

Cloning and Expression of Class I Major Histocompatibility Complex Genes of the Rat

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Summary

Little is known about the organization of class I genes in the rat although there is prima facie evidence that it is distinct from that of the mouse. We report the cloning of 61 nonclassical rat class I genes into cosmid clusters with a total mapped length of 1,264 kb. It is certain that the total number of class I genes in the rat must exceed this number. From restriction maps it is possible to identify substantial regions of duplication. By transfection of cosmids into mouse L cells, it has been possible to demonstrate at least seven different nonclassical rat class I genes that are expressible on the cell surface. Crossreaction of a single mouse monoclonal antibody with all of these class I molecules is consistent with sequence homogenization within the rat nonclassical system. Attempts to find rat homologues of the mouse *Tla* genes by crosshybridization of rat cosmids with a range of different *TLa*-specific probes were unsuccessful, suggesting that this large group of divergent class I genes is absent or nearly so from the rat. The large number of class I genes in the rat appears to have arisen by expansion of genes more closely related to the classical sequence.

The general outline of rat MHC structure was established by classical genetic methods to be similar to that of the mouse, with genes specifying class I glycoproteins distributed on either side of a region specifying class II glycoproteins (1, 2). For the time being, only these two members of the same subfamily (*Murinae*) are known to have MHCs organized in this way (3). The apparent close similarity between MHCs of these two closely related species has been emphasized recently by the cloning of the rat class II region into overlapping cosmid clusters showing a high degree of concordance in both genetic and functional organization with the *A* and *E* regions of *H-2* (4). At a superficial level, the genomic organization of class I seems also to be similar in the two species, since analysis of Southern blots of rat genomic DNA with promiscuously hybridizing mouse class I probes has shown that the vast majority of rat class I genes map to the (conventional) *D* end of the MHC (5). A natural conclusion has been that these abundant *D*-end genes represent the rat homologues of *D*, *L*, *Qa*, and *Tla*, while the few class I genes at the *K* end presumably represent the homologues of *H-2K* region genes. Three observations complicate this conclusion. First, the rat expresses no functional analogue of *H-2D* or *H-2L* at the right-hand side of its MHC. The key properties of classical class I MHC molecules, namely high and ubiquitous expression, extensive polymorphism, strong alloantigenicity, and, above all, the ability to act as a restriction element for foreign antigens, are all invested in

the product of *RT1.A*, a class I gene mapping to the left of the rat MHC (6), in the position apparently homologous to that of *H-2K*. Second, the sequence of an *RT1.A* rat classical class I allele shows a closer similarity to other rat class I genes than to any mouse class I gene, including *H-2K* alleles (7), and, among mouse comparisons, is closest to the *K*-end nonclassical gene *K1^k* (8). Finally, when *Tla* region-specific probes of mouse origin were used to analyze genomic Southern blots of rat DNA, little or no crosshybridization was found (9). Thus, the most abundant and most distinctive family of genes in *H-2* was apparently missing from the rat MHC.

The impression left by these three observations is that the resemblance between the genomic organization of rat and mouse class I genes may be largely superficial, in contrast to the case of class II. To understand this complicated situation better, we have begun to analyze the genomic organization of the rat class I system in detail, extending the preliminary work reported earlier (10). This paper describes the analysis of a rat genomic cosmid library using mouse class I probes. A total of 61 distinct class I genes, most of them organized in clusters, have been identified from a single haplotype in this study, and there are reasons to believe this number is not exhaustive. It is therefore clear that the rat class I system is substantially larger than that of the mouse. In addition, despite the large number of class I genes present in the rat, we have confirmed that class I genes corresponding to typical mouse *Tla* are essentially absent from this close relative.

Both of these findings suggest that class I gene organization is unexpectedly dynamic in short periods of evolutionary time, and indicate that the divergent sequence of *Tla* genes probably does not imply a distinctive and highly evolved function of great antiquity.

Materials and Methods

Cosmid Library Construction. The cosmid library was described previously (4, 11). Briefly, genomic DNA was prepared from a PVG-RT1^{av1} strain rat and partially digested with the enzyme MboI. This DNA was size selected (35–45 kb) on a sucrose gradient and ligated into the BglII site of the cosmid vector pTL5 (12) (Fig. 1). 5×10^5 clones were plated on the host bacteria, 490A.

Plasmid and cosmid DNA was prepared either by cesium chloride/ethidium bromide banding or by using a modified form of the alkaline lysis mini-prep method. The probe insert was released from the vector by restriction enzyme digestion and separated by gel electrophoresis. Insert bands were either eluted from the gel or were directly labeled in gel slices (13). Probes were labeled using [³²P]dCTP (Amersham) by nick translation or hexanucleotide priming (13). Southern blots were made onto nylon filters (GeneScreen Plus; DuPont, or Hybond-N; Amersham). Many gels were not transferred but were used in hybridization as “unblots” (14). Hybridization conditions were standard (15). All restriction enzymes were used under conditions recommended by the suppliers.

