Proc. Natl. Acad. Sci. USA
Vol. 89, pp. 1463–1467, February 1992
Immunology

Polymorphism in a second ABC transporter gene located within the class II region of the human major histocompatibility complex

(HLA/antigen processing)

STEPHEN H. POWIS*†, IAN MOCKRIDGE*, ADRIAN KELLY*, LESLEY-ANNE KERR‡, RICHARD GLYNNE*, UZI GILEADI§, STEPHAN BECK*, AND JOHN TROWSDALE*

*Imperial Cancer Research Fund Laboratories, Lincoln's Inn Fields, London WC2A 3PX, United Kingdom; ‡Molecular Immunogenetics, United Medical and Dental Schools of Guy's and St. Thomas' Hospitals, Guy's Campus, London SE1 9RT, United Kingdom; and §Imperial Cancer Research Fund Laboratories, Institute of Molecular Medicine, University of Oxford, John Radcliffe Hospital, Oxford OX3 9DU, United Kingdom

Communicated by Avrion Mitchison, November 12, 1991 (received for review August 30, 1991)

ABSTRACT **Recent studies have identified genes within the major histocompatibility complex (MHC) that may play a role in presentation of antigenic peptides to T cells. We have previously described *RING4*, a gene within the human MHC class II region that has sequence homology with members of the ABC ("ATP-binding cassette") transporter superfamily. We now report the nucleotide sequence of *RING11*, a second ABC transporter gene located approximately 7 kilobases telomeric to *RING4*. *RING11* is γ-interferon inducible, a property shared with other genes involved in antigen presentation. Comparison between the amino acid sequences of RING11 and RING4 reveals strong homology. We propose that they form a heterodimer that transports peptides from the cytoplasm into the endoplasmic reticulum. We have identified two *RING11* alleles, which differ in the length of their derived protein sequence by 17 amino acids. The more common of these alleles is present in a Caucasoid population at a frequency of 79%.**

T lymphocytes recognize foreign antigen when it is presented bound to class I or class II major histocompatibility complex (MHC) molecules (1–3). Until recently, the mechanism by which antigen first associates with MHC molecules was poorly understood (4). In the case of class I antigens, short peptides are initially bound in the endoplasmic reticulum (5). Once bound, peptides confer stability upon the class I molecule (6). However, as most peptides are derived from the cytoplasm, they must first be transported across the ER membrane (7). Evidence to support this has been provided by a number of mutant cell lines with abnormalities of class I assembly, possibly due to defective peptide transport (8, 9). As these mutations map to the class II region of the MHC, it was of great interest that a gene encoding a putative peptide transporter was found in this location (10–13). This gene, which we have called *RING4*, has sequence homology with the ABC ("ATP-binding cassette") transporter superfamily, whose members are involved in the transport of a wide variety of substrates across cell membranes (14). Members of the superfamily include the oligopeptide permease system in bacteria, the multidrug resistance system in eukaryotes, and the human cystic fibrosis gene product (15–17).

To confirm that *RING4* was involved in antigen processing, Spies and DeMars (18) transfected their gene *PSF* (for peptide supply factor, the sequence of which is identical to *RING4*) into LCL721.134, a mutant cell line with an isolated defect in *RING4*. Normal class I expression was restored. However, transfection into LCL721.174, a mutant with a large deletion over the class II region, failed to restore function. This suggests that other antigen-processing gene(s) are present in this region (19).

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

In this paper we describe the characterization of *RING11*, a second ABC transporter gene within the MHC class II region, and demonstrate that it is polymorphic.¶

MATERIALS AND METHODS

Isolation of cDNA Clones. cDNA libraries were constructed in a derivative of the CDM8 vector as described by Seed (20). Libraries were screened with fragments of genomic cosmids and secondary screening of positive clones was performed as previously described (21). DNA from positive clones was prepared by using standard protocols.

PCR Amplification. Genomic DNA samples (0.1–1 μg) were amplified in 100-μl reaction mixtures containing each oligonucleotide primer at 700 nM, 200 μM dNTPs, 1× *Taq* DNA polymerase buffer, and 1 unit of *Taq* DNA polymerase (Promega). Reaction conditions were 95°C for 10 min, 40 cycles of 94°C for 1 min, 57°C for 2 min, 72°C for 2 min, and a final step of 72°C for 10 min.

Sequence Analysis. cDNA clones were initially sequenced by the M13/dideoxynucleotide chain termination method (22). Subsequently, 20-mer oligonucleotide primers were synthesized over the first full-length clone at approximately 200-base-pair intervals in both directions. These were then used to sequence further cDNA clones. Solid-phase DNA sequencing from PCR products was performed according to a method adapted from Hultman *et al.* (23). PCR amplification was performed as described above, except that primer concentrations were reduced to 250 nM and a biotinylated 5′ primer was used. The PCR product was incubated with 200 μg of Dynabeads M-280 streptavidin (Dynal, Oslo) for 30 min at room temperature. DNA was denatured by washing once with 0.15 M NaOH and once with 0.15 M NaOH/0.1 M NaCl. Beads were then resuspended in 7 μl of distilled water and the DNA was sequenced.

