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29. G_t proteins purified from bovine retina (*24*) were mixed with the lipid vesicles for 10 min on ice before addition of the enzyme. The following protein concentrations were used: p110γ, 10 pM; p110α, 10 pM; $G\alpha_t$-GDP, 2 μM; and $G\beta\gamma_t$, 1 μM. The activated $G\alpha_t$-GDP–AlF_4^- was produced by incubation of Gα-GDP with 20 μM $AlCl_3$ and 10 mM NaF in assay buffer for 1 hour at room temperature (*25*). AlF_4^- alone did not activate the enzyme. The content of Gβγ in the $G\alpha_t$ preparations was estimated to be approximately 2% (mol/mol) by immunoblotting with Gβ antibodies and different amounts of $G\beta\gamma_t$ for calibration. An involvement of βγ impurities in the stimulatory effect of $G\alpha_t$ can be excluded because half-maximal activation by $G\alpha_t$-GDP–AlF_4^- and $G\beta_t$ was observed at concentrations of about 2 μM and 200 nM, respectively.
30. $G\alpha_t$-GTP-γ-S was produced by means of Blue Sepharose (Pharmacia) chromatography (*26*) in the presence of 100 μM GTP-γ-S.
31. Before addition of the enzyme, both Gα proteins were incubated for 1 hour on ice in the presence of 100 μM GTP-γ-S and 5 mM $MgCl_2$, mixed with the PtdIns-containing lipid vesicles, and incubated again for 10 min on ice. Activity was assayed as described (Fig. 3). After separation of extracted lipids by thin-layer chromatography, [^{32}P]PI-3P spots were located with the use of a phosphorimager (Bio-Rad) and were quantitated according to the manufacturer's instructions. In three independent experiments, maximal stimulatory concentrations of $G\alpha_{i1}$–GTP-γ-S (2 nM) and $G\alpha_t$–GTP-γ-S (1 μM) induced 1.5 to 3 times the base-line rate of p110γ activity. Heat denaturation of the Gα proteins (10 min at 100°C) completely abolished this stimulation. GTP-γ-S alone (100 μM) and a separate preparation of $G\alpha_i$-GDP did not stimulate enzyme activity.
32. We thank J. Thorner for unpublished sequence information and A. Ullrich in whose laboratory a partial clone of p110γ was isolated by B.S. This work was supported by grants from Deutsche Forschungsgemeinschaft (SFB 197), the British Council, and Deutscher Akademischer Austauschdienst. B.V. was supported in part by the Belgian NFWO.

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A Gene Outside the Human MHC Related to Classical HLA Class I Genes

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By presenting antigenic peptides to T lymphocytes, major histocompatibility complex (MHC) class I molecules play important roles in the human immune system. Knowledge is limited on the evolutionary history of human MHC class I–related molecules. An expressed class I gene, *MR1*, has now been identified on human chromosome 1q25, outside the MHC. In contrast to other known human divergent class I genes, *MR1* encodes peptide-binding domains similar to those encoded by human leukocyte antigen (HLA) class I genes on chromosome 6 and by nonmammalian classical MHC class I genes. This gene may thus contribute to understanding the evolution of the MHC.

MHC class I molecules present antigenic peptides to $CD8^+$ T cells (*1*). In humans, the HLA class I gene family consists of six members: polymorphic *HLA-A*, *-B*, and *-C*, and oligomorphic *HLA-E*, *-F*, and *-G* (*2*). The functions of HLA-E, -F, and -G molecules are unknown, although HLA-G is thought to play a role in the maternal-fetal interaction (*3*). Several groups of human class I–related molecules—including the CD1 family (*4*), Zn-α2-glycoprotein (Znα2gp) (*5*), MICA (MHC class I chain-related molecule A) (*6*), and a human homolog of the rat neonatal Fc receptor (FcRn) (*7*)—have also been identified. Studies on nonmammalian MHC-related systems suggest a tendency to expand the number of genes in various animals (*8–10*). Even in humans, it is possible that only a fraction of MHC class I–related molecules have been identified to date. To examine this possibility, we have attempted to isolate unknown human class I genes. We now describe a close relative of classical HLA class I genes in the human genome.