Probes Used to Isolate and Analyze Rat MHC Class I Genes. The cosmid library was screened with the mouse class I probe, pH2-IIa. This cDNA contains exons 4–8 of a class I gene (16) and hybridizes to most if not all mouse class I genes (17–20) and to numerous class I genes of other species, including rat (5, 9, 20–24). Characterization of the cosmid clones isolated involved several mouse class I probes. In addition to pH2IIa, two probes that hybridize to all mouse class I genes were used: pH2III (16), a cDNA that includes exons 2 and 3 of a class I gene; and Pst 8.8, a 5' flanking probe (25). *Tla* gene-specific probes were also used. Three genomic probes derived from the *T13^c* gene were used: pTLA.4 (including exon 2 and half of exon 3) and pTLA.5 (exons 5–6) detect 1–2 and 3–4 *Tla* genes, respectively (dependent on MHC haplotype) (26), and pTLA.3 (exon 3), which has essentially the same specificity as pTLA.4 (D. Fisher, personal communication); 5' *Tla* is a 5' flanking probe from *T1^b* and hybridizes to 1–3 *H-2* class I genes (depending on MHC haplotype) (26).

Mapping Cosmid Clones. The protocol was based on the partial digest method for mapping λ phage and cosmid clones (27, 28). Cosmids were linearized with ClaI or Sall, which have single restriction sites in the vector (Fig. 1) and rarely cut in the insert. Aliquots of each clone were partially digested with each mapping enzyme and resolved by electrophoresis through separate 0.4% agarose gels. The gels were dried (14), hybridized with the appropriate pair of end probes (as described in Fig. 1), and autoradiographed. The radioactive bands were digitized and their molecular weights calculated from the markers using computer interpolation. The “ladder” of partial digest products detected was used to generate the restriction maps, as described by Rackwitz et al. (27). A small number of cosmids had two or more sites for both ClaI and Sall, and could not be mapped by the procedure described here.

Transfection and FACS[®] Analysis. This was carried out as described (29, 30). Briefly, thymidine kinase-deficient mouse L cells were cotransfected using the calcium phosphate precipitation method, with cosmid DNA and a plasmid pTKA carrying the HSV Tk gene (31). Transfectants were selected by growth in HAT medium. Three groups of anti-rat class I antibodies were used;

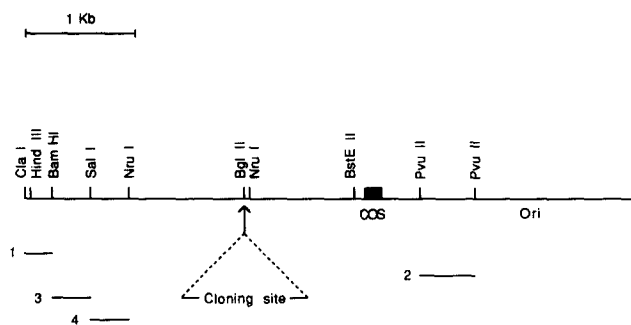


Figure 1. Restriction enzyme map of the cosmid vector pTL5. Also shown are the fragments of pTL5 used as probes for partial digest mapping of cosmid clones. Most clones were linearized with ClaI, in which case probes 1 and 2 were used for mapping. Other cosmids were linearized with Sall and were mapped with probes 3 and 4.

(a) anti-RT1.A^{av1}, a cocktail of six rat alloantibodies (AFRC MAC 161, R2/10P, R3/47, R2/15S, AFRC MAC210, and YR1/100) against different epitopes of the RT1.A^{av1} classical class I molecule (32, 33) (G. W. Butcher, unpublished data); (b) anti-RT1.C^{av1}, a cocktail of six rat alloantibodies (AFRC MAC104, 105, 106, 107, 108, and 110) against the RT1.C^{av1} nonclassical class I antigen (33, 34) (G. W. Butcher, unpublished data); (c) MRC OX18, a mouse mAb against a monomorphic determinant on RT1.A (35) and other rat class I antigens (11, 36, 37). The rat antibody R1-21.2 (38) binds to several mouse classical class I antigens, including H-2D^k and H-2K^k expressed on L cells, and is used here as a positive control. The negative control antibody DB3 is a mouse IgG1 specific for progesterone (39). Binding of rat and mouse first-stage mAbs was detected using FITC-conjugated rabbit anti-mouse Ig (Dako, Bucks, UK). This reagent crossreacted strongly between mouse and rat Ig. Labeled cell populations were analyzed using a FACS 420[®] or FACScan[®] (Becton Dickinson & Co., Mountain View, CA). Cells expressing rat class I molecules were selected by FACS[®] sorting to achieve homogeneous high expression. In some cases, expressing cells were also cloned by limiting dilution.