Oligonucleotide Typing. Typing was performed according to the method of Bugawan *et al.* (24), with modifications. PCR products, immobilized on Hybond N+ membranes (Amersham), were hybridized with biotinylated oligonucleotide probes at 56°C. Unbound probe was removed by washing at 59°C.

Northern Analysis. Total cellular RNA was prepared by acid phenol extraction and transferred to Hybond-N membrane (Amersham) by using standard protocols. Hybridization was performed overnight at 42°C and membranes were washed at high stringency. Autoradiography was for 16 hr at −70°C with XAR-5 film (Kodak).

Abbreviations: Hmdr, human multidrug resistance gene product; MHC, major histocompatibility complex.

†To whom reprint requests should be addressed.

¶The sequence reported in this paper has been deposited in the GenBank data base (accession no. M84748).

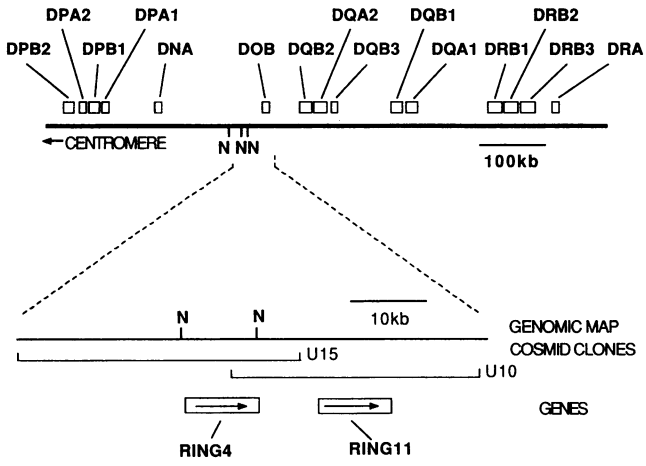

FIG. 1. Location of the *RING11* gene within the human MHC. The upper map shows the organization of the class II region on the short arm of chromosome 6. The lower map shows the position of *RING4* and *RING11*. N designates a *Not* I restriction enzyme site. The map is based on the published map of cosmids U10 and U15 (13). The position of *RING4* was determined by sequencing of genomic DNA and the position of *RING11* by genomic sequencing and restriction enzyme mapping.

RESULTS AND DISCUSSION

Isolation of *RING11* cDNA. The *RING11* cDNA was initially obtained from a library made from the T-cell line CEM,

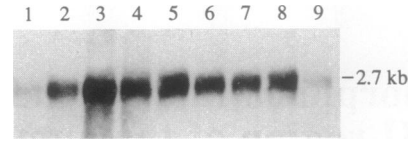

FIG. 3. Effect of γ-interferon on *RING11* expression. Lanes 1–8, 10 μg of denatured total cellular RNA extracted from normal human keratinocytes after incubation with recombinant γ-interferon at 200 units/ml for 2, 6, 24, 48, 72, 96, 120, or 168 hr, respectively; lane 9, 20 μg of total cellular RNA from uninduced keratinocytes. The filter was hybridized with the CEM-derived *RING11* cDNA shown in Fig. 2.

which was probed with a fragment of cosmid U10 (10, 13). *RING11* is located between the class II genes *DNA* and *DOB*, approximately 7 kilobases (kb) telomeric to *RING4* (Fig. 1). The organization of the gene will be described elsewhere (S.B., A.K., S.H.P., and J.T., unpublished results). The nucleotide sequence of the CEM *RING11* cDNA is shown in Fig. 2. The longest open reading frame encodes a derived peptide sequence of 686 amino acids with a molecular mass of 75.7 kDa and a pI of pH 7.99. On Northern analysis, *RING11* was shown to be up-regulated by γ-interferon (Fig. 3), an observation common to *RING4* and other genes involved in antigen processing (25, 26).

