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Chromosomal Locations of the Murine T-Cell Receptor Alpha-Chain Gene and the T-Cell Gamma Gene

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Abstract. *Two independent methods were used to identify the mouse chromosomes on which are located two families of immunoglobulin (Ig)-like genes that are rearranged and expressed in T lymphocytes. The genes coding for the α subunit of T-cell receptors are on chromosome 14 and the gamma genes, whose function is yet to be determined, are on chromosome 13. Since genes for the T-cell receptor β chain were previously shown to be on mouse chromosome 6, all three of the Ig-like multigene families expressed and rearranged in T cells are located on different chromosomes, just as are the B-cell multigene families for the Ig heavy chain, and the Ig kappa and lambda light chains. The findings do not support earlier contentions that genes for T-cell receptors are linked to the Ig heavy chain locus (mouse chromosome 12) or to the major histocompatibility complex (mouse chromosome 17).*

Recent studies have revealed similarities and differences between the antigen-specific receptors of the B- and T-lymphocyte populations, which, together, make up the bulk of the cells of the immune system. The receptors on B cells have long been known to be immunoglobulins (Ig). Those on T cells consist of Ig-like integral membrane glycoproteins containing two polypeptide subunits (α and β) of similar molecular weight, 40 to 45 kD in the mouse (*1*, *2*) and 40 to 55 kD in the human (*3*). Like Ig's on B cells, each T-cell receptor subunit has, external to the cell membrane, an amino terminal variable domain (V) and a carboxyl terminal constant (C) domain (*4–6*). The genes that encode these subunits have been identified (*7–13*) and, like Ig genes, are assembled from gene segments (*14–18*). There are at least four segments for β chains—variable (V), diversity (D), joining (J), and constant (C)—and three, so far, for α chains (V, J, and C).

During the search for the T-cell receptor genes, Saito *et al.* (*11*, *19*) identified in T lymphocytes another Ig-like gene, called γ. Like the α and β genes, the γ gene is also assembled in T cells (but not in B cells) from gene segments that are homologous to the Ig V, J, and C segments. The function of the γ gene is not known, but the fact that it is assembled from gene segments in T cells (*20*) and shows clonal diversity in V region sequences (*21*) suggests that its product is (or is part of) a second T-cell receptor that helps determine T-cell specificity. The possibility that there are two receptors on T cells, rather than one, has long been debated, because of a difference in the way B and T cells recognize antigens: B cells (like antibody molecules)

can recognize antigens alone, but T cells characteristically recognize antigens only on those target cells that also have the appropriate surface glycoproteins encoded in the major histocompatibility complex (MHC), a property that is generally referred to as MHC-restricted antigen-recognition.

In this report we describe another way of comparing the B- and T-cell molecules that are responsible for antigen recognition, namely the chromosomal location of the genes that encode them. The three gene families for the Ig subunits [heavy (H), κ, and λ] are each located on a different chromosome (*22–25*). The T-cell β-chain gene family is located on mouse chromosome 6 (*26, 27*), but the chromosomal location of the α and γ genes has not been known (until now). To identify the α and γ locations, we used the corresponding complementary DNA (cDNA) clones as probes to analyze DNA from mouse-hamster cell hybrids and to carry out in situ hybridization on mouse cell metaphase spreads. We now report that the α gene is located on chromosome 14 and the γ gene is located on chromosome 13. Thus, like the H, κ, and λ gene families, the α, β, and γ gene families are also each located on a different chromosome. Our findings with the α, β, and γ genes are also noteworthy because they do not support earlier contentions that genes for T-cell receptors are linked to the Ig H-chain locus on mouse chromosome 12 or to the major histocompatibility complex on mouse chromosome 17 (*28*).

The T-cell receptor α-chain gene is on mouse chromosome 14. Chromosome mapping of the α-chain gene was performed by the analyses (*29*) of mouse-hamster hybrids (*22, 23, 25, 26*). DNA from 12 mouse–Chinese hamster hybrid cell lines was examined by Southern blot analysis (*29*) with a probe corresponding to the combined V and C regions of the rearranged gene for the α chain of cytotoxic T-lymphocyte clone 2C [cDNA clone pHDS 58 (*11*)]. This probe hybrid-