Immunoprecipitation of Antigens from Transfectant L Cells. L cell transfectants were surface radio-iodinated by the glucose oxidase-lactoperoxidase method. After preclearing, antigens were precipitated either by antibodies conjugated directly to Sepharose immunoabsorbents (MRC OX18, R1-21.2), or else by a two-stage method using anti-Ig reagents conjugated to Sepharose (anti-RT1.C^{av1}-specific AFRC MAC antibodies). Samples were run in 5–20% linear gradient SDS-polyacrylamide gels and autoradiographed for 28 d at -70°C .

Results

Identification and Mapping of Cosmids Containing 61 Rat Class I Genes. The mouse 3' class I probe, pH2-IIa, detected 202 colonies in the PVG-RT1^{av1} genomic cosmid library. Of these, a total of 115 were recovered for analysis, the remainder being lost at rescreening. 95 clones were fully mapped for the sites of at least three restriction enzymes. The remainder were unsuitable for mapping, either because they contained multiple Sall and ClaI sites (four cosmids), or because of persistent deletion (16 cosmids). The clones were then organized into 21 groups of connected genomic DNA on the basis of their restriction maps, represented in Fig. 2. Since multiple class I genes were often found in these groups, they were

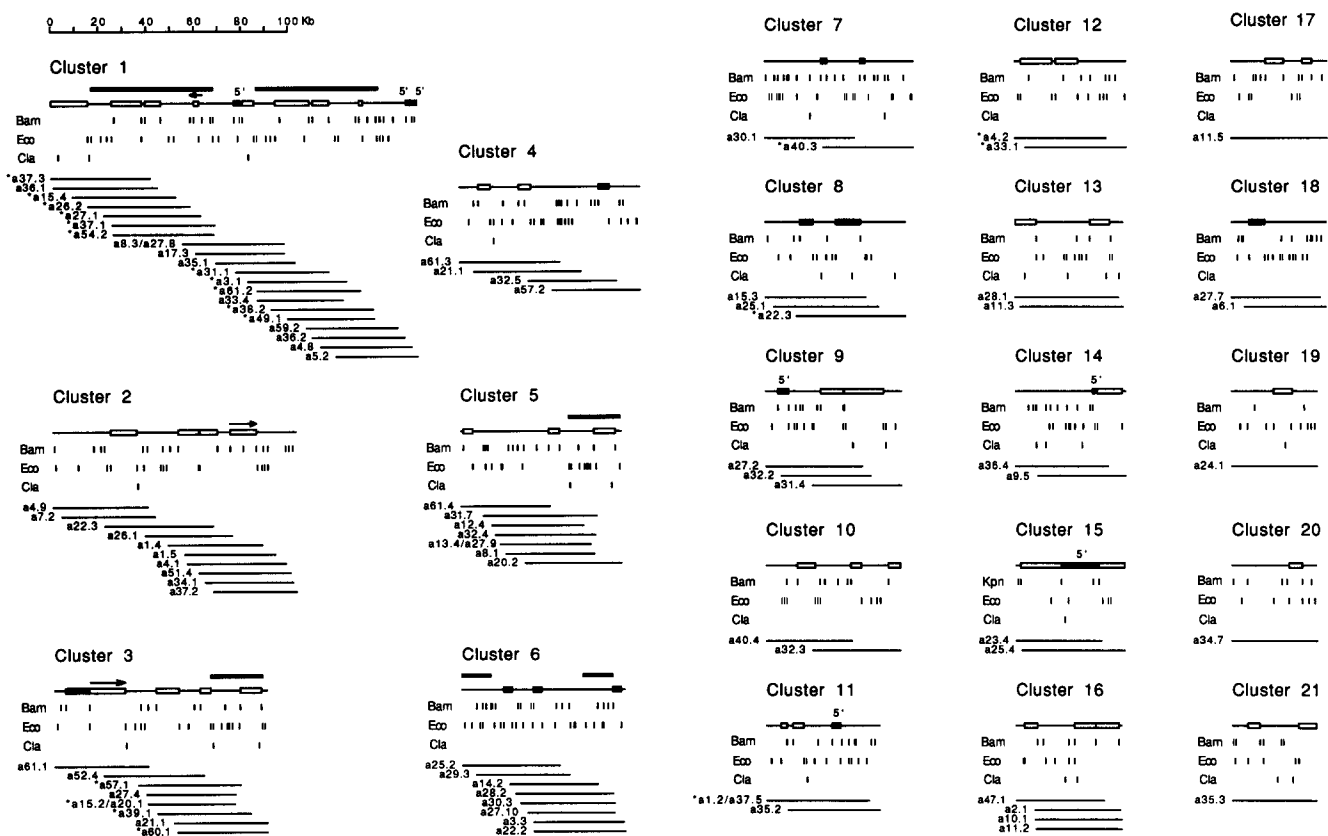


Figure 2. Restriction maps and positions of cosmid clones containing rat class I genes. The relationships between cosmids that contain overlapping sections of rat DNA are indicated by the arrows beneath the composite restriction map. The position of class I genes and gene segments is shown above the restriction map: open boxes represent fragments hybridizing to both 5' and 3' gene probes; fragments that hybridized to the 3' gene probe only are shown by filled boxes; diagonally hatched boxes show hybridization to the 5' gene probe only. In some cases the orientation of a gene could be deduced from the hybridization data, shown as an arrow above the gene. Gene duplications are shown as bars above the genes: within cluster 1 (filled bars), cluster 6 (vertical hatched bars) and between clusters 3 and 5 (open bars). Asterisks by the cosmid name indicate that L cell transfectants of these clones express rat class I molecules (see text).