Sequence Analysis. Comparisons of RING11 with RING4 and other members of the ABC transporter family are shown in Fig. 4 and Table 1. Typically, each ABC transporter

```
                                                                1
    GCTGCGGTCTCCCCGCCGCGGCTGAGCCATG CGGCTCCCTGACCTG AGACCCTGGACCTCC CTGCTGCTGGTGGAC GCGGCTTTACTGTGG CTGCTTCAGGGCCCT
                  *     M  R  L  P  D  L  R  P  W  T  S  L  L  L  V  D  A  A  L  L  W  L  L  Q  G  P
  79 CTGGGGACTTTGCTT CCTCAAGGGCTGCCA GGACTATGGCTGGAG GGGACCCTGCGGCTG GGAGGGCTGTGGGGG CTGCTAAAGCTAAGA GGGCTGCTGGGATTT
  27 L  G  T  L  L  P  Q  G  L  P  G  L  W  L  E  G  T  L  R  L  G  G  L  W  G  L  L  K  L  R  G  L  L  G  F
 184 GTGGGGACACTGCTG CTCCCGCTCTGTCTG GCCACCCCCCTGACT GTCTCCCTGAGAGCC CTGGTCGCGGGGGCC TCACGTGCTCCCCCA GCCAGAGTCGCTTCA
  62 V  G  T  L  L  L  P  L  C  L  A  T  P  L  T  V  S  L  R  A  L  V  A  G  A  S  R  A  P  P  A  R  V  A  S
 289 GCCCCTTGGAGCTGG CTGCTGGTGGGGTAC GGGGCTGCGGGGCTC AGCTGGTCACTGTGG GCTGTTCTGAGCCCT CCTGGAGCCCAGGAG AAGGAGCAGGACCAG
  97 A  P  W  S  W  L  L  V  G  Y  G  A  A  G  L  S  W  S  L  W  A  V  L  S  P  P  G  A  Q  E  K  E  Q  D  Q
 394 GTGAACAACAAAGTC TTGATGTGGAGGCTG CTGAAGCTCTCCAGG CCGGACCTGCCTCTC CTCGTTGCCGCCTTC TTCTTCCTTGTCCTT GCTGTCTTGGGTGAG
 132 V  N  N  K  V  L  M  W  R  L  L  K  L  S  R  P  D  L  P  L  L  V  A  A  F  F  F  L  V  L  A  V  L  G  E
 499 ACATTAATCCCTCAC TATTCTGGTCGTGTG ATTGACATCCTGGGA GGTGATTTTGACCCC CATGCCTTTGCCAGT GCCATCTTCTTCATG TGCCTCTTCTCCTTT
 167 T  L  I  P  H  Y  S  G  R  V  I  D  I  L  G  G  D  F  D  P  H  A  F  A  S  A  I  F  F  M  C  L  F  S  F
 604 GGCAGCTCACTGTCT GCAGGCTGCCGAGGA GGCTGCTTCACCTAC ACCATGTCTCGAATC AACTTGCGGATCCGG GAGCAGCTTTTCTCC TCCCTGCTGCGCCAG
 202 G  S  S  L  S  A  G  C  R  G  G  C  F  T  Y  T  M  S  R  I  N  L  R  I  R  E  Q  L  F  S  S  L  L  R  Q
 709 GACCTCGGTTTCTTC CAGGAGACTAAGACA GGGGAGCTGAACTCA CGGCTGAGCTCGGAT ACCACCCTGATGAGT AACTGGCTTCCTTTA AATGCCAATGTGCTC
 237 D  L  G  F  F  Q  E  T  K  T  G  E  L  N  S  R  L  S  S  D  T  T  L  M  S  N  W  L  P  L  N  A  N  V  L
 814 TTGCGAAGCCTGGTG AAAGTGGTGGGGCTG TATGGCTTCATGCTC AGCATATCGCCTCGA CTCACCCTCCTTTCT CTGCTGCACATGCCC TTCACAATAGCAGCG
 272 L  R  S  L  V  K  V  V  G  L  Y  G  F  M  L  S  I  S  P  R  L  T  L  L  S  L  L  H  M  P  F  T  I  A  A
 919 GAGAAGGTGTACAAC ACCCGCCATCAGGAA GTGCTTCGGGAGATC CAGGATGCAGTGGCC AGGGCGGGGCAGGTG GTGCGGGAAGCCGTT GGAGGGCTGCAGACC
 307 E  K  V  Y  N  T  R  H  Q  E  V  L  R  E  I  Q  D  A  V  A  R  A  G  Q  V  V  R  E  A  V  G  G  L  Q  T
1024 GTTCGCAGTTTTGGG GCCGAGGAGCATGAA GTCTGTCGCTATAAA GAGGCCCTTGAACAA TGTCGGCAGCTGTAT TGGCGGAGAGACCTG GAACGCGCCTTGTAC
