Proc. Natl. Acad. Sci. USA
Vol. 88, pp. 4666–4670, June 1991
Evolution

Transmembrane domain length variation in the evolution of major histocompatibility complex class I genes

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Communicated by D. Bernard Amos, March 7, 1991

ABSTRACT **The fifth exons of major histocompatibility complex (MHC) class I genes encode a transmembrane domain (TM) that is largely responsible for class I antigen cell-surface expression usually through conventional hydrophobic amino acid–membrane interactions or, less often, through phosphatidylinositol linkage. In this report we show that *Peromyscus leucopus*, a Cricetidae rodent, has MHC class I genes (*Pele-A* genes) encoding three distinct sizes of TMs. Increases in TM lengths were due to tandem duplications of sequences similar to human hypervariable minisatellite repeats and the λ *chi* site. We discerned remnants of a similar duplication event in comparable rodent and primate MHC class I genes. Furthermore, several duplications and deletions appear to have occurred independently in *H-2*, *RT1*, *Pele-A*, and *ChLA* genes in near-identical positions. Accumulated data suggests that sequences in the fifth exon of MHC class I genes may, therefore, constitute a mutational or recombinational hot spot that is mediated by minisatellite- and *chi*-like sequences imbedded within the coding region. The MHC class I genes may thus have recruited "selfish" DNA in their evolution to encode cell surface proteins. Expression of *Pele-A* genes was examined by the polymerase chain reaction (PCR) using oligonucleotide primers specific for exon 4 and 5 sequences. The PCR product sizes indicated that genes encoding each TM domain length are ubiquitously transcribed.**

Antigen-binding molecules such as T-cell receptors, immunoglobulins, and major histocompatibility complex (MHC) class I and II proteins exhibit a diversity that is unparalleled in mammalian genetics. For MHC genes, in particular those encoding class I molecules, the diversity is apparent at three levels. (*i*) There is a high degree of allelic variability (polymorphism) in MHC "classical" class I genes (1) indicative of the role of these MHC class I proteins in antigen presentation (2, 3). (*ii*) There is multiformity of class I loci within a species. Paralogous MHC class I loci in both humans (e.g., *HLA-A* and *HLA-E*) and mice (e.g., *H-2D* and *H-2T18*) are quite distinct in sequence and expression (4–6). (*iii*) There is heterogeneity in MHC class I proteins among species to the extent that orthologous MHC class I loci in distantly related species, such as mice and humans, are difficult or impossible to discern (7, 8).

Details of the molecular events generating diversity in MHC class I genes, inferred by DNA sequence comparison, still remain sketchy (9–11). It appears, however, that unconventional mutation mechanisms like segmental exchange (controversially referred to as gene conversion) are operative in *HLA* and *H-2* systems. For *HLA* class I genes, interallelic segmental exchange predominates; interloci exchange is more evident in *H-2* class I genes (10–12). Moreover, some of the events that led to MHC allelic variation are ancient—i.e., they may antedate speciation of some primates and murine rodents (9–11, 13–15).

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The myomorph rodent *Peromyscus leucopus* (family Cricetidae) provides an ideal model from which to view the evolution of MHC gene diversity since it is quite divergent from *Rattus* and *Mus* (family Muridae) but contains an MHC (*MhcPele*) with loci clearly homologous to the major *H-2* class I and II gene subtypes (16, 17). To understand the evolution of MHC class I loci, we have initially focused upon the transmembrane domain (TM)-encoding exon (exon 5). This region exhibits relatively low allelic polymorphism and the sequence of the TM is distinctive of various class I subtypes in *H-2* and *HLA* systems (5, 6, 18–22), perhaps reflecting unique biological functions associated with each subtype. These features have formed the basis for categorizing class I genes of *P. leucopus*—based on exon 5 sequences: *Pele-A* genes correspond to *H-2K,D,L,Q* genes, and six *T* genes; *Pele-B* genes correspond to several *Tl* region genes (*Tl-3*, *T6*, and *T16-18*); and *Pele-C* genes correspond to *Hmt* region gene *H-2M1* (ref. 16; *H-2* nomenclature from ref. 23). Interspecies homology of orthologous MHC loci (e.g., *H-2D* versus *Pele-A52a*) is greater than the homology between paralogous loci within a species (e.g., *H-2D* versus *H-2T3*, and *Pele-A52a* versus *Pele-B40b* genes; ref. 16).