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The *MR1* gene was discovered by an approach based on our previous polymerase chain reaction (PCR)–based strategy (*11–13*). A candidate human class I gene fragment was amplified from human genomic DNA with the use of two primers that correspond to the two conserved regions in the α3 domain of class I molecules (*14*). The DNA sequence of this PCR product did not correspond to that of any previously described human class I sequence. Because expression of this DNA sequence was detected by Northern (RNA) analysis (Fig. 1) (*15*), a human thymus complementary DNA (cDNA) library was screened with the PCR fragment, and the nucleotide sequence and predicted amino acid sequence (Fig. 2) of the new gene, *MR1*, were determined. Similar to a typical class I molecule, the MR1 protein comprises a signal sequence, three extracellular domains (α1, α2, and α3), a transmembrane domain, and a cytoplasmic domain. The chromosomal location of *MR1* was determined by fluorescence in situ hybridization (FISH) analysis (*16*, *17*) as the distal portion of 1q25 (1q25.3) (Fig. 3A), outside the human MHC (6p21.3). The location of *MR1* on chromosome 1 was also supported by the results of PCR amplification of the *MR1* fragment from somatic cell hybrid DNA (*18*) (Fig. 3B).

The predicted amino acid sequence of MR1 was compared with the sequences of various class I molecules (Fig. 4). The three extracellular domains (α1, α2, and α3) of a class I molecule can be divided into two structurally distinct components: the peptide-binding symmetrical α1 and α2 domains, which contain characteristic α helices, and the α3 domain, which adopts the structure of the immunoglobulin fold. In the α3 domain, the percentage amino acid identity between MR1 and the HLA class I molecules is similar to that between the HLA class I molecules and either other human divergent class I molecules or nonmammalian class I molecules (Fig. 4).

However, comparison of the functionally important peptide-binding domains (α1 and α2) revealed that MR1 exhibits ~40 to 50% amino acid identity with the classical MHC class I molecules of human, mouse, and nonmammalian (for example, chicken) species in both these domains (Fig. 4). Polymorphic

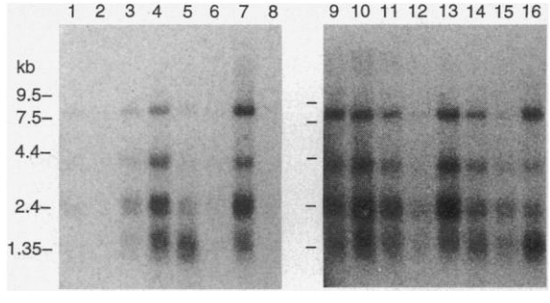

Fig. 1. Northern analysis of *MR1* expression. A 1.15-kb fragment prepared from the *MR1* cDNA clone C7 was hybridized to membranes containing polyadenylated RNA from human heart, brain, placenta, lung, liver, skeletal muscle, kidney, pancreas, spleen, thymus, prostate, testis, ovary, small intestine, colon, and peripheral blood leukocytes (lanes 1 to 16, respectively) (*15*). Size markers (in kilobases) are indicated on the left.

Fig. 2. The predicted amino acid sequence of the MR1 protein. The putative signal peptide is underlined. (▼), the predicted boundaries of three extracellular domains. The arrows above the α3 domain indicate the locations of the two oligonucleotide primers used for PCR. (▽), a potential N-glycosylation site. Of eight amino acid positions in HLA classical class I molecules that are important for interaction with antigenic peptide termini (*27*), MR1 shares two with the HLA-A2 molecule (●). (■), the glutamine residue that is highly conserved within the CD8 binding site in the α3 domain. (□), a potential phosphorylation site (*28*) in the cytoplasmic region. Residue numbers are shown on the right. The percentage sequence identities between MR1 and HLA-A2 in the extracellular domains are shown in Fig. 4; that in the transmembrane and cytoplasmic regions is 22%. The sequence of *MR1* cDNA (clone C7) has been deposited with GenBank (accession number, U22963). Abbreviations for the amino acid residues are: A, Ala; C, Cys; D, Asp; E, Glu; F, Phe; G, Gly; H, His; I, Ile; K, Lys; L, Leu; M, Met; N, Asn; P, Pro; Q, Gln; R, Arg; S, Ser; T, Thr; V, Val; W, Trp; and Y, Tyr.