 342 V  R  S  F  G  A  E  E  H  E  V  C  R  Y  K  E  A  L  E  Q  C  R  Q  L  Y  W  R  R  D  L  E  R  A  L  Y
1129 CTGCTCGTAAGGAGG GTGCTGCACTTGGGG GTGCAGATGCTGATG CTGAGCTGTGGGCTG CAGCAGATGCAGGAT GGGGAGCTCACCCAG GGCAGCCTGCTTTCC
 377 L  L  V  R  R  V  L  H  L  G  V  Q  M  L  M  L  S  C  G  L  Q  Q  M  Q  D  G  E  L  T  Q  G  S  L  L  S
1234 TTTATGATCTACCAG GAGAGCGTGGGGAGC TATGTGCAGACCCTG GTATACATATATGGG GATATGCTCAGCAAC GTGGGAGCTGCAGAG AAGGTTTTCTCCTAC
 412 F  M  I  Y  Q  E  S  V  G  S  Y  V  Q  T  L  V  Y  I  Y  G  D  M  L  S  N  V  G  A  A  E  K  V  F  S  Y
1339 ATGGACCGACAGCCA AATCTGCCTTCACCT GGCACGCTTGCCCCC ACCACTCTGCAGGGG GTTGTGAAATTCCAA GACGTCTCCTTTGCA TATCCCAATCGCCCT
 447 M  D  R  Q  P  N  L  P  S  P  G  T  L  A  P  T  T  L  Q  G  V  V  K  F  Q  D  V  S  F  A  Y  P  N  R  P
1444 GACAGGCCTGTGCTC AAGGGGCTGACGTTT ACCCTACGTCCTGGT GAGGTGACGGCGCTG GTGGGACCCAATGGG TCTGGGAAGAGCACA GTGGCTGCCCTGCTG
 482 D  R  P  V  L  K  G  L  T  F  T  L  R  P  G  E  V  T  A  L  V  G  P  N  G  S  G  K  S  T  V  A  A  L  L
1549 CAGAATCTGTACCAG CCCACAGGGGGACAG GTGCTGCTGGATGAA AAGCCCATCTCACAG TATGAACACTGCTAC CTGCACAGCCAGGTG GTTTCAGTTGGGCAG
 517 Q  N  L  Y  Q  P  T  G  G  Q  V  L  L  D  E  K  P  I  S  Q  Y  E  H  C  Y  L  H  S  Q  V  V  S  V  G  Q
1654 GAGCCTGTGCTGTTC TCCGGTTCTGTGAGG AACAACATTGCTTAT GGGCTGCAGAGCTGC GAAGATGATAAGGTG ATGGCGGCTGCCCAG GCTGCCCACGCAGAT
 552 E  P  V  L  F  S  G  S  V  R  N  N  I  A  Y  G  L  Q  S  C  E  D  D  K  V  M  A  A  A  Q  A  A  H  A  D
1759 GACTTCATCCAGGAA ATGGAGCATGGAATA TACACAGATGTAGGG GAGAAGGGAAGCCAG CTGGCTGCGGGACAG AAACAACGTCTGGCC ATTGCCCGGGCCCTT
 587 D  F  I  Q  E  M  E  H  G  I  Y  T  D  V  G  E  K  G  S  Q  L  A  A  G  Q  K  Q  R  L  A  I  A  R  A  L
1864 GTACGAGACCCGCGG GTCCTCATCCTGGAT GAGGCTACTAGTGCC CTAGATGTGCAGTGC GAGCAGGCCCTGCAG GACTGGAATTCCCGT GGGGATCGCACAGTG
 622 V  R  D  P  R  V  L  I  L  D  E  A  T  S  A  L  D  V  Q  C  E  Q  A  L  Q  D  W  N  S  R  G  D  R  T  V
1969 CTGGTGATTGCTCAC AGGCTGCAGACAGTT CAGCGCGCCCACCAG ATCCTGGTGCTCCAG GAGGGCAAGCTGCAG AAGCTTGCCCAGCTC TAGGAGGGACAGGAC
 657 L  V  I  A  H  R  L  Q  T  V  Q  R  A  H  Q  I  L  V  L  Q  E  G  K  L  Q  K  L  A  Q  L  *
2074 CTCTATTCCCGCCTGGTGCAGCAGCGGCTGATGGACTGAGGCCCCAGGGATACTGGGCCCTCTTCTCAGGGGCGTCTCCAGGACCCAGAGCTGTTCCTGCTTTGAGTTTCC
2185 CTAGAGCTGTGCGGCCAGATAGCTGTTCCTGAGTTGCAGGCACGATGGAGATTTGGACACTGTGTGCTTTTGGTGGGGTAGAGAGGTGGGGTGGGGTGGGGTGGGGGCTGT
2296 CTGTGTCCAGGAAACTTAATTCCCTGGTGACTAGAGCTTTGCCTGGTGATGAGGAGTATTTTGTGGCATAATACATATATTTTAAAATATTTTCCTTCTTACATGAACTGT
2407 ATACATTCATATAGAAAATTTAGACAATATAAAAAAGTAC
```