In this report, the nucleotide sequence† of exon 5 from a large number of *Pele-A* genes are presented. By comparison with class I genes from different species, we show that short-tract duplications within this exon are a common feature of mammalian class I gene evolution and that the generation of these tandem repeats may be mediated by sequences homologous to hypervariable minisatellite repeats and the λ *chi* site. We further show that in *P. leucopus* genes encoding each TM domain size are ubiquitously expressed.

MATERIALS AND METHODS

DNA Sequencing and Comparisons. The isolation and initial characterization of 40 genomic clones harboring 43 *Pele-A*, *-B*, and *-C* genes has been described (16). *Pele-A* gene exon 5 sequences were determined by automated dideoxynucleotide sequencing at the (University of California, Los Angeles) Sequenator Core Facility using plasmid subclones of *Pele-A* gene genomic clones. *H-2*, *RT1*, *Hm1.6* (a class I gene from Syrian hamster), and *ChLA-B1* sequences were drawn from refs. 15, 18, 20, 22, and 24–28. Sequence comparisons were facilitated by programs on the University of Wisconsin software package (29).

Polymerase Chain Reaction (PCR) Analysis. Genomic DNA (250 ng), phage DNA (1 pg), or cDNA (2.5 ng) was amplified using 17- or 22-base-pair (bp) oligonucleotides primers and AmpliTaq DNA polymerase according to the recommendations of the supplier (Perkin–Elmer Cetus). The primers were as follows: ex4, ACCTTCCAGAAGTGGGC; 52a1, CCT-

Abbreviations: MHC, major histocompatibility complex; TM, transmembrane.

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†The sequences reported in this paper have been deposited in the GenBank data base (accession nos. M33983 and M59218–M59226).

CAGTCCACTGTCCCCATC; 52a2, CTCCTCCTCCACCACACAACA; 11b1, CCTCAGTCCATGGTCACCATT; 11b2, CCTCCTCATCTTCACAACAGC. After initial denaturation, annealing, and extension (95°C for 2.5 min, 55°C for 1 min, and 72°C for 3 min, respectively), 30 or 40 cycles of denaturation, annealing, and extension (95°C for 40 sec, 55°C for 1 min, and 72°C for 1 min, respectively) were performed followed by incubation at 72°C for 5 min. One-fourth of each reaction mixture was then electrophoresed through a 12% polyacrylamide gel [acrylamide/*N,N'*-methylenebisacrylamide, 30:1 (wt/vol)] and the DNA was visualized by ethidium bromide fluorescence. Bacteriophage λ DNA and genomic DNA from an individual inbred (f10) *P. leucopus* was isolated using standard techniques (30). For mRNA analysis, cDNAs were prepared from polyadenylylated RNA derived from tissues of an individual inbred (f8) *P. leucopus* using Moloney murine leukemia virus reverse transcriptase and oligo(dT), essentially as described (31).

RESULTS

Interspecies Comparison of *Pele-A* Gene Exon 5 Sequences. Exon 5 sequences from *Pele-A*, *RT1*, and *H-2* genes were compared to a consensus exon 5 sequence derived from *H-2* class I genes (Fig. 1*a*). The most striking feature of this comparison was the presence of direct repeats in several genes that increase TM lengths by 6 amino acids. The sequences in shaded boxes (Fig. 1) are to be read twice—i.e., only half of each direct repeat is shown. These short-tract tandem duplications are not unique to *P. leucopus*. They are also observed in *H-2Q1* and in many *RT1* genes. Examination of the *H-2* consensus sequence reveals remnants of a similar duplication in all rodent MHC class I genes (Fig. 1*a*, first and last lines, in bold letters), which is also found in *HLA-B* genes (Fig. 1*b*), implying that its reiteration was an early event in the evolution of the classical transplantation antigens. Due to this "primordial repeat," the duplicated sequences in several genes cannot be unambiguously defined (i.e., the shaded boxes in Fig. 1*a* can be moved to any position within the range demarcated by brackets, to yield identical values of similarity between repeated units in the same gene).

Several observations indicate that at least some of the duplications have arisen independently. (*i*) The same sequence duplicated in *Pele-A* genes is triplicated in a rat gene, RT.BM1 (26). (*ii*) A duplication of 15 bp (rather than 18 bp) is found in several rat MHC class I genes (RT16 in Fig. 1*a*; RT21 and RTIA1, refs. 24 and 25). (*iii*) *H-2Q1* and *RT1.A*^{a} are clearly metalogous loci being found telomeric and centromeric, respectively, of class II genes (27, 32). Thus the comparative analysis of exon 5 sequences implies that duplication events in this region are rather frequent occurrences in the evolution of rodent class I genes.