called clusters. The figure shows sites for the restriction enzymes BamHI, ClaI, and EcoRI, (except in cluster 15, mapped for KpnI in place of BamHI). Most of the clones were also mapped for HindIII and KpnI sites (these data are available upon request). Also shown in Fig. 2 are the positions of the detected rat class I genes. These were identified using the two mouse probes pH2IIa and pH2III, which derive from the 3' and 5' ends, respectively, of a class I cDNA (16). The probes were hybridized to BamHI and EcoRI digests of each cosmid clone. Some restriction fragments hybridized to only one of the two cDNA probes. If it was apparent from the maps that an immediately adjacent fragment hybridized to the other cDNA probe, it was assumed that the restriction enzyme separated the 5' and 3' ends (Fig. 2, arrows). Otherwise, fragments that hybridized to one cDNA probe but not the other were presumed to be either divergent genes or isolated gene segments.

Gene duplications are apparent both within and between some of the clusters by examination of the restriction maps (Fig. 2). Thus, cluster 1 possesses an internal duplication involving ~50 kb of DNA and three genes/gene segments, whereas the duplicated region within cluster 6 is smaller (~12

kb) and involves no detected class I genes. Between clusters 3 and 5 there appears to be a duplication of just over 20 kb. There are some similarities also between clusters 3, 4, and 11 (not shown), but this is based on few restriction sites and may be fortuitous.

Not presented here are the restriction maps of four cosmids that had regions of identity with sections of clusters 1, 2, 3, and 14. The maps of these clones suggested that they may be double ligation artifacts generated during construction of the library.

Identification of Expressible Rat Class I Genes. All 115 cosmid clones were transfected into mouse L cells. Stable transfectants were assayed by FACS[®] for cell surface expression of products recognized by mAbs to rat class I antigens. Allele-specific antibodies against the RT1.A^{av1} and the RT1.C^{av1} products, and the mouse antibody MRC OX18, which detects a monomorphic rat class I epitope (11, 36), were used. Transfectants of 21 cosmid clones caused surface expression of products detected by the mouse anti-rat class I mAb MRC OX18 (Fig. 3 and Table 1). Of these, 11 also expressed products detected by the rat anti-RT1.C^{av1} alloantibodies. No transfectant bound the allele-specific mAbs directed against

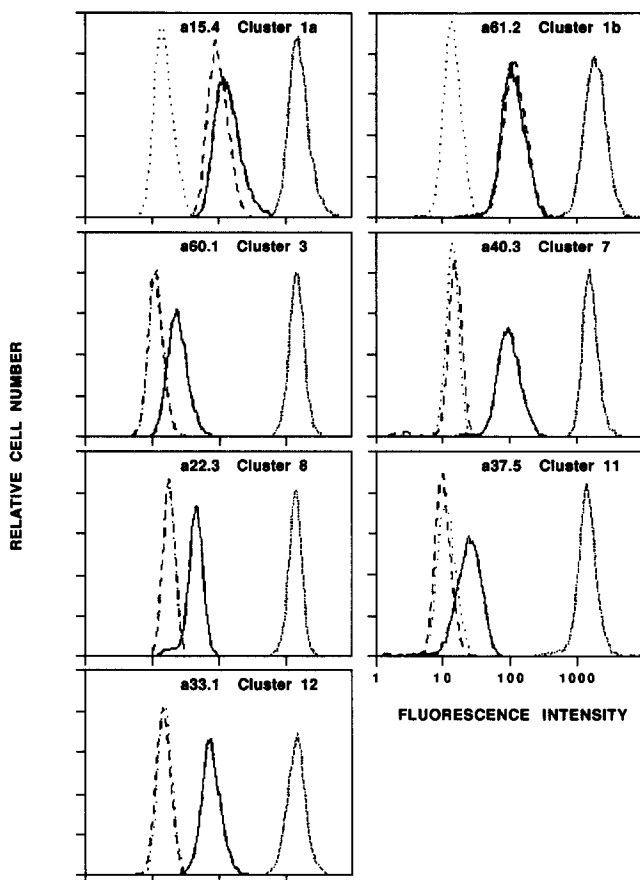


Figure 3. Flow cytometric analysis of L cells transfected with seven cosmid clones. Mouse L cells stably transfected with the indicated cosmid DNA were analyzed for expression of rat class I antigens using the mAbs MRC OX18 (—) (specific for a monomorphic determinant on rat class I antigens) and a cocktail of six mAbs specific for RT1.C^{av1} (---) (AFRC MAC 104–110). R1-21.2 (·····) detects class I molecules expressed by L cells, and DB3 (· · · ·) is an antiprogesterone antibody.