FIG. 2. Sequence of the *RING11* cDNA and derived amino acid sequence. The sequence of the longest clone obtained from the CEM library is shown. The open reading frame encodes a protein of 686 amino acids. A stop codon (*) is shown just before the potential initiating methionine.

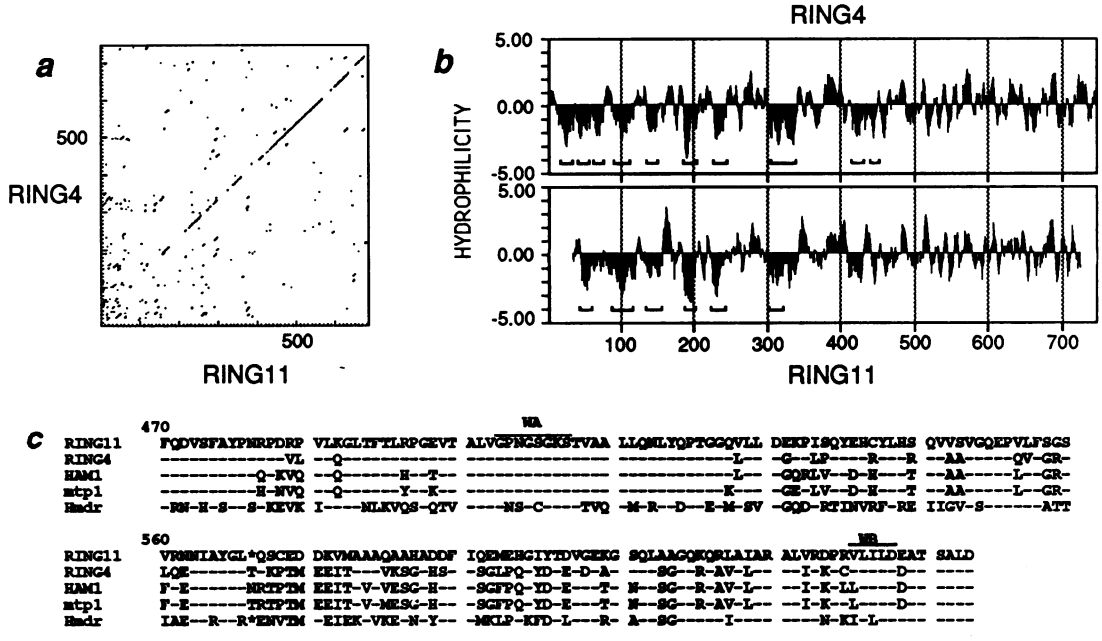

FIG. 4. (*a*) Dot matrix plot between the amino acid sequences of RING11 and RING4. The comparisons were run using the Similarity Investigation Programme in the Staden package with an odd span length of 11, proportional score of 132, and gap penalty of 10 (27). Homology is greatest over the C-terminal ATP-binding domains. (*b*) Hydrophilicity plots of RING4 and RING11. The sequences are aligned over the G residue of the Walker A nucleotide-binding motif (residue 508 of RING11). Numbering refers to RING4. Plots were produced by using the method of Kyte and Doolittle (28) with an amino acid window of 7. Potential transmembrane regions are marked by horizontal bars and were identified as previously described (10). Note it may be possible that other hydrophobic stretches form transmembrane regions but were not identified as such in our computer analysis. (*c*) Alignment of the ATP-binding domain of RING11 with RING4, HAM1, mtp1 (respectively, the murine and rat homologues of RING4). The N-terminal ATP-binding domain of the human multidrug resistance gene product (Hmdr) is also included for comparison. Single-letter amino acid code is used; numbering refers to RING11. A hyphen represents a residue identical to that in RING11; an asterisk represents a gap that has been introduced to optimize the alignment. The Walker A (WA) and B (WB) nucleotide-binding motifs are indicated (29). More extensive alignments of ABC transporters can be found in refs. 10, 12, 30, and 31.

requires two hydrophobic domains and two ATP-binding domains (14, 31, 34). In the oligopermease system, each of the four domains is encoded by a separate polypeptide chain, but in other systems, domains are frequently fused together (15). For example, all four domains of Hmdr (P-glycoprotein) are formed by a single polypeptide (16). RING11 and RING4 each consist of one hydrophobic domain and one ATP-binding domain and, as such, are similar in organization to the HlyB hemolysin A transporter system of *E. coli* (33).

In general, amino acid sequence identity between the domains of a single ABC transporter is greater than that between the domains of different transporters (14). ATP-binding domains are usually more similar than hydrophobic domains. When the ATP-binding domains of RING11 and RING4 are compared with each other, the sequence identity is about 60%, whereas when they are compared with the ATP-binding domains of other transporters, the identity falls to below 50% (Table 1). Similarly, comparison of the hydro-

Table 1. Identity between RING11, RING4, and other members of the ABC transporter superfamily

	RING11	RING4	Hmdr N terminus	Hmdr C terminus	HlyB	OppB	OppC	OppD
RING11		29.8	19.2	21.6	18.5	21.0	19.6	—
RING4	*61.3*		21.1	22.2	16.8	16.9	23.0	—
Hmdr N terminus	*44.4*	*42.5*		30.0	21.7	21.3	19.0	—
Hmdr C terminus	*49.3*	*45.0*	*60.2*		19.2	16.1	16.1	—
HlyB	*43.3*	*41.2*	*50.5*	*47.7*		19.7	17.1	—
OppB	—	—	—	—	—		26.6	—
OppD	*27.1*	*29.2*	*26.5*	*26.9*	*29.5*	—	—	
OppF	*26.6*	*30.7*	*31.4*	*27.4*	*32.7*	—	—	*40.3*