Independently derived short-tract deletions were also observed in *H-2*, *RT1*, and *Pele-A* class I genes. A total of 5 amino acids are lost in the deletions (12 bp and 3 bp) seen in *Pele-A42c*. A 13-bp deletion found in *H-2Q10* genes is displaced from the *Pele-A42c* deletion by 3 nucleotides, and a 13-bp deletion in a rat MHC class I gene (RTBS.3.3; ref. 27, not shown in Fig. 1) starts 18 bp upstream of the *Pele-A42c* deletion. Unlike these rat and mouse genes, the protein sequence of *Pele-A42c* is not predicted to terminate prematurely to yield a soluble protein.

Interspecies comparison of MHC class I exon 5 sequences supports the hypothesis that gene conversion or segmental exchange is a feature of MHC class I gene evolution giving rise to species-specific DNA sequences and homogenization of multigene families (10, 27, 33). A putative example of this are the nucleotides sequences at positions 96–100 in *Pele-A* genes (Fig. 1*a*). Three *Pele-A* genes that contain an 18-bp duplication (*Pele-A34c*, *-A24*, and *-A38b*) have two different sequences in this region (either TGGTG or AAGAT). Both sequences are observed in several other *Pele-A* genes that do not contain tandem repeats. Thus, either independent duplication events occurred in the evolution of *Pele-A* genes or, more likely, gene conversion events have generated the "patchwork homology" observed in this region.