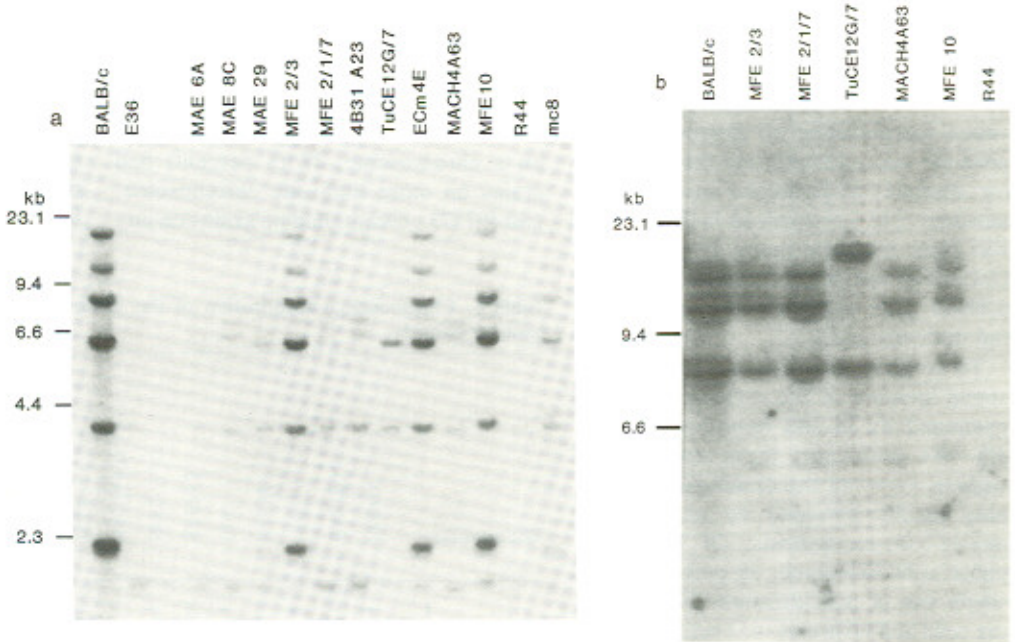

Fig. 1. Southern blot analyses (*29*) of DNA from representative mouse-hamster hybrid cell lines. DNA from mouse (BALB/c), hamster (E36), and mouse-hamster hybrid cell lines (*22, 23, 25, 26*) was digested with Eco RI, subjected to electrophoresis through a 0.8 percent agarose gel and blotted onto nitrocellulose. The blot was hybridized with ^{32}P-labeled, nick-translated probes: (a) insert of cDNA clone pHDS58 (*11*) corresponding to the V plus C regions of the α-chain gene from mouse and (b) insert of cDNA clone pHD54 (*19*) corresponding to the C region of the γ gene from mouse. Hybridization was carried out at 42°C in 50 percent formamide and 5× SSC. Filters were washed at 65°C in 0.2× SSC. The hamster DNA pattern with the γ-gene probe (b) was identical to that of DNA from mouse-hamster hybrid line R44.