The percent identity was calculated after alignment of amino acid sequences using the gene analysis program in the Genetics Computer Group package (32). A representative sample is shown. For this analysis, polypeptides were split into their individual hydrophobic and ATP-binding domains. Hmdr was split into four as the entire transporter is present on a single polypeptide. HlyB is an *Escherichia coli* ABC transporter that transports hemolysin toxin. It has a single ATP-binding domain and a single hydrophobic domain and is believed to function as a homodimer. Comparison of hydrophobic domains is shown in the upper right half of the table (percentages not italic); sequences compared were RING11 (1–469), RING4 (1–504), Hmdr N terminus (1–394), Hmdr C terminus (639–1036), HlyB (1–459), and the entire sequences of OppB and OppC, which are *Salmonella typhimurium* peptide transporters (10, 15, 16, 33). Comparison of ATP-binding domains is shown in the lower left part of the table (percentages italicized); sequences compared were RING11 (470–686), RING4 (505–748), Hmdr N terminus (395–639), Hmdr C terminus (1037–end), HylB (460–690), and the entire OppD and OppF sequences (10, 15, 16, 33).

```
          1969
RING11A   CTGGTGATTGCTCAC AGGCTGCAGACAGTT CAGCGCGCCCACCAG ATCCTGGTGCTCCAG GAGGGCAAGCTGCAG
RING11B   --------------- ---------G----- --------------- -------------------------------
          657
RING11A   L  V  I  A  H   R  L  Q  T  V   Q  R  A  H  Q   I  L  V  L  Q   E  G  K  L  Q
RING11B   -  -  -  -  -   -  -  -  A  -   -  -  -  -  -   -  -  -  -  -   -  -  -  -  -

          2044
RING11A   AAGCTTGCCCAGCTC TAGGAGGGACAGGAC CTCTATTCCCGCCTG GTGCAGCAGCGGCTG ATGGACTGA
RING11B   ---------------C--------------- --------------- --T------------ ---------
          682
RING11A   K  L  A  Q  L   *
RING11B   -  -  -  -  -   Q  E  G  Q  D   L  Y  S  R  L   V  Q  Q  R  L   M  D  *
```

FIG. 5. RING11 polymorphism. Sequence variation between RING11A and -B in the 3′ coding region is shown. A hyphen represents an identical nucleotide or amino acid. The single-letter amino acid code is used. *RING11B* also had a single base pair substitution of T for C at position 489 (which did not result in an amino acid change) and additional variation in the 3′ untranslated region of the cDNA clone (data not shown).

phobic domains of RING11 and RING4 reveals approximately 30% identity, whereas comparison of RING11 and RING4 with the hydrophobic domains of other transporters reveals identity of about 20% or less. This implies that RING11 and RING4 function as a heterodimer, although direct experimental evidence to support this is not yet at hand. So far, only circumstantial evidence has been provided by the cell line LCL721.134, which is deficient in *RING4* and shows abnormal class I assembly and peptide presentation (18). This suggests that RING11 does not function as a homodimer, but it does not exclude this possibility for RING4.

Consistent with their proposed role in hydrolysis of ATP, it is assumed that the ATP-binding domains of ABC transporters are located on the cytoplasmic side of cell membranes (35). This would also be predicted from the hydrophilic nature of the domains, which contain no potential membrane-spanning regions. If this observation is consistent throughout the family, we would expect the putative RING11/RING4 dimer to be positioned in the endoplasmic reticulum membrane with its ATP-binding domains within the cytoplasm.

Sequence analysis of the hydrophobic domains from different transporters suggests that each domain usually contains 6 transmembrane regions, although some may contain 8 (15, 36–38). When hydrophilicity plots of RING11 and RING4 are compared, the hydrophobic regions are strikingly similar in shape and position (Fig. 4*b*). At least 6 potential transmembrane regions are predicted in each hydrophobic domain, although there may be up to 10 in RING4.

RING11 Polymorphism. To determine whether RING11 was polymorphic, we sequenced cDNA clones from additional cell lines. One such clone had several base pair substitutions, including two in the 3′ region of the gene that resulted in amino acid substitutions. One of these changed the putative stop codon and lengthened the protein by 17 amino acids (Fig. 5). The allele encoding the shorter protein was named *RING11A*, and the longer allele, *RING11B*. To determine the allele frequencies of *RING11A* and *RING11B*, we constructed oligonucleotide primers either side of the region containing the observed amino acid polymorphism. We then analyzed PCR products amplified from the genomic DNA of a number of homozygous HLA typing cell (HTC) lines and a panel of random Caucasoid controls. Allele frequencies are shown in Table 2.

Class I and class II MHC antigens are one of the most polymorphic families of proteins known, and it is intriguing that a putative peptide transporter located within the class II region also exhibits polymorphism. So far we have sequenced only a small number of full-length *RING11* cDNA clones, and it is possible that other polymorphisms exist. Nevertheless, we have already identified two major alleles of *RING11*. At present, we do not know whether these are functionally polymorphic with respect to peptide transport. In other transporter systems, mutations within both the hydrophobic and ATP-binding domains have been shown to affect function (17, 39–42). The RING11A/11B polymorphism lies at the extreme C terminus of the ATP-binding domain, outside its conserved core. When different transporters are compared, this part of the molecule is highly variable in sequence and length (43). Its function, if any, is not known, but it is of interest that RING11A has one of the shortest C-terminal regions within the transporter family.

