutions (Li *et al.*, 1985), we attempted to determine the phylogenetic relationship within the γ2 gene family. Evolutive studies must take into account that immunoglobulin gamma genes have evolved dynamically through gene duplication and accumulation of point mutations associated with possible chromosomal exchanges either by double unequal crossing-over or by gene conversion (Miyata *et al.*, 1980; Yamawaki-Kataoka *et al.*, 1980, 1981; Schreier *et al.*, 1981; Ollo and Rougeon, 1983; Hayashida *et al.*, 1984). With the exception of the CH2 domain, only the flanking and intervening regions present some divergence between BALB/c and MAI γ2a genes. As shown in Table II, the ks and ka in the CH1, CH2 and CH3 domains as well as the ki rate in flanking and intervening regions between MAI γ2c and C57BL/6 Igh-1[b] genes were similar or just slightly higher than those observed between MAI and BALB/c Igh-1[a]. In spite of the total length conservation of the hinge region between MAI γ2c and C57BL/6 Igh-1[b], the divergence in this region and in the neighbouring IVS2 is 3- to 10-fold higher than in the other regions. Of the 15 differences observed, 14 led to amino acid changes. Indeed, in rapidly evolving genes such as immunoglobulins, amino acid exchanges are accepted to a much greater extent and even radical replacements occur at a substantial rate (Li *et al.*, 1985). Alignment of nucleotide sequences from the hinge region of immunoglobulin gamma gene isotypes from mouse (Hayashida *et al.*, 1984) or man (Ellison *et al.*, 1982) also showed a strong difference both in size and in composition. This hinge region variability

suggests the absence of selective pressure in this region which could evolve freely in a more accelerated process. It was also proposed that the hinge region substitutions are more rapidly fixed by selection leading to the generation of new and diverse effector functions carried out by IgG subclasses (Ellison *et al.*, 1982). Alternatively, as proposed to explain the similarities among different γ1, γ2b and γ2a domains (Miyata *et al.*, 1980; Yamawaki-Kataoka *et al.*, 1980) we may also suppose that a double unequal crossing-over event has taken place during MAI γ2c or C57BL/6 Igh-1[b] evolution resulting in a segmental transfer of the DNA region covering the hinge and IVS2 regions from an as yet undescribed haplotype.

The divergence between MAI γ2a and γ2c isotypes is similar to that observed between BALB/c and C57BL/6 γ2a genes. The ks/ka ratio ranges from 0.6 to 1.6 in the CH1, CH2 and CH3 domains, indicating that these regions are not under a strong selective constraint and are mainly evolving with accumulation of replacement substitutions almost equivalent to silent ones. In contrast, the hinge region shows a very low ks/ka ratio (0.03−0.08), indicating a strong prevalence of replacement substitutions.

In order to trace back the evolutionary history of a gene family it is advantageous to analyse gene segments that have been unaffected by recombinational events (Hayashida *et al.*, 1984). Comparisons of mouse nucleotide sequences have shown marked segmental homology between γ1/γ2a/γ2b/γ3 in the CH1 region, γ2a/γ2b in the IVS1−IVS3 regions and γ3/γ2b/γ2a in the CH2−IVS3 region, suggesting that DNA

Fig. 5. Aligned nucleotide sequences of MAI γ2a and γ2c with published BALB/c (Ba 2a) and C57BL/6 (C57 2a) γ2a, drawn from the EMBL data base with mnemonic accession code MMIG13 and MMIGCF respectively. Only the nucleotides that differ from those of MAI γ2a are shown for the other genes, slashes indicate deletions inserted to maximize homology. The acceptor and donor splicing signals, the termination codon TGA and the polyadenylation site are underlined.

segment exchanges occurred during the evolution of gamma genes (Miyata et al., 1980; Yamawaki-Kataoka et al., 1981; Hayashida et al., 1984). However, the CH3 domain seems to be free of such events (Hayashida et al., 1984) and was

used to estimate the divergence time within the γ2 gene family. The evolutive rate between γ2 genes was estimated using the average of the ks values obtained by comparing rat and mouse γ2 genes, considering the rat−mouse

Table I. Percentage identity in coding and non-coding regions

	5'F % n	CH1 % n	CH1 % aa	IVS1 % n	Hinge % n	Hinge % aa	IVS2 % n	CH2 % n	CH2 % aa	IVS3 % n	CH3 % n	CH3 % aa	3'UT/3'F % n
MAI γ2a × BALB/c γ2a	99.2	100	(100)[a]	99.3	100	(100)	99	98.5	(97.3)	98.2	100	(100)	98.2
MAI γ2c × C57BL/6 γ2a	96.7	100	(100)	98.7	76.2	(52.4)	90.8	99.1	(99.1)	96.4	96.3	(91.6)	96.8
MAI γ2a × MAI γ2c	89.3	97.9	(94.8)	94.8	78.0	(50.0)	90.8	96.7	(93.6)	96.4	85.7	(71.9)	89.2
BALB/c γ2a × C57BL/6 γ2a	88.4	97.9	(94.8)	94.5	80.0	(61.1)	95.4	96.7	(93.6)	93.7	83.8	(69.1)	87.3

[a]Number in parentheses corresponds to percentage identity in amino acid composition. Percentage identity was calculated as: (number of identical positions)/(total number of nucleotides compared) × 100.
Deletions and insertions were considered as one mutational event independent of the nucleotide number.