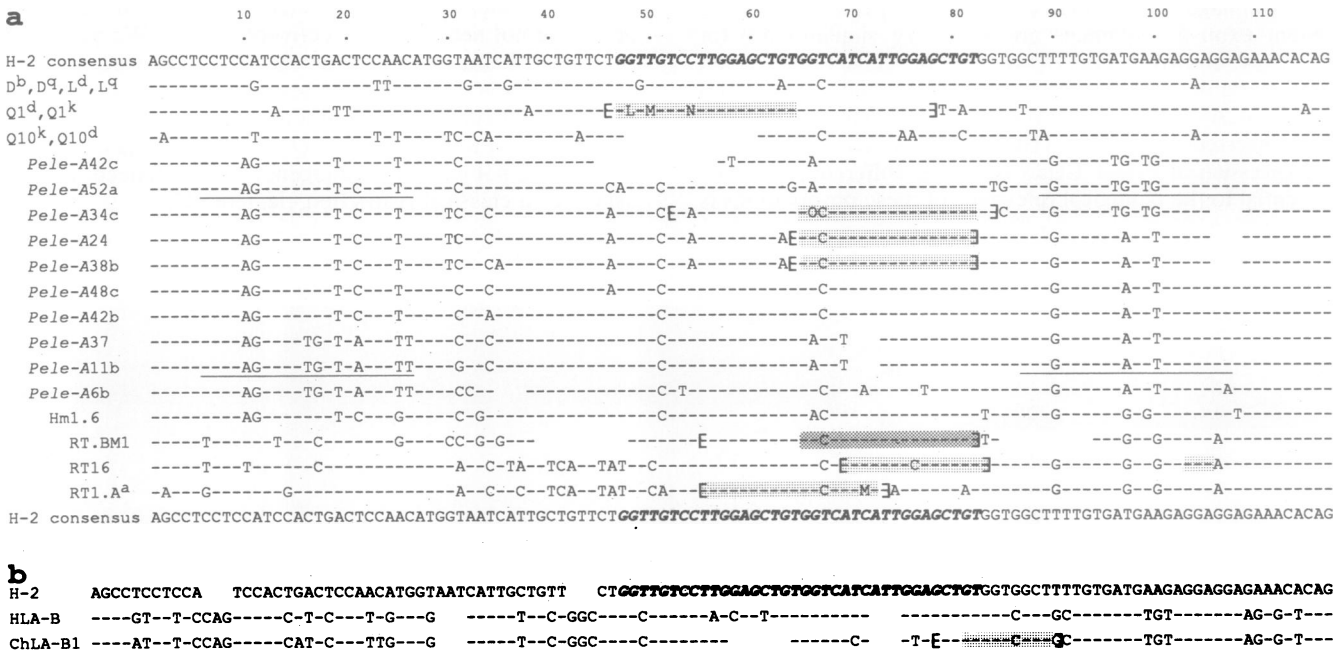

FIG. 1. (*a*) Comparison of mouse, rat, and *P. leucopus* class I exon 5 sequences. Dashes indicate identity to an *H-2* consensus sequence (top line), gaps are indicated by spaces, double repeats are lightly shaded, and triple repeats are heavily shaded. Only the first repeated unit is shown. The letters L–O indicate positions that differ in the repeats: L, G/A (the first repeat has G/, the second has A at this position); M, G/C; N, C/A; O, G/T. Brackets indicate the area in which the duplicated sequence could be placed with the highest percent similarity between repeats. The primordial repeat (see text) is in bold italic letters. Oligonucleotide primers (see Fig. 3) are underlined. (*b*) Comparison of *H-2* and *HLA-B* genes. Consensus sequences of *H-2* "group 1" genes and *HLA-B* genes are from refs. 18 and 22, respectively.

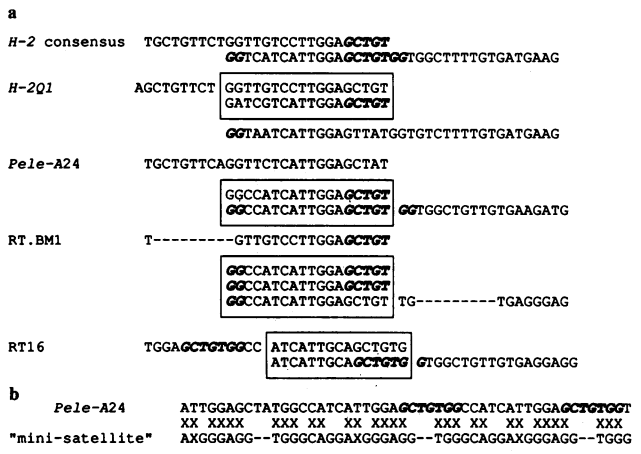

FIG. 2. Similarity of tandem repeats to the λ *chi* site and human hypervariable minisatellite repeats. (*a*) Comparison of exon 5 repeated sequences. Several repeats shown in Fig. 1*a* are compared and include sequences 5′ and 3′ of the repeated units. The first base of the second (and, when present, third and fourth) repeated unit begins the next line aligned under the preceding repeat. *chi*-like sequences (GCTGTGG) are in bold type. Boxed sequences denote the shaded regions depicted in Fig. 1. Dashes indicate gaps inserted to optimize alignment. (*b*) Similarity of exon 5 tandem repeats to human hypervariable minisatellite repeats. A portion of *Pele-A24* exon 5 containing tandem repeats is compared to tandem repeats of a human hypervariable repeat consensus sequence (GGAG-GTGGGCAGGAXG, ref. 34) in which a 2-nucleotide gap has been inserted (GGAGG--TGGGCAGGAXG, where dashes indicate a space).

Homology of Exon 5 Tandem Repeats to the λ *chi* Site and Hypervariable Minisatellite DNA. DNA regions within and adjacent to tandem repeats contain sequences similar to the general recombination site of λ (the *chi* site) and human hypervariable minisatellite DNA (Fig. 2). *chi*-like sequences in the fifth exon of MHC class I genes (GCTGTGG) differ by a 1-bp deletion from the *chi* site (GCTGGTGG, ref. 35) of bacteriophage λ (Fig. 2*a*). The tandem repeats observed in rodent exon 5 sequences are also very similar (75% for *Pele-A24*, shown in Fig. 2*b*) to tandem repeats of a human hypervariable repeat core consensus sequence (GGAG-GTGGGCAGGAXG, ref. 34) in which a 2-nucleotide gap has been inserted (GGAGG--TGGGCAGGAXG).