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MGELMAFLLPLIIVLMVKHSDSRTHSLRYFRLGVSDPIHGVPEFISVGYV    50
DSHPITTYDSVTRQKEPRAPWMAENLAPDHWERYTQLLRGWQQMFKVELK   100
RLQRHYNHSGSHTYQRMIGCELLEDGSTTGFLQYAYDGQDFLIFNKDTLS   150
WLAVDNVAHTIKQAWEANQHELLYQKNWLEEECIAWLKRFLEYGKDTLQR   200
TEPPLVRVNRKETFPGVTALFCKAHGFYPPEIYMTWMKNGEEIVQEIDYG   250
DILPSGDGTYQAWASIELDPQSSNLYSCHVEHCGVHMVLQVPQESETIPL   300
VMKAVSGSIVLVIVLAGVGVLVWRRRPREQNGAIYLPTPDR            341
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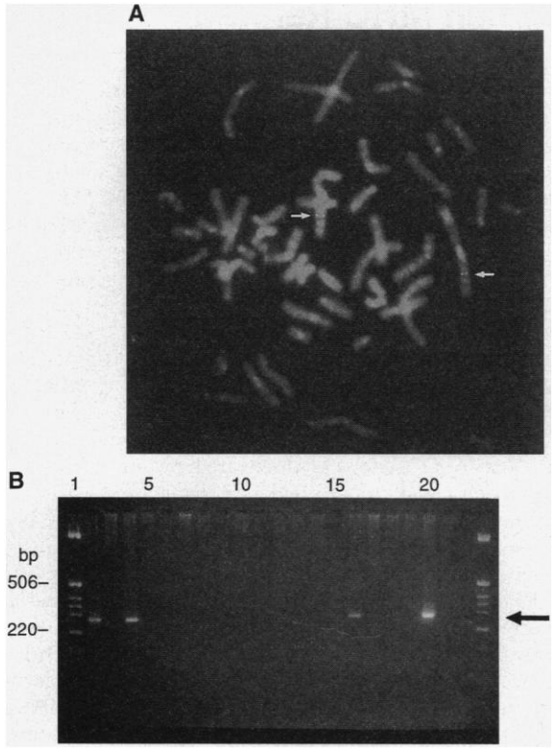
Fig. 3. (**A**) FISH analysis of human chromosomes with a biotinylated *MR1* probe (*16, 17*). The R-banded metaphase plate reveals hybridization signals at the distal portion of 1q25 (1q25.3) (arrows). (**B**) PCR amplification of the α1 domain of *MR1* from human-hamster somatic cell hybrid DNA (*18*). PCR products were separated by electrophoresis on a 4% agarose gel (SepRate-SDF; Amersham). Lanes 1 and 23, DNA size markers (pBR322 DNA digested with Hinf I); representative DNA sizes (in base pairs) are indicated on the left. Lanes 2 to 22, PCR products (one-fifth of the reaction mixture) amplified from various templates. Arrow, the position of the expected PCR product (278 base pairs). Template DNA was as follows: human-hamster somatic cell hybrid DNA (lanes 2 to 19), human DNA (lane 20), hamster DNA (lane 21), and no-template control (lane 22). Somatic cell hybrids used in lanes 2 and 16 are reported to contain human chromosome 1 on the basis of cytogenetic examination (BIOS). Cell hybrids used in lanes 5 and 7 are reported to contain human chromosome 6, on which MHC is located.



classical class I and closely related molecules from various vertebrates also share ~40 to 50% amino acid identity in their peptide-binding domains (Fig. 4); however, with the exception of the α2 domain of Znα2gp, this degree of identity is not achieved by other divergent class I molecules. Thus, on the basis of the amino acid sequence identity in the peptide-binding domains, MR1 is a close relative of typical vertebrate classical class I molecules. Accordingly, MR1 is overall significantly more related to HLA class I molecules than any other known human divergent class I molecule, including MICA, whose gene is located within the MHC; the CD1 family, whose genes are located on the same chromosome as *MR1*; and Znα2gp, a soluble serum protein devoid of transmembrane and cytoplasmic domains.