Table II. Frequencies of synonymous (ks or ki) and non-synonymous (ka) substitutions in coding and non-coding regions

	5' Fl ki	CH1 ka	CH1 ks	IVS1 ki	Hinge ka	Hinge ks	IVS2 ki	CH2 ka	CH2 ks	IVS3 ki	CH3 ka	CH3 ks	3'UT=3'Fl ki
MAI γ2a × BALB/c γ2a	0.0083	0	0	0.0097	0	0	0.0092	0.0117	0.0307	0.0181	0	0	0.0180
MAI γ2c × C57BL/6 γ2a	0.0338	0	0	0.0127	0.3766	0.0449	0.0978	0.0039	0.0299	0.0366	0.0375	0.0443	0.0328
MAI γ2a × MAI γ2c	0.1159	0.0237	0.0136	0.0430	0.2820	0.0092	0.0978	0.0276	0.0454	0.0357	0.1420	0.1860	0.1166
BALB/c γ2a × C57BL/6 γ2a	0.1257	0.0237	0.0136	0.0566	0.2920	0.0242	0.0473	0.0276	0.0457	0.0653	0.1544	0.2115	0.1253

The frequencies of substitution at synonymous and non-synonymous site were calculated using the program of Li et al. (1985). Corrected frequencies of substitutions in non-coding regions were calculated as $K = 3/4 \ln(1 - 4/3ki)$ where ki + (no. of different positions)/(total number of nucleotides compared).

speciation to be between 9 and 12 myr (Bonhomme, 1986). In rats only the γ2b isotype is homologous to mouse γ2b and γ2a (Brüggermann, 1988). Surprisingly, the evolutive rate obtained (17.7 × 10⁻⁹/site/year) was the same when calculated either in relation to mouse γ2b or mouse γ2c and γ2a, suggesting that all γ2 isotypes are submitted to the same selective pressure since rat and mouse separation. This evolutive rate is almost 4-fold higher than that observed for immunoglobulin genes when mouse and man are compared (Li et al., 1985). However, rates of nucleotide substitutions in rodents are estimated to be 4−8 times higher than those in primates, suggesting that differences in the number of DNA replications per year are the primary cause of rate differences in mammals (Li et al., 1987).

In Figure 6 we propose an evolutionary model for the mouse γ2 gene family that could explain why we can now find animals carrying a γ2b-γ2c haplotype and others carrying γ2b-γ2a or γ2b-γ2c-γ2a haplotypes. Firstly, the γ2b−γ2a/c divergence took place in parallel with rat− mouse speciation. Then, a new duplication of the γ2a/c ancestral locus took place leading to populations carrying the γ2b-γ2a/c haplotype and/or populations carrying the γ2b-γ2c-γ2a haplotype. These duplication/deletion events could be favoured by the highly homologous regions localized 3' of the γ2a genes (Fukui et al., 1984). More recently, the γ2b-γ2a (like in BALB/c or other M.m.domesticus) and γ2b-γ2c haplotypes could arise by differential deletion of the γ2c or γ2a isotypes, either by looping out or by double unequal crossing-over between the two circulating haplotypes (γ2b-γ2a/c and γ2b-γ2c-γ2a). It is curious that no mice containing γ2b-γ2c haplotype, like C57BL/6 strain, could be identified in a serological survey of natural populations belonging to M.m.domesticus or M.m.musculus species (Jouvin-Marche et al., 1989) which suggests a better fixation of γ2b-γ2c-γ2a and γ2b-γ2a haplotypes in natural populations.
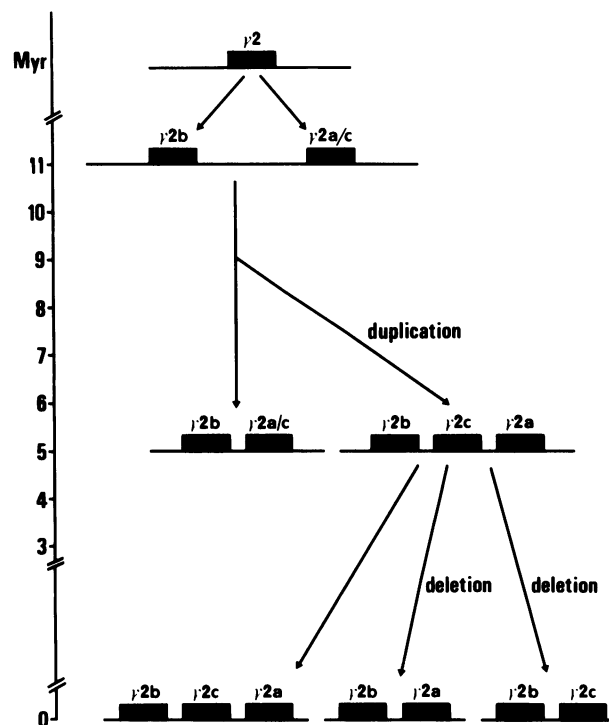