epitopes of the classical RT1.A^a molecule (data not shown). All the clones expressing RT1.C^{av1} specificities were transfectants of cosmids from cluster 1. However, the cosmids generating these expressed products come from both ends of the cluster: thus, a15.4 and a61.2 (Fig. 3, *a* and *b*), for example, clearly cover different regions of cluster 1. These data suggest that at least two genes whose products express RT1.C epitopes must be present in cluster 1. Transfectants of another 10 cosmids were recognized by MRC OX18, but not by either of the pools of rat alloantibodies (Fig. 3, *c–g* and Table 1). These cosmids derived from five different clusters with unrelated restriction maps and therefore certainly contain distinct expressible genes. Preliminary results further suggest that cosmid a47.1 (cluster 16) may also determine the expression of a sixth class I gene detected only by MRC OX18 (data not shown).

In some cases, cosmids that appeared to possess the same genes did not show the same expression pattern after transfection. For example, the clone a60.1 generated an MRC OX18 signal after transfection, whereas the overlapping clone a21.1

did not. Multiple transfections with both these clones produced the same results making it unlikely that this effect is due to an artifact of the transfection system. This result raises the possibility that such clones are not truly overlapping but show similarity due to an undetected duplication.

In summary, analysis of transfectants revealed at least seven distinct loci capable of expressing rat class I-like products.

Immunoprecipitation of an RT1.C^{av1} Product from Transfectants of Cosmid a15.4. As a preliminary to a more extensive analysis of expressed nonclassical class I genes, we sought information about the nature of the expressed polypeptides by immunoprecipitation. The RT1.C^{av1} antigen is the only serologically recognizable polymorphic nonclassical class I antigen in the rat that has been localized to a specific gene and is therefore of special interest. A transfectant clone, 15.4.3.1.1, expressing high levels of the RT1.C^{av1} product, was obtained by positive FACS[®] selection with MRC OX18, followed by limiting dilution cloning. Class I molecules were immunoprecipitated from these cells after surface radioiodination, using both MRC OX18- and RT1.C^{av1}-specific mAbs. With varying efficiency, three of the six anti-RT1.C antibodies (Fig. 4, lanes E, G, and H) all precipitated a heavy chain of ~42 kD and a presumed β_2 -microglobulin (β_2 m) chain <17.5 kD (Fig. 4; in the gel shown, β_2 m migrated with the gel front). The size of the heavy chain was identical to that immunoprecipitated from rat tissue using the same antibodies (data not shown). MRC OX18 apparently also immunoprecipitated the same molecule, but in addition bound to at least four smaller heavy chain species. This result is currently under further investigation.

Analysis with Mouse Class I Region Probes. A panel of mouse class I region probes was used to analyze the cloned rat genes (see Materials and Methods). Probes were hybridized to dot blots of a canonical set of the cosmids described in the legend to Fig. 5. The 43 cosmids chosen were the minimum required to cover the entire cloned region. All clones hybridized with the 3' coding sequence probe pH2IIa (data not shown). Some clones, however, did not hybridize to the 5' coding sequence probe pH2III (not shown) or to a 5' flanking sequence probe, pst 8.8 (Fig. 5). The hybridization of four *Tla* (*T13^c*) probes from the 5' coding (pTLA.3, pTLA.4), 5' flanking (5' Tla), and 3' coding region (pTLA.5) is shown in Fig. 5. With one exception, none of the cosmids hybridized to more than one of the *Tla* probes and most failed to hybridize at all. At higher stringency washes (0.1× SSC, 65°C), the *Tla* probes were completely removed (data not shown), while the Pst 8.8 and pH2IIa probes still hybridized strongly (Fig. 5). One cosmid, a23.4 (cluster 15) hybridized at low stringency to both the pTLA3 and 5' TLA probes (row 3, clone 10). However, neither this cosmid nor a25.4, which derives from the same cluster, hybridized to any of the other coding region *Tla* probes. Analysis with probes 48.1BS and 48.1RS from *T8^c* (9) similarly failed to hybridize strongly to cosmids in any cluster; however, clear but weak hybridization was seen on gene 2 of cluster 12 (data not shown), which also hybridizes strongly to pH2III. There was thus no clear identification of rat *Tla*-like genes.