Expression of *Pele-A* Genes Encoding Different-Size TMs. Essential to the biological relevance of these observations is whether or not such TM length-altering duplications and deletions occur in nonexpressed (and, therefore, unselected) genes. The most abundant class I mRNAs in *P. leucopus* tissues are detected using probes specific for *Pele-A* genes; most *Pele-B* and *-C* genes are weakly expressed if at all (unpublished observations). Oligonucleotide primers based on the sequence of *Pele-A* genes were, therefore, used to examine class I expression by the PCR. The location of the primers and predicted size of PCR amplification products (based on the sequence of *Pele-A52a*) are shown in Fig. 3*a*. The 52a1/52a2 primer pair was first tested on *P. leucopus* genomic DNA and *Pele-A* gene clones (Fig. 3*b*). In genomic DNA from inbred *P. leucopus*, bands of three sizes were observed using 52a1 and 52a2 primers: ≈120, 105, and 90 bp (Fig. 3*b*, lane 1) consistent with the sizes predicted from *Pele-A* gene exon 5 sequences and corresponding to the fragment sizes observed using various *Pele-A* genomic clones as PCR targets (examples from Fig. 1 shown in Fig. 3*b*, lanes 2–4). The 11b1/11b2 primer pair amplified a single fragment (≈105 bp) from genomic DNA and several *Pele-A* genomic clones (data not shown).

The 52a1/52a2 and 11b1/11b2 primer pairs were then used to amplify exon 5-containing portions of cDNAs synthesized using mRNA from a variety of tissues (Fig. 3*c*). In every tissue tested (kidney, liver, spleen, and testes in Fig. 3*c*, and data not shown), the amplified fragments were equivalent in size to those observed using genomic DNA and genomic clone targets. This data indicates that genes encoding each TM size are transcribed. Expression in liver, spleen, and kidney was ≈10-fold higher than in testes, in rough agreement with Northern blot analysis (data not shown). To rule out the possibility that the products were derived from contaminating genomic DNA, a primer derived from exon 4 was employed with the 52a2 primer. The sizes of the PCR products obtained using these primers (≈240, 215, and 200 bp) are in exact accordance with the sizes predicted for MHC class I transcripts with appropriately spliced exons 4 and 5. Thus, the duplications and deletions observed in *Pele-A* genes are not necessarily selectively neutral. We can not at this time exclude the possibility that *Pele-A* genes containing the duplications are pseudogenes with mutations elsewhere that would preclude their function. In this regard, however, it is important to note that *H-2Q1* and the *RT1* genes shown in Fig. 1 do not exhibit pseudogene criteria. Indeed, *RT1.A*^{a} serves as a classical restriction element (27).

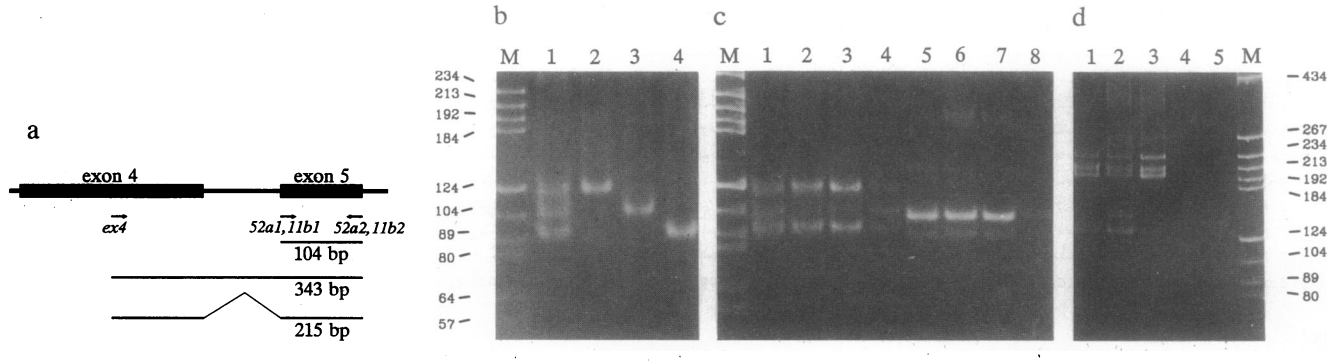

FIG. 3. PCR analysis of MHC class I exon 5 size and expression in *P. leucopus*. (*a*) Location of primers and expected DNA sizes after PCR amplification. Arrows indicate the location of each primer. Sizes of the expected PCR amplification products using 52a1/52a2 or 11b1/11b2 primer pairs (top line) and ex4/52a2 primers on genomic DNA and spliced mRNA (cDNA) targets (second and third lines, respectively) are shown below the gene structure schematic. (*b–d*) Polyacrylamide gel electrophoretic analysis of PCR-amplified products. (*b*) The products of PCR amplification of genomic DNA (lane 1) and three genomic clones (lanes 2–4 contain clones 34c, 52a, and 42c, respectively) using the 52a1/52a2 primers are shown. Markers were pBR322 digested with *Hae* III (lane M in b–d) and the sizes (in bp) are given at the right and left. (*c*) Amplification products of cDNA derived from kidney (lanes 1 and 5), liver (lanes 2 and 6), spleen (lanes 3 and 7), and testes (lanes 4 and 8) using 52a1/52a2 primers (lanes 1–4) or 11b1/11b2 primers (lanes 5–8). (*d*) Amplification of cDNA derived from kidney, liver, spleen, and testes mRNA (lanes 1–4, respectively) by using the ex4/52a2 primers. Lane 5 is a negative control (no cDNA in the PCR).