Fig. 6. Model proposed for the evolution of the γ2 gene family. The γ2a/c duplication time was calculated based on the ks values obtained by comparing the CH3 domains of MAI γ2a and γ2c. Scale is indicated in millions of years (Myr).

Materials and methods

Construction and screening of a genomic library

A genomic library was constructed from M.m.musculus MAI strain DNA partially digested with MboI. Fragments of 15−20 kb were separated by gel electrophoresis, electroeluted and ligated to BamHI λJ1 vector arms (Mullins et al., 1984). After in vitro packaging (Gipapack plus, Stratagene) the recombinant phages were screened by in situ hybridization using

*Xho*I−*Pst*I probe that covers the γ2a[a] CH1−IVS1 region (Jouvin-Marche *et al.*, 1989). This probe was derived from pBG2a-4 BALB/c genomic clone (Ollo *et al.*, 1981).

Restriction mapping of DNA cloned in λ phage

The recombinant phages were mapped by single and double digestion with several restriction enzymes. The phages were also mapped using the cos oligomer probes according to Rackwitz *et al.* (1984). Briefly, partially digested λ DNA was hybridized with the dodecamer ON-R (5'-d GGGCGGCGACCT-3') (New England Biolabs) complementary to the right cohesive λ DNA terminus labelled with [γ-^{32}P]ATP (3000 Ci/mmol). After gel electrophoresis and autoradiography the restriction map was directly determined.

DNA probes

All nucleotidic probes used in this paper are specified in Figure 1 and were labelled by random priming (Feinberg and Vogelstein, 1983) to 1−2 × 10^8 c.p.m./μg DNA specific activity. The ε probe is a 1-kb *Hind*III−*Xba*I fragment isolated from a cosmid containing γ2a and ε constant regions kindly supplied by Dr F.Rougeon.

Isolation of DNA and Southern blot analysis

High mol. wt DNA was obtained from the tails of individual mice according to Brinster *et al.* (1985) with minor modifications. Briefly, after obtaining the tails, they were quick-frozen in dry ice, crushed and suspended in 5 ml of a solution containing 50 mM Tris−HCl, pH 8, 10 mM EDTA, 200 mM NaCl, 0.5% SDS and 100 μg/ml proteinase K. After mixing the suspensions were incubated at 37°C overnight. DNA samples were extracted with phenol (saturated with 1 M Tris−HCl, pH 7.5) for 1 h, phenol/chloroform (1:1) for 1 h, chloroform and precipitated with 2 vol of ethanol, dried and dissolved in water. DNA (15 μg) was digested to completion with various restriction endonucleases, electrophoresed through 0.8% agarose gels and blotted onto nylon membrane (Amersham) overnight according to standard protocols (Southern, 1975). Hybridization conditions were followed as previously reported (Hüppi *et al.*, 1985). After hybridization the filters were washed with 3 × SSC, 0.05% SDS at room temperature for 15 min, then washed in 1 × SSC, 0.05% SDS at 65°C for 15 min and in a final high-stringency wash with 0.1 × SSC, 0.05% SDS at 65°C for 30 min. The filters were autoradiographed at −80°C in the presence of intensifying screens by exposure to Kodak XAR-5 film.

DNA sequencing and sequence analyses

The *Eco*RI fragments containing γ2a and γ2c constant region genes were isolated from MAI 66 and MAI 63 phage clones (fragments 10 and 4 from Figure 1) respectively and subcloned in Bluescribe plasmid for detailed mapping analyses. Appropriate fragments were subcloned into M13 mp20 or mp21 and sequenced by the dideoxynucleotide chain termination method (Sanger *et al.*, 1977) in both directions. Nucleotide sequences were aligned by the NUCALN program (Wilbur and Lipman, 1983). Percentage of identity was calculated as number of identities/total number of compared positions × 100. Multiple contiguous insertions/deletions were considered as unique events. The rate of divergence for silent (ks) or replacement (ka) substitutions was calculated as described (Li *et al.*, 1985).

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