Table 1. Transfectants of 21 Cosmids Show Cell Surface Expression of Rat Class I Products

Stained by	Cluster	Cosmid transfectants
MRC OX18 only	3	a57.1*, a15.2, a39.1, a60.1
	7	a40.3
	8	a22.3
	11	a1.2, a37.5
	12	a4.2, a33.1
MRC OX18 and anti-RT1.C ^{av1}	1	
	(Left side) [‡] (Right side) [‡]	a37.3, a15.4, a26.2, a27.1, a37.1, a54.2 a31.1, a3.1, a61.2, a38.2, a49.1

* The name of the cosmids used in transfection, and the clusters from which they derive are given.

[‡] Left and right sides refer to the location of the clones in cluster 1, as shown in Fig. 2.

Discussion

The data presented here describe cosmid cloning and initial characterization of nonclassical class I genes from the *RT1^{av1}* haplotype of the laboratory rat. 61 class I genes have been identified in a total of 1,264 kb cloned DNA (Fig. 2). Thus, the *RT1^{av1}* class I region is about double the size of the mouse class I regions that have been studied at the

molecular level (17, 18). This factor may be even greater since the data described here probably underestimate the true size of the rat class I region. For instance, we were unable to detect the classical class I product RT1.A in this study, although we have already reported cloning of this gene at the cDNA level and its expression on transfected L cells (7). An oligonucleotide probe made from this cDNA failed to hybridize to any of the cloned genes at high stringency (C. Rada and J.C.H. unpublished data), and hence, we assume *RT1.A^a* was not recovered from the library. We also failed to find a rat homologue of the mouse *H-2M3* gene in our existing class I cosmid set using a mouse probe known to hybridize at high stringency to three bands in rat genomic DNA (40). The rat homologue of *H-2M3*, like *RT1.A^a*, has been identified and sequenced at the level of cDNA (41). Furthermore, several cosmids that we could not map for technical reasons were obtained during screening, and these constitute at least one additional cluster of class I genes.

It is apparent from the maps in Fig. 2 that *RT1^{av1}* class I genes are arranged in dense clusters and many are located in recently duplicated segments. This structural organization is superficially reminiscent of the mouse class I region (17, 18). In contrast, the class I genes of humans (42) and the mole rat (23) are less closely grouped together, and recent gene duplications are less obvious. Moreover, the mouse Q region, in at least one strain, contains duplicated genes encoding Qa-2 antigens (43). In *RT1^{av1}*, cluster 1 (Fig. 2) also contains clustered, duplicated class I genes of which at least two genes encode antigens bearing RT1.C determinants (Table 1 and Fig. 3). Immunological and biochemical similarities between Qa and RT1.C antigens have been discussed previously (44). However, the arrangement of genes in cluster 1 apparently involves a repeat unit of three genes, rather than the characteristic two gene repeat of the mouse Q region, clearly reflecting a distinct history. This precludes a simple alignment of cluster 1 with the mouse Q region.

It is probable that the majority of genes reported here derive from the *RT1.C* region. Analysis of recombinant strains has shown that most class I genes (at least 80%) map to *RT1.C*

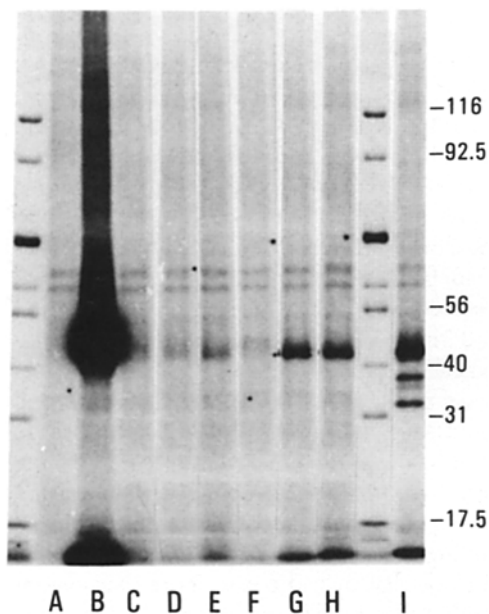


Figure 4. Immunoprecipitation of rat class I antigens from L cells transfected with cosmid a15.4. L cell transfectants were surface labeled using ¹²⁵I and immunoprecipitated with the following antibodies: lane A, negative control (normal mouse serum); lane B, R1-21.2; six C-H mAbs against RT1.C^{av1}: lane C, AFRC MAC 104; lane D, AFRC MAC 105; lane E, AFRC MAC 106; lane F, AFRC MAC 107; lane G, AFRC MAC 108; lane H, AFRC MAC 110; lane I, MRC OX18. The immunoprecipitates were resolved on a 5–20% linear gradient SDS-polyacrylamide gel with ³⁵S markers of the indicated molecular weights. The dried gel was autoradiographed for 28 d at –70°C.