The identification of a homolog of *MR1* in the mouse genome (*19*) implies that the ancestor of *MR1* was present in the primordial mammalian species. The existence of the *MR1* lineage in mammals as well as the tendency of the MHC system to increase the number of its members suggest that there may be close relatives (not necessarily related to the *MR1* lineage) of classical MHC genes in other vertebrate orders (*20*). From an evolutionary standpoint, the coexistence of multiple classical class I–related genes suggests that species at different evolutionary stages may adopt distinct gene lineages for their MHC. In a New World primate (the cotton-top tamarin), the most broadly expressed class I gene lineage is *HLA-G* (*21*), which is expressed only in a highly restricted manner and is oligomorphic in humans. The discovery of *MR1* suggests that selection for the major polymorphic class I lineages similarly may have occurred over a much longer evolutionary time scale. In this context, the relation between the mammalian and chicken classical class I genes may be revealing because the organization of the chicken MHC differs markedly from that of mammals (*10, 22*). Chicken may have adopted a clas-

Fig. 4. Similarities of amino acid sequences between various class I molecules. The percentage amino acid identity is shown for each extracellular domain (α1, α2, and α3). Percentage values of ≥40 in the α1 or α2 domains are boxed. Comparisons of class I molecules within the same group (for example, HLA-A versus HLA-B, or CD1A versus CD1B) or in closely related groups (for example, HLA-A versus H-2K^b) are also included to indicate the conservation in the α1 and α2 domains. References for the amino acid sequences are as follows: HLA-A2 (A*0201) and HLA-B27 (B*2705) (*29*), H-2K^b (*30*), CD1A and CD1B (*31*), FcRn (*7*), MICA (*6*), Znα2gp (the gene for which is located on human chromosome 7) (*32*), chicken (BF-12) (*22*), lizard (Amieva lizard, LC1) (*33*), frog (*Xenopus laevis*, *Xela-UAA1^f*, class Ia) (*26*), and salmon (Atlantic salmon, p30) (*25*).

		HLA-A	HLA-B	H-2K^b	Znα2gp	MICA	FcRn	CD1A	CD1B	Chicken	Lizard	Frog	Salmon
MR1	α1	[40]	[42]	[44]	26	22	26	16	13	36	[41]	[42]	34
	α2	[48]	[45]	[42]	[41]	27	26	32	26	[47]	[42]	35	[42]
	α3	32	34	36	36	35	33	30	26	40	33	38	29
HLA-A2	α1		[82]	[69]	30	27	26	15		38	[41]	[45]	33
	α2		[84]	[76]	[40]	23	20	25		[52]	[45]	38	37
	α3		92	73	34	34	34	28		33	37	30	29
Znα2gp	α1					22	25	18		25	26	33	21
	α2					27	25	24		38	38	32	32
	α3					32	28	31		34	30	38	26
MICA	α1						21	11		27	26	25	21
	α2						22	18		23	27	27	24
	α3						23	25		34	30	28	28
FcRn	α1							17		25	33	28	26
	α2							21		22	24	25	19
	α3							25		35	30	27	27
CD1A	α1								[46]	10	14	12	17
	α2								[51]	23	24	20	24
	α3								89	32	27	32	25
Chicken	α1										[49]	38	[42]
	α2										[49]	[51]	[45]
	α3										43	40	28
Lizard	α1											[44]	[41]
	α2											[45]	[47]
	α3											24	24
Frog	α1												[43]
	α2												[40]
	α3												30

sical class I lineage distinct from that of mammals. MR1 does share some characteristics, which are not found in HLA class I molecules, with the chicken MHC and other class I molecules including the CD1 family; examples include deletions and insertions of amino acids in the α1 and α2 domains, and the amino acid similarity around residue 252 (Fig. 2).

The location of *MR1* on chromosome 1 may have a rational evolutionary history rather than be a result of a minor accidental translocation event, because a divergent MHC class I–related *CD1* gene family exists on human chromosome 1q22-23 (*23*). In addition, many other immunoglobulin gene superfamily members—including CD2, LFA-3, Blast-1, the Fc receptors for immunoglobulins G and E, and the polymeric immunoglobulin receptor—are also located on chromosome 1 (*24*). It is conceivable that other MHC class I– and also class II–related genes may be located near *MR1* on chromosome 1. The relative proximity of *MR1* and *CD1* is the first example in which distinct families of MHC class I–related genes are found on the same chromosome outside the MHC.