The region between *DNA* and *DOB* is proving to be densely populated with genes. In addition to *RING11* and *RING4*, we have identified three other genes in the region, *RING9, -10*, and *-12* (26, 44). *RING10* and *-12* appear to correspond to the low molecular weight polypeptides (LMPs), originally described in the mouse, which are thought to be involved in the degradation of protein antigens into small peptides prior to transportation into the endoplasmic reticulum (25, 45). It seems remarkable that such a tightly linked cluster of antigen-processing genes should exist within the MHC. We suggest two possible explanations. First, their location may reflect a requirement for coregulation, perhaps by γ-interferon. Second, there may be an evolutionary advantage for the clustering of polymorphic genes within the MHC, thus ensuring that particular alleles are kept in combination.

An association between MHC antigens and various diseases, such as diabetes mellitus and celiac disease, is well described (46). As many of the genes within the MHC are in linkage disequilibrium, the possibility arises that some of these associations may primarily be with previously unknown MHC genes, such as *RING11* and *RING4*. It is important to investigate whether polymorphisms within *RING11* and other genes in the region are associated with MHC-linked disease.

Table 2. Frequencies of *RING11A* and *RING11B* alleles

Allele	HTCs (n = 36)	Caucasoids (n = 54)
RING11A	60/72 (83%)	85/108 (79%)
RING11B	12/72 (17%)	23/108 (21%)

Frequencies were determined from the PCR products of an HLA-homozygous typing cell (HTC) line panel and a panel of normal Caucasoid control individuals. The oligonucleotide primers GGGGATCGCACAGTGCTGGTG and CTGGAATTCAGGAACAGCTAT were used to amplify the region between positions 1954 and 2024, which contained the polymorphic residues shown in Fig. 5. HTCs were analyzed by using direct solid-phase sequencing. No other sequence variation was observed by using this method. Caucasoid controls were analyzed by oligonucleotide typing, using the following pairs of oligonucleotide probes: AGGCTGCAGACAGTTCAG and AGGCTGCAGGCAGTTCAG to detect variation at position 1993; CCTCCTAGAGCTGGGCAA and CCTCCTGGAGCTGGGCAA to detect variation at position 2059; and ATTCCCGCCTGGTGCAGC and ATTCCCGCCTGGTTCAGC to detect variation at position 2091.

We thank D. Simmonds for cDNA libraries and G. Blanck for the cosmids U10 and U15. We are grateful to C. Higgins for helpful discussion and access to unpublished information. S.H.P. is a Medical Research Council Clinician Scientist Fellow; L.-A.K. is funded by the Wellcome Trust.