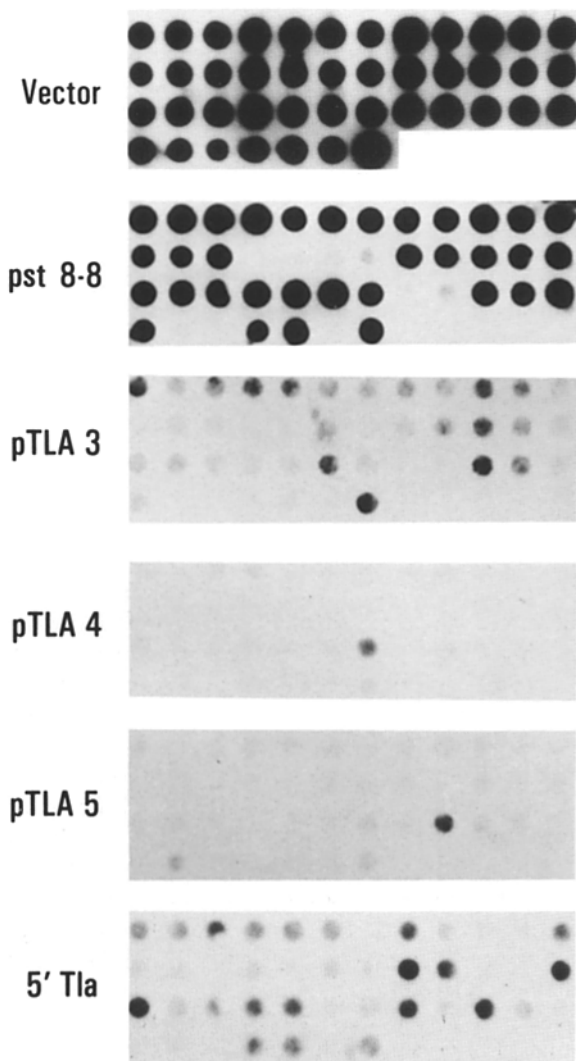


Figure 5. Hybridization of cosmid DNA to mouse class I MHC probes. A canonical set of cosmids was designed from the clusters shown in Fig. 2. The 43 clones used are listed in order below. Cosmid DNA was applied to replicate nylon filters using a dot blot apparatus. After hybridization with the indicated probes, high stringency washes were as follows: $0.1\times$ SSC 68°C for "vector" and "Pst 8-8", $1\times$ SSC at 68°C for the others. Exposure for autoradiography with enhancing screens was for 4–16 h. The cosmid clones analyzed were organized into four rows: row 1: a37.3, a37.1, a17.3, a61.2, a5.2, a4.9, a22.3, a37.2, a61.1, a57.1, a60.1, a61.3; row 2: a57.2, a61.4, a20.2, a25.2, a22.2, a30.1, a40.3, a27.2, a31.4, a15.3, a22.3, a40.4; row 3: a32.3, a1.2, a35.2, a4.2, a33.1, a28.1, a11.3, a36.4, a9.5, a23.4, a25.4, a47.1; row 4: a11.2, a27.7, a6.1, a11.5, a24.1, a34.7, a35.3.

(5, 45), and we can formally localize cluster 1 to this region since expressed products of genes in this cluster were detected with C region-specific alloantibodies. Further mapping awaits the isolation of appropriate low-copy probes from the cloned regions. Refinement in the definition of the C region has come from recent studies of recombinants and deletion mutants in the *RT1^I* haplotype (45). Assuming that it is possible to relate the *RT1^{av1}* genes described here to the *I* haplotype, we should be able to determine the relative order of many of the class I genes cloned. However, we were unable

to detect any relationship between our material and the few cloned *RT1.C* region genes from other haplotypes (45, 46), by comparing the available restriction maps.

The capacity of the cloned genes to encode cell surface products was assayed by FACS[®] analysis of transfectants, using mAbs to monomorphic and polymorphic portions of rat class I antigens. By this means seven class I antigens were identified (Fig. 3 and Table 1). As discussed above, two loci in cluster 1 encode antigens bearing determinants for the *RT1.C^{av1}* region. The antigens encoded by the other five loci were identified only by the xeno antibody MRC OX18, which detects a monomorphic rat determinant. These therefore represent novel class I proteins in the rat. The ability of a single mouse anti rat mAb to detect seven different rat class I genes from different clusters (eight including *RT1.A^a* itself) is consistent with the observation that different rat class I cDNA sequences share characteristic features by which they differ from mouse class I sequences (7). In addition to these data, we have evidence that the gene encoding the soluble serum class I protein described by Spencer and Fabre (36) is probably located in cluster 2. We recently described a cDNA that appears to encode this soluble protein (7), and an oligonucleotide probe generated from the distinctive transmembrane segment hybridizes uniquely to gene 4 of cluster 2 (Fig. 2) (unpublished observations). Furthermore, immunoprecipitation from cells transfected with a15.4 (cluster 1) using the MRC OX18 antibody revealed several products in addition to the *RT1.C^{av1}* antigen (Fig. 4). These could be alternatively spliced products, or even proteolytically degraded forms of the *RT1.C* antigen that retain the MRC OX18 epitope but lose the *RT1.C^{av1}*-specific determinants. On the other hand, these products may represent additional class I antigens encoded in this cosmid. Clearly, it will now be interesting to see whether any of the novel class I-like products described here correspond to the T cell-defined *RT1.C* region antigen systems, "CT" (47, 48), and "H" (49).