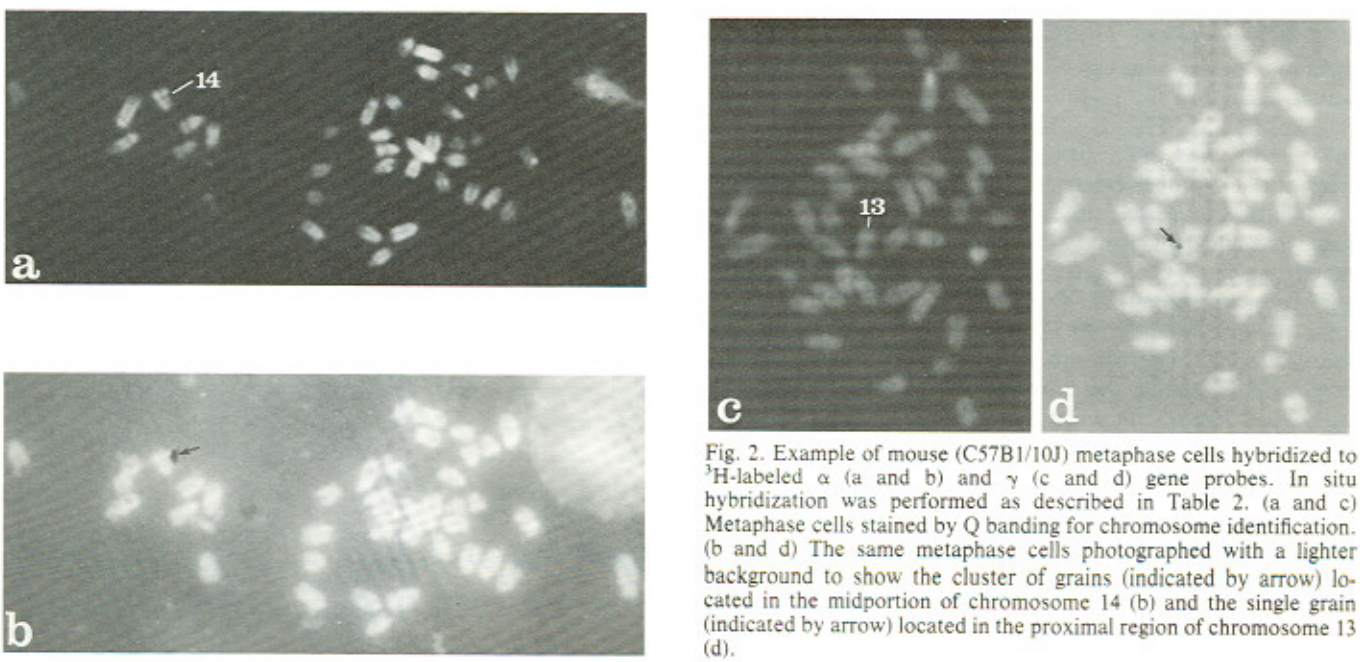

Fig. 2. Example of mouse (C57B1/10J) metaphase cells hybridized to ^{3}H-labeled α (a and b) and γ (c and d) gene probes. In situ hybridization was performed as described in Table 2. (a and c) Metaphase cells stained by Q banding for chromosome identification. (b and d) The same metaphase cells photographed with a lighter background to show the cluster of grains (indicated by arrow) located in the midportion of chromosome 14 (b) and the single grain (indicated by arrow) located in the proximal region of chromosome 13 (d).

ized strongly with six Eco RI fragments (about 18, 11, 8, 6, 4, and 2.2 kb) from BALB/c embryo DNA and weakly with five Eco RI fragments (about 18, 7.2, 6.5, 4.0, or 1.9 kb) from Chinese hamster cell DNA (Fig. 1a). Five of the somatic cell hybrids contained all six mouse-derived Eco RI fragments. The exceptional hybrid line (TuCE12G/7) contained only the 6-kb fragment derived from the mouse parent. We have observed a similar pattern of deleted bands in DNA from mouse T cells and, as shown below, the TuCE12G/7 line also exhibits a rearrangement of the T-cell γ gene. Because this line was isolated from a hybridization between the mouse sarcoma CM54 passaged in vivo and the Chinese hamster cell line E36, it is likely that this hybrid line is the product of a fusion between a mouse T cell present in the tumor cell suspension and a parent Chinese hamster cell. The discordancies between the presence (or absence) of the gene for the α subunit and the presence (or absence) of particular mouse chromosomes are summarized in Table 1. It was clear from the many discordancies (≥4) for all but one chromosome that the α-chain gene maps to chromosome 14 (no discordancies).

To confirm this finding, the chromosomal location of the α-chain gene was examined by in situ hybridization (*30, 31*). We performed these experiments without knowing the chromosome mapping results obtained by analyses of mouse-hamster hybrids. Of 709 metaphase cells examined, 28 percent had a single site of hybridization. The rest of the cells had no grains (453 cells) or more than one site of hybridization (56 cells). Among 123 informative metaphase cells, 34 sites of hybridization (or 23 percent) were on chromosome 14 (Table 2). One grain or a cluster of grains was predominantly located in the middle portion of chromosome 14 (Fig. 2, a and b). Because there was often more than one grain at the site of hybridization, it was not possible to assign this site to one single chromosomal band. It was estimated that the α-chain gene was probably located in regions C or D of chromosome 14, with bands D1 or D2 the more likely sites (*32*). Therefore the results of in situ hybridization and Southern blot analyses of mouse-hamster hybrids indicate that the α-chain gene is located on mouse chromosome 14.

The T-cell γ gene is on mouse chromosome 13. DNA from 14 mouse–Chinese hamster hybrid cell lines was examined by Southern blot analyses with a probe corresponding to the C region of the γ gene—cDNA clone pHDS4 (*19*). This probe hybridized with three Eco RI fragments (7.5, 10.5, and 13.4 kb) from BALB/c embryo DNA, but hybridized only weakly under the same conditions, with a 6-kb hamster DNA fragment (Fig. 1b) (Table 1). Of the 14 somatic cell hybrids, 6 contained Eco RI fragments derived from the mouse cell DNA. As indicated above with the α-chain gene, hybrid TuCE12G/7 displayed a pattern (bands at 7.5 and 16 kb) that was similar to the rearranged pattern observed in most T cells. From the number of discordancies observed for each chromosome (Table 1), it appears that the γ gene is located on chromosome 13 (no discordancies).