1. Zinkernagel, R. M. & Doherty, P. C. (1974) *Nature (London)* **251,** 547–548.
2. Bjorkman, P. J., Saper, M. A., Samraoui, B., Bennett, W. S., Strominger, J. L. & Wiley, D. C. (1987) *Nature (London)* **329,** 512–518.
3. Brown, J. H., Jardetzky, T., Saper, M. A., Samraoui, B., Bjorkman, P. J. & Wiley, D. C. (1988) *Nature (London)* **332,** 845–850.
4. Neefjes, J. J., Stollorz, V., Peters, P. J., Geuze, H. J. & Ploegh, H. L. (1990) *Cell* **61,** 171–183.
5. Falk, K., Rotzschke, O., Stevanovic, S., Jung, G. & Rammensee, H. (1991) *Nature (London)* **351,** 290–296.
6. Townsend, A., Ohlen, C., Bastin, J., Ljunggren, H. G., Foster, L. & Karre, K. (1989) *Nature (London)* **340,** 443–448.
7. Townsend, A. & Bodmer, H. (1989) *Annu. Rev. Immunol.* **7,** 601–624.
8. Cerundolo, V., Alexander, J., Anderson, K., Lamb, C., Cresswell, P., McMichael, A., Gotch, F. & Townsend, A. (1990) *Nature (London)* **345,** 449–452.
9. DeMars, R., Rudersdorf, R., Chang, C., Petersen, J., Strandtmann, J., Korn, N., Sidwell, B. & Orr, H. T. (1985) *Proc. Natl. Acad. Sci. USA* **82,** 8183–8187.
10. Trowsdale, J., Hanson, I., Mockridge, I., Beck, S., Townsend, A. & Kelly, A. (1990) *Nature (London)* **348,** 741–744.
11. Deverson, E. V., Gow, I. R., Coadwell, W. J., Monaco, J. J., Butcher, G. W. & Howard, J. C. (1990) *Nature (London)* **348,** 738–741.
12. Monaco, J. J., Cho, S. & Attaya, M. (1990) *Science* **250,** 1723–1726.
13. Spies, T., Bresnahan, M., Bahram, S., Arnold, D., Blanck, G., Mellins, E., Pious, D. & DeMars, R. (1990) *Nature (London)* **348,** 744–747.
14. Higgins, C. F., Hyde, S. C., Mimmack, M. M., Gileadi, U., Gill, D. R. & Gallagher, M. P. (1990) *J. Bioenerg. Biomembr.* **22,** 571–592.
15. Hiles, I. D., Gallagher, M. P., Jamieson, D. J. & Higgins, C. F. (1987) *J. Mol. Biol.* **195,** 125–142.
16. Chen, C.-J., Chin, J. E., Ueda, K., Clark, D. P., Pastan, I., Gottesman, M. M. & Roninson, I. B. (1986) *Cell* **47,** 381–389.
17. Riordan, J. R., Rommens, J. M., Kerem, B.-S., Alon, N., Rozmahel, R., Grzelczak, Z., Zielenski, J., Lok, S., Plasvic, N., Chou, J.-L., Drumm, M. L., Iannuzzi, M. C., Collins, F. S. & Tsui, L.-C. (1989) *Science* **245,** 1066–1073.
18. Spies, T. & DeMars, R. (1991) *Nature (London)* **351,** 323–324.
19. Parham, P. (1991) *Nature (London)* **351,** 271–272.
20. Seed, B. (1987) *Nature (London)* **329,** 840–842.
21. Kendall, E., Sargent, C. A. & Campbell, R. D. (1990) *Nucleic Acids Res.* **18,** 7251–7257.
22. Sanger, F., Nicklen, S. & Coulson, A. R. (1977) *Proc. Natl. Acad. Sci. USA* **74,** 5463–5467.
23. Hultman, T., Stahl, S., Hornes, E. & Uhlen, M. (1989) *Nucleic Acids Res.* **17,** 4937–4946.
24. Bugawan, T. L., Begovich, A. B. & Erlich, H. A. (1990) *Immunogenetics* **32,** 231–241.
25. Parham, P. (1990) *Nature (London)* **348,** 674–675.
26. Glynne, R., Powis, S. H., Beck, S., Kelly, A., Kerr, L.-A. & Trowsdale, J. (1991) *Nature (London)* **353,** 357–360.
27. Staden, R. (1990) *CABIOS* **6,** 387–393.
28. Kyte, J. & Doolittle, R. F. (1982) *J. Mol. Biol.* **157,** 105–132.
29. Walker, J. E., Saraste, M., Runswick, M. J. & Gay, N. J. (1982) *EMBO J.* **1,** 945–951.
30. Mimura, C. S., Holbrook, S. R. & Ames, G. F.-L. (1991) *Proc. Natl. Acad. Sci. USA* **88,** 84–88.
31. Hyde, S. C., Emsley, P., Hartshorn, M. J., Mimmack, M. M., Gileadi, U., Pearce, S. R., Gallagher, M. P., Gill, D. R., Hubbard, R. E. & Higgins, C. F. (1990) *Nature (London)* **346,** 362–365.
32. Devereux, J., Haeberli, P. & Smithies, O. (1984) *Nucleic Acids Res.* **12,** 387–395.
33. Felmlee, T., Pellett, S. & Welch, R. A. (1985) *J. Bacteriol.* **163,** 94–105.
34. Kerppola, R. E., Shyamala, V. K., Klebba, P. & Ames, G. F.-L. (1991) *J. Biol. Chem.* **266,** 9857–9865.
35. Gallagher, M. P., Pearce, S. R. & Higgins, C. F. (1989) *Eur. J. Biochem.* **180,** 133–141.
36. Froshauer, S., Green, G. N., Boyd, D., McGovern, K. & Beckwith, J. (1988) *J. Mol. Biol.* **200,** 501–511.
37. Wang, R., Seror, S. J., Blight, M., Pratt, J. M., Broome-Smith, J. K. & Holland, I. B. (1991) *J. Mol. Biol.* **217,** 441–454.
38. Pearce, S. R., Mimmack, M. L., Gallagher, M. P., Gileadi, U., Hyde, S. C. & Higgins, C. F. (1992) *Mol. Microbiol.* **6,** 45–57.
39. Higgins, C. F., Haag, P. D., Nikaido, K., Ardeshir, F., Garcia, G. & Ames, G. F.-L. (1982) *Nature (London)* **298,** 723–727.
40. Payne, G., Spudich, E. N. & Ames, G. F.-L. (1985) *Mol. Gen. Genet.* **200,** 493–496.
41. Cutting, G. R., Kasch, L. M., Rosenstein, B. J., Zielenski, J., Tsui, L.-C., Antonarakis, S. E. & Kazazian, H. H. (1990) *Nature (London)* **346,** 366–369.
42. Safa, A., Stern, R. K., Choi, K., Agresti, M., Tamai, I., Mehta, N. D. & Roninson, I. B. (1990) *Proc. Natl. Acad. Sci. USA* **87,** 7225–7229.
43. Higgins, C. F., Hiles, I. D., Salmond, G. P. C., Gill, D. R., Downie, J. A., Evans, I. J., Holland, I. B., Gray, L., Buckel, S. D., Bell, A. W. & Hermondson, M. A. (1986) *Nature (London)* **323,** 448–450.
44. Kelly, A., Powis, S. H., Glynne, R., Radley, E., Beck, S. & Trowsdale, J. (1991) *Nature (London)* **353,** 667–668.
45. Monaco, J. J. & McDevitt, H. O. (1986) *Hum. Immunol.* **15,** 416–426.
46. Tiwari, J. L. & Terasaki, P. I. (1985) *HLA and Disease Associations* (Springer, New York).