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Isolation and characterization of yeast artificial chromosome clones linking the HLA-B and HLA-C loci

(human major histocompatibility complex class I genes/gene mapping/CpG islands)

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ABSTRACT **A 290-kilobase-pair chromosomal segment containing the genes encoding the human class I major histocompatibility complex molecules HLA-B and HLA-C as well as a class I pseudogene has been isolated on three overlapping yeast artificial chromosome (YAC) clones. One YAC clone contains both the HLA-B and HLA-C genes. These loci are located ≈85 kilobase pairs apart, each in close association with a CpG island. Southern blotting and nucleotide sequencing showed no evidence of alteration of the structure of the cloned DNA in the YACs. End fragments from the YAC inserts have been isolated and used to confirm the overlaps between clones. These fragments can also serve as polymorphic markers for structural analysis of the major histocompatibility complex. Our data show that YAC cloning offers an attractive alternative for analysis of the structures of large gene complexes such as HLA.**

The human major histocompatibility complex (MHC) is located on the short arm of chromosome 6 and is estimated to span 3.5–4.0 million base pairs of genomic DNA (1–3). The class I region of the MHC encodes a family of structurally related, 44-kDa glycoproteins that associate with the non-MHC molecule β_2-microglobulin. The most extensively studied molecules of the class I family are the classical transplantation antigens, HLA-A, -B, and -C. These highly polymorphic molecules can be detected serologically and are found on the surfaces of essentially all nucleated cells, where they serve as the restricting elements for antigen recognition by cytotoxic T lymphocytes (4–6). Southern blotting of genomic DNA with a class I gene probe detects 18 cross-hybridizing restriction fragments (7). All of these sequences have been cloned and characterized, describing a group of related sequences including intact genes, full-length pseudogenes, and gene fragments. Among these, in addition to the classical transplantation antigens, three more functional class I molecules have been identified, HLA-E (8), -F (9), and -G (10). They show little polymorphism and their expression is limited in a highly tissue-specific manner (8–12).

The arrangement of the class I loci within the MHC has been studied by analysis of recombination within informative pedigrees (13) and of irradiation-induced HLA-loss mutants (14) as well as by pulsed-field gel electrophoresis (1–3). These studies predict that the class I region spans at least 1000 kilobase pairs (kb). The centromeric boundary of the class I region is usually defined by the HLA-B gene. The telomeric boundary is not precisely defined.

The isolation of cosmid clones containing the class I genes and pseudogenes has allowed some molecular linkages to be determined. For example, HLA-B and a class I pseudogene, HLA-1.7p, are found on a single cosmid clone (7). However, most of the class I sequences, including the functional class I loci, appear to be widely spaced within the complex. Pulsed-field mapping studies of genomic DNA indicate that the most closely linked functional loci, HLA-B and HLA-C, are separated by 80–130 kb (1–3, 15, 16). While pulsed-field mapping studies may establish the physical relationship of linked loci, they do not provide access to the genomic DNA between the loci themselves. Additionally, because of the variable degree of methylation of genomic DNA, the restriction maps that they provide identify only a subset of the relevant restriction sites. Because of the wide spacing of the known loci in the class I region, conventional cloning with bacteriophage or cosmid vectors may not be the ideal method for isolation and detailed mapping of the intergenic regions. Yeast artificial chromosome (YAC) cloning affords an attractive alternative for analysis of the structure of the class I region of the MHC because of the potential for YAC clones to carry large inserts (17).**

MATERIALS AND METHODS

Reagents and Cell Lines. Restriction enzymes were from New England Biolabs. *Thermus aquaticus* (*Taq*) DNA polymerase was from Perkin–Elmer/Cetus. Lyticase was from Sigma. CGM1 is an Epstein–Barr virus-transformed B-lymphoblastoid cell line derived from the YAC library donor. MCH6 (18) is a human chromosome 6-containing mouse microcell hybrid (kindly provided by Sherman M. Weissman, Yale University School of Medicine). Cell line 9022 was from the American Society of Histocompatibility and Immunogenetics (Boston) cell panel.

Isolation of YAC Clones. Clones were isolated from the YAC library of the Center for Genetics in Medicine at Washington University School of Medicine (St. Louis, MO) (19, 20). This library was constructed using the pYAC4 vector (17) and partially *Eco*RI-digested high molecular weight DNA from peripheral blood leukocytes of a healthy male Caucasian donor. HLA typing of this donor and informative family members by the Barnes Hospital Histocompatibility Laboratory (St. Louis, MO) provided the haplotype assignments A3, B8, C−, DR3, DQw2, DRw52 and A29, B14, C−, DR7, DQw2, DRw53.

The YAC library has been successfully screened both by colony hybridization and by polymerase chain reaction (PCR) (21). In these experiments, class I-positive clones were identified by colony hybridization at moderate stringency (22) with an HLA-B cDNA probe (kindly provided by Ben-

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Abbreviations: YAC, yeast artificial chromosome; MHC, major histocompatibility complex; PCR, polymerase chain reaction.

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**The sequences reported in this paper have been deposited in the GenBank data base (accession nos. M59840, M59841, and M59865).

jamin D. Schwartz, Washington University School of Medicine, St. Louis, MO).

Preparation and Analysis of Yeast DNA. DNA of yeast strains carrying YACs were prepared as described by Treco (23). This DNA was suitable for analysis by conventional Southern blotting (24) and by PCR (25). For long-range restriction fragment analysis, high molecular weight YAC DNA was prepared in agarose plugs (19, 20). Plugs containing ≈10 μg of this DNA were digested with 100 units of the indicated restriction enzymes under the conditions recommended by the suppliers for ≈18 hr. Digested DNAs were fractionated by contour-clamped homogeneous electric field (CHEF) gel electrophoresis (26) and transferred to nylon membranes. Gels were prepared for transfer by exposure to a short-wavelength UV light source for 90 sec, followed by denaturation in 0.4 M NaOH/0.6 M NaCl and neutralization in 1 M Tris·HCl, pH 7.5/0.6 M NaCl. Transfer was in 6× SSC (1× SSC = 0.15 M NaCl/0.015 M sodium citrate). DNA probes were labeled to high specific activity with ^{32}P by nick-translation (22). Hybridization was as described (27) and washing was in 0.2× SSC/0.1% $NaDodSO_4$ at 25°C prior to autoradiography.

Nucleotide Sequencing of YAC-Derived HLA Class I Genes. HLA-B and HLA-C gene sequences were amplified from their respective YACs by using PCR and an HLA-B/C crossreacting primer (CCGGAATTCTCGGGCGGGTCTGAGCCCCT) at the 