The discovery of *MR1* suggests that additional close relatives of MHC genes may be present in the human genome. Some of those genes may possess unusual functions and others may serve as a reservoir for future classical polymorphic MHC genes. The identification of *MR1* and other MHC-related genes should aid in our understanding of the evolution of the MHC.

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14. Approximately 82–base pair DNA fragments were amplified by PCR [R. K. Saiki *et al.*, *Science* **239**, 487 (1988); (*11*)] from human placenta genomic DNA with two primers that correspond to the two highly conserved regions in the MHC class I α3 domain (Fig. 2). Various primer sequences with slight modifications were tested, and a candidate for a new class I gene fragment was revealed after analysis of PCR products with restriction enzymes such as Alu I and Sau 3AI. Southern (DNA) hybridization with this PCR fragment detected a single positive band in Hind III– or Bam HI–digested human placenta DNA (~6 and 4.5 kb, respectively). A human thymus cDNA library in λgt11 [oligo(dT)- and random-primed, and amplified once (Clontech)] was screened with the PCR fragment, and 14 positive clones (nine independent clones) out of 2.4×10^6 clones were obtained. The DNA sequence of one of the positive clones (C7, ~2 kb) was determined [J. Sambrook, E. F. Fritsch, T. Maniatis, *Molecular Cloning: A Laboratory Manual* (Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, ed. 2, 1989)]. The two relevant primers were 5′-TCAGGATCCT-GYCGNGCNCAYGRNTTYTAYCC (N = A, T, G, or C; Y = T or C; R = G or A) and 5′-GCAGAATTC-NRYYTGR(A/T)ANGTNCCRTC, with Bam HI and Eco RI sites in their 5′ regions, respectively. The primers were synthesized on a DNA synthesizer (Applied Biosystems). The former primer was based on the chicken class I sequence (*22*); this region had been first used to isolate carp class I and class II genes (*11*). The latter primer region is highly conserved, even in the shark sequence (*12*), and had been used to isolate salmon MHC genes (*25*).
15. Human multiple tissue Northern blot membranes were obtained from Clontech. Polyadenylated RNA (2 μg) from various tissues was probed with a 1.15-kb Bam HI–Hinc II fragment of *MR1* cDNA clone C7, which contains more than five-sixths of the α1 domain, all of the α2, α3, transmembrane, and cytoplasmic domains, and the 3′ untranslated region. Hybridization and washing were performed according to the manufacturer's recommendations. Hybridized membranes were exposed to x-ray film at −70°C for 9 days. Another probe, the original *MR1* PCR product (*14*) containing the middle region of the α3 domain, also detected the same bands as those apparent in Fig. 1, although the signals were much weaker.
16. A 5-kb *MR1* α3 domain exon–containing genomic DNA probe (Hind III site at one end) was prepared from a phage clone that had been isolated from a human placenta genomic library (produced by partial digestion with Sau 3AI) with the *MR1* PCR fragment, and the chromosomal location of *MR1* was determined by FISH analysis (*17*). The probe was labeled with biotin-14–dUTP (deoxyuridine triphosphate) with the use of a nick translation kit (Gibco BRL). Hybridization signals were detected with rabbit antibodies to biotin and fluorescein isothiocyanate–conjugated goat antibodies to rabbit immunoglobulin G (ENZO). The 1.15-kb probe (Bam HI–Hinc II fragment) prepared from *MR1* cDNA clone C7 was also used in FISH analysis, and the same result was obtained.