Analysis of the cloned rat class I genes with mouse probes produced two main findings. First, the majority of genes hybridized to probes defining the mouse classical class I and Q region genes. This includes the genes encoding the serologically detected *RT1.C* products and also seven of the novel class I products defined with MRC OX18. One expressed gene, contained in cosmid a40.3 (cluster 7), does not hybridize to these probes nor to any of the other mouse 5' gene probes used. This suggests that the functional gene within a40.3 is significantly divergent. Second, very few, if any, genes were found with homology to mouse *Tla* genes. We did not detect any cosmid clone that hybridized to more than one of the 5' coding region *Tla* probes used (Fig. 5). This suggests that the hybridization seen with these probes is detecting fragments of genes or noncoding sequences with limited homology to *Tla* genes, and this is supported by the loss of hybridization signal under high stringency washes. This result is probably not due to inappropriate probes used during the original library screening, since pH2IIa derives from a well-conserved portion of class I genes, and hybridizes to all known *Tla* region genes (17, 18, 50). Some putative *Tla*-like

genes could be absent from the library, although it seems unlikely that a region the size of the mouse *Tla* locus would be entirely absent.

A significant number of rat class I cDNA sequences has now been accumulated, and it is clear that the deficit in *Tla*-related sequences is also apparent at that level. The unique exception is the DA (*RT1^{av1}*)-derived *RT.BM1* sequence of Parker et al. (51), which shows a significant relationship with the BALB/c *Tla* gene 37^d. It is proposed on the basis of Southern blot hybridization that the *RT.BM1* gene is duplicated in the rat (51), and we have recently found a cDNA clone in a DA rat cDNA library that is almost identical to *RT.BM1* but differs at the 3' end of the 3' untranslated region (unpublished results). However, this exceptional case proves the rule, since 37^d, although located in the mouse *Tla* region, is more closely related to Q region and classical class I sequences than to typical *Tla* sequences (52). There thus appears to be a remarkable disparity in the representation of class I genes of the *Tla* family between rat and mouse, confirming the original observations of Rogers (9) based on genomic Southern blot analysis. The deficit of *Tla*-related genes in the rat is more striking in that a recent study in the rodent *Peromyscus leucopus*, (53) in which a genomic λ library was analyzed with the same mouse class I probe (pH2IIa) used in the present study, revealed a substantial group of class I genes with exon 5 sequences typical of mouse *Tla* genes on the basis of the Brorson et al. classification (54).

It has been proposed that the mouse *Tla* locus represents a well-conserved, ancient family diverging from the *H-2/Qa* region genes before mammalian speciation (3). From our results and those of Rogers (9), this theory would require that the majority of the *Tla*-like genes have either diverged extensively or been lost in the rat (and several other species).

An alternative to this hypothesis is that typical *Tla* genes arose more recently, since speciation of the mouse, and hence would not be clearly homologous to class I genes in other species. In view of the recovery of abundant putatively *Tla*-like genes from *Peromyscus*, a cricetid rodent separated from the murines by ~50 Myr, the first possibility appears more likely. In parallel with the apparent loss of typical *Tla* genes, the majority of class I genes of the rat seem to have arisen after divergence from the mouse by extensive duplication of genes of the classical and Q-related groups.

The recent expansion of the *H-2/Qa* group of rat class I genes may have favored the interlocus segmental sequence exchange documented elsewhere (7). Duplication and segmental exchange in this group prohibit the identification of specific orthologous loci between rat and mouse (7). Nevertheless, some divergent nonclassical class I loci seem to have remained outside this process: a close rat homologue of the mouse *M3* (*Hmt*) (55, 56) gene has recently been identified (41), and the rat *RT.BM1* gene is closely homologous to the mouse 37^d gene (57).

In summary, our data suggest that class I loci are subject to rapid evolutionary changes in number. These changes seem to affect groups of related loci, and presumably arise by duplication and deletion after unequal crossovers. In view of the distinctive characteristics of the *Tla* genes in the mouse, and their probable presence in *Peromyscus*, it is surprising that this group should be one that is apparently absent or greatly reduced in number in the rat, while single genes such as 37^d and *M3* seem to have persisted. Whether this pattern of behavior is governed by natural selection operating through function (as, for example, the *N*-formyl specificity of the peptide binding cleft in H-2M3 [58]) or due to random genetic drift is not yet clear.

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