Because the number of discordancies for several chromosomes was low (chromosomes 2 and 8 had one discordancy each), the chromosomal location of the γ gene was also examined by in situ hybridization. Of the 559 metaphase cells examined, 137 (24 percent) had one grain on a chromosome, 15 (3 percent) had grains on more than one chromosome, and 407 (73 percent) had no grains on any chromosome. In 111 informative cells, 38 percent of the total number of grains was located on chromosome 13; the remaining grains were scattered on other mouse chromosomes (Table 2). Of the grains located on chromosome 13, 83 percent were located in the proximal region (region A2 or A3) (*32*) (Fig. 2, c and d). Therefore, the results of in situ hybridization and Southern blot analyses of mouse-hamster hybrids indicate that the γ gene is located on mouse chromosome 13.

Our results, together with the recently determined chromosomal location of the T-cell β-chain gene (*26, 27*), show that the known Ig-like genes (α, β, and γ), which rearrange in T cells, are each located on a different mouse chromosome: α on 14, β on 6, and γ on 13. The three Ig gene families are similarly located on different mouse chromosomes: the genes for H, κ, and λ chains are on chromosomes 12, 6, and 16, respectively (*22–25*). That β and κ genes are both on the same mouse chromosome is probably coincidental, since in humans the

Table 1. Chromosomal localization of the murine T-cell α- and γ-gene families in mouse-hamster hybrids.

Chromosome	MAE6A	MAE29	MAE32	MAE8C	CeC	MFE2/3	MFE2/1/7	MACH4B31A23	TuCE12G/7	ECm4e	MACH4A63	MFE10	R44[a]	mc8	Discordancies α-gene	Discordancies γ-gene
1	+	−	−	−	−	+	+	−	−	−	−	+	−	+	5	3
2	−	−	−	−	−	+	+	+	+	−	+	+	−	+	5	1
3	−	−	−	−	−	+	+	−	−	−	−[b]	−	−	−	6	4
4	−	−	−	−	−	+	−	−	−	−	+[b]	−	−	+	5	3
5	−	−	−	−	−	−	−	−	+	−	−	+	−	−	4	4
6	−	−	−	−	2/3[c]	+	+	−	+[b]	−	−	+	−	−	4	2
7	−	−	−	−	−	+	+	+	−	−	+	+	−	+	6	2
8	−	−	−	−	−	+	+	−	+[b]	−[b]	+[b]	+	−	−	5	1
9	−	−	−	−	−	−	+	−	−	−	−	+	−	+	5	3
10	−	−	−	−	−	+	−	−	+	−	−	−	−	−	4	4
11	−	−	−	−	−	+	−	−	−	−	−	+	−	−	4	4
12	+	−	−	+	−	+	+	+	+	+	+	+	−	−	7	4
13	−	−	−	−	−	+	+	−	+	−	+	+	−	+	4	0
14	−	+	−	−	−	+	−	−	+	+	−	+	−	+	0	4
15	−	−	−	−	−	+	+	+	+	+	+	+	−	−	5	3
16	−	−	+	−	−	−	−	+	+	−	+	+	−	−	6	5
17	−	−	−	−	−	+	+	−	+	−	−	+	+	−	5	3
18	−	−	−	−	−	+	−	−	+	−	+	+	+	−	5	3
19	−	−	−	−	−	+	+	+	+	−	+	−	−	+	6	2
X	+	+	+	+	+	+	+	−	+	−	−	+	−	+	4	6
α-gene	−	+	ND[d]	−	ND	+	−	−	+[e]	+	−	+	−	+		
γ-gene	−	−	−	−	−	+	+	−	+[e]	−	+	+	−	+		

[a]Cell line R44 contains chromosomes 17 and 18 as part of translocations together with some other unidentified chromosomal material. [b]These chromosomes were not identified by cytogenetic analyses, but were positive based on isoenzyme markers. [c]Cell line CeC contains a translocation between the X chromosome and the distal two-thirds of chromosome 6. [d]Not determined. [e]Cell line TuCE12G/7 contains mouse-derived α and γ genes that appear to be rearranged from the germline configuration.

two are located on different chromosomes—β on chromosome 7, and κ on chromosome 2 (*27*). An obvious implication of this finding (α, β, and γ genes on different chromosomes) is that each gene family must utilize its own V, (D), J, and C gene segments and its own regulatory elements.