DISCUSSION

This report describes the occurrence of short-tract duplications and deletions in rodent MHC class I gene exon 5 sequences. In several instances, they arose independently during the course of evolution, suggesting that the TM-encoding exon of MHC class I genes is a mutational or recombinational hot spot.

Mayer *et al.* (15) noted a 9-bp perfect repeat in the fifth exon of *ChLA-B1*, a chimpanzee MHC class I gene. The sequence of this repeat overlaps with the repeats shown in Fig. 1*a* by 7 bp or less (Fig. 1*b*). Though observed in another *ChLA-B* allele (*ChLA-B39*), this 9-bp repeat does not appear to be found in any *HLA-B* genes (22). Three possibilities exist that could account for this. (*i*) It may be that not enough *HLA-B* alleles have been sequenced. (*ii*) The event giving rise to this duplication may have occurred recently in primate evolution, subsequent to the *Pan/Homo* split. (*iii*) The 9-bp repeat may in fact represent an ancient polymorphism that was lost during the course of human evolution. In any case, it is clear that similar duplication events occurred independently in both primate and rodent species and corroborate the hypothesis that the fifth exon of MHC class I genes are (or have been) a hot spot for such mutations. It will be important to determine whether MHC class I TM length variation in rodents represents an allelic polymorphism (as it apparently does for *ChLA-B* alleles) or whether in *Peromyscus* and *Rattus* short tract duplications in exon 5 are peculiar for all alleles of a particular loci (as appears to be the case for the *H-2Q1* gene).

The functional ramifications of length variability in the MHC class I TMs is currently unknown. Several genes that display duplicated sequences also possess regions that seem deleted when compared to other MHC class I exon 5 sequences. This suggests that MHC class I proteins function optimally with transmembrane domains of about 40 amino acids. The fact that exon 5 length has been conserved between mammals and chickens lends support to this hypothesis (1, 36–38).

Significantly, sequences conspicuously similar to hypervariable repeat satellite sequences and the *chi* site of λ are present within and adjacent to repeated units, perhaps rendering the DNA in these areas more susceptible to tandem duplications, deletions, or gene conversion events. Wheeler *et al.* (39) demonstrated microconversion (short-tract gene conversion) during meiosis of nonallelic *H-2* class I genes transfected into yeast. The endpoints of several conversions (but not all) were close to sequences similar to the *chi* site. Furthermore, a recombination hot spot in the murine I-A region has been found to map close to a repeated sequence of hypervariable satellite-like and *chi*-like sequences (40). Along these lines, hypervariable minisatellite DNA appears to stimulate homologous recombination in human cells (41) and the polymorphisms of hypervariable satellites are often due to germ-line amplification of repeated units probably by unequal exchange during meiosis (42).

Other recombination mechanisms exist that do not involve minisatellite- or *chi*-like sequences and may be operative in the generation of short-tract repeats. For example, a slippage event during replication was proposed to explain the repeat in *ChLA-B1* (15). It is not clear whether such a mechanism would be influenced by hypervariable minisatellite- or *chi*-like sequences.

Similarity between the hypervariable repeat core consensus sequence and a region of the TM-encoding exon suggests that the former sequences were recruited early in the phylogeny of MHC class I genes. If hypervariable repeat satellite-like sequences are imbedded within and intrinsic to the evolutionary history of MHC class I gene exon 5 sequences, then in a sense "selfish" DNA may have been a fundamental contributor to cellular immunity.

We thank Drs. Rita Effros, Jonathan Braun, and Mitchell Kronenberg for their helpful comments on the manuscript. This work was supported by U.S. Public Health Service Grants AG04419-06 and AG08936-01 from the National Institute on Aging. M.D.C. was supported by U.S. Public Health Service Training Grant T32 AG00121-01A1.

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