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18. Human-hamster somatic cell hybrid DNA (BIOS) was subjected to PCR (30 cycles of 1 min at 94°C, 1 min at 55°C, and 1 min at 72°C) with primers that amplify the α1 domain of *MR1*. The two primers were 5′-GCAGAATTCGGACGCACTCTCTGAGATA and 5′-GACAAGCTTTGAGTGATTGTAGTGCCTCTG, with Eco RI site and Hind III sites in their 5′ regions, respectively. PCR products of the expected size were amplified with control human DNA but not with control hamster DNA as template. Human-hamster somatic cell lines used for the gel shown in Fig. 3B are numbers 867, 854, 423, 860, 803, 909, 1006, 811, 967, 734, 968, 683, 507, 750, 1099, 324, 940, and 983 (lanes 2 to 19, respectively). In addition to chromosome 1, the cell hybrids used in lanes 2 and 16 also contain other human chromosomes (lane 2, chromosomes 5, 13, 14, 18, and 19; lane 16, chromosomes 5, 13, 19, 21, and 22). However, these chromosomes are also present in other cell lines, and discordance analysis showed that *MR1* is not located on these chromosomes. Relevant chromosomes and cell lines (indicated by lane number) that contain them are as follows: chromosome 5, lanes 2, 3, 5 to 8, 10 to 16, 18, and 19; chromosome 13, lanes 2, 8, 12, 15, and 16; chromosome 14, lanes 2, 7, and 13 to 15; chromosome 18, lanes 2, 9, 11, and 17; chromosome 19, lanes 2, 5 (45%), 8, 13, 15, and 16; chromosome 21, lanes 5, 8, 13, and 16; and chromosome 22, lanes 6, 13, 14 (25%), and 16. (Percentages after lane numbers refer to the percentage of the cell population that contains the relevant chromosome; for lane numbers with no percentage value, >75% of the cell population contains the relevant chromosome.) PCR fragments of the expected size were also amplified in lanes 4 and 13 (faint in lane 13). The cell line used in lane 4 is reported to contain only chromosome 3, but the cell lines used in lanes 5 and 14 also contain chromosome 3 (lane 5, 15%) and were negative for *MR1* by PCR. Therefore, chromosome 1 or fragments thereof may be present in cell line 423 (lane 4) but cannot be detected by cytogenetic examination. Lane 13 is reported to contain chromosomes 5, 11, 12 (45%), 14, 19, 21, and 22. These chromosomes are contained in the following cell lines (indicated by lane number): chromosome 11, lanes 6 (15%) and 13; chromosome 12, lanes 13 (45%) and 14; other chromosomes, see above. With the exception of chromosome 11, each of these chromosomes can be readily excluded by discordance analysis; with regard to chromosome 11, no differences in PCR products were apparent between lane 6 and chromosome 11–negative lanes, such as lane 7, even after 50 cycles of PCR. From 5 to 30% of recent samples of cell line 683 (lane 13) are reported to contain chromosome 1 (BIOS). Our result with lane 13 may be explained by the presence of a small amount of chromosome 1 in the sample of cell line 683 used in our study.
19. The nucleotide sequence of a PCR product amplified from BALB/c mouse liver DNA with the two primers described in (*14*) was also determined (clone mp18; GenBank accession number, U24235). Several of the predicted amino acid residues of the mouse sequence differ from those of the human sequence as follows: Y233 (human) → S (mouse), V244 → A, I247 → V, D251 → G, and I252 → V. The percentage amino acid identity between human and mouse sequences in this region is 81%.
20. In bony fish, in addition to the carp divergent class I genes (*8*), a salmon class I gene, which is more similar to the classical class I genes of other species than are the carp genes, has been described (*25*). The authentic classical class I gene in bony fish remains to be elucidated. In *Xenopus*, an MHC classical class I gene (*26*) as well as a large family of non–MHC-linked class I genes [the XNC family (*9*)] have been identified. Although the *Xenopus* classical class I α3 domain is more closely related to the XNC α3 domains than to class I α3 domains of any other species, the peptide-binding domains are not well conserved between the *Xenopus* classical class I and XNC molecules. *Xenopus* class I molecules, whose peptide-binding domains are more similar to that of *Xenopus* classical class I than are those of XNC molecules, may exist. In chickens, a second polymorphic MHC-like system, *Rfp-Y*, has been identified (*10*). The *Rfp-Y* genes appear to be highly similar to the chicken MHC (*B* complex) genes. Close relatives of chicken MHC class I genes distinct from those in *B* and *Rfp-Y* may also exist.
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