In addition, the localization of the three Ig and the three Ig-like gene families on different chromosomes is probably not fortuitous. Although sequence homology between the Ig genes (expressed by B cells) and the Ig-like genes (expressed by T cells) is low, there are striking similarities between them. These include organization into gene segments (*14–18, 20, 33*), preservation of the flanking sequences that appear to be involved in gene segment assembly (*14–18, 20, 33*), and conservation of the amino acids necessary for the characteristic three-dimensional folding of Ig domains (*34, 35*). These remarkable similarities suggest that all six gene families are derived from a common ancestral sequence that replicated and became disseminated through the genome. The presence of two (or more) not yet extensively diverged families on the same chromosome would, on the other hand, have presented numerous opportunities for detrimental crossing-over events between the families, possibly resulting in instability of genetic elements in the vicinity of these genes and the loss of the distinctive identity of each of the gene families. The presence of β and κ gene families on mouse chromosome 6 is not in conflict with this view, since the nucleotide sequence homology between them, as indicated by representative V_β and V_κ gene segments and between C_β and C_κ segments, is very limited. Thus, it seems likely that the evolutionary precursors of these gene families had become diversified before becoming located on the same chromosome.

A discussion of the chromosomal location of T-cell receptor genes must address the many studies that resulted in the assignment of these genes to chromosomes other than those indicated here. Many of these earlier studies (*36, 37*), in which antisera to Ig variable regions (idiotypes) were used, indicated that T cells and antibodies that are specific for the same antigen can share idiotypes, particularly those associated with variable regions of antibody H chains (V_H idiotypes). Moreover, the ability of antisera to certain inbred mouse strain-specific V_H idiotypes to stimulate T cells of only those mouse strains having the appropriate set of H-chain genes, suggested that T-cell receptors are encoded by genes in the Ig H-chain linkage group, located on mouse chromosome 12. Other studies (*38*) have indicated that antigen-specific suppressor factors released by T cells carry antigenic determinants encoded in the I-J region of the major histocompatibility complex, located on mouse chromosome 17. However, as shown here for α and γ, and previously for β, these genes are clearly not on chromosomes 12 and 17. One obvious explanation for the difference between the previous findings and ours is that the earlier ones were simply in error because of difficulties inherent in the use of heterogeneous antisera and heterogeneous cell populations. Indeed, more recent studies with monoclonal antibodies to idiotypes have not confirmed sharing of idiotypes by T and B cells of the same specificity (*39*). However, it is also possible that T-cell suppressor factors, which have been shown to contain VH and I-J determinants, may be encoded by genes completely distinct from those encoding the α and β chains of the T-cell receptor on helper and cytotoxic T lymphocytes.

Table 2. Distribution of autoradiographic grains resulting from the in situ hybridization of ^{3}H-labeled α and γ gene to mouse chromosomes. Chromosome preparations were obtained from short-term cultures of mouse (C57B1/10J) embryo cells (*30*). In situ hybridization was performed by a modification of the procedure of Harper *et al.* (*31*). Probes (see Fig. 1) for the α-chain gene and the γ gene were labeled (with three ^{3}H-labeled nucleotides by nick-translation) to a specific activity of 5×10^7 to 1×10^8 cpm/μg. After treatment with ribonuclease, the slides were denatured in 75 percent formamide in double strength saline sodium citrate (2× SSC) for 2.5 minutes at 70°C, dehydrated through an alcohol series, then stored at −20°C until hybridization. The hybridization mixture consisted of 10 percent dextran sulfate, 2× SSC, 50 percent formamide, 10× Denhardt solution, sheared salmon sperm DNA at 100 μg/ml, transfer RNA at 100 μg/ml (*p*H 7), and probe at a final concentration of 0.005 to 0.01 μg/ml. After incubation at 42°C overnight, the slides were washed three times for 2 minutes in 2× SSC at room temperature, three times for 2 minutes in 50 percent formamide in 2× SSC at 39°C, and twice for 2 minutes in 2× SSC at 39°C. After being coated with nuclear track liquid emulsion (Kodak, NTB 2), the slides were exposed for 7 to 10 days at −4°C, then developed and stained with quinacrine mustard (500 μg/ml for 10 minutes). Metaphase cells were photographed for chromosome identification by Q banding and for grain localization by increasing the background transmitted light.

Chromosome	Grains (No.)	
	α Gene	γ Gene
1	8	9
2	5	5
3	6	3
4	5	3
5	7	9
6	6	4
7	9	5
8	4	3
9	6	1
10	5	6
11	6	2
12	9	3
13	5	46
14	34	1
15	11	3
16	6	4
17	4	3
18	4	2
19	5	6
X	4	2
Y	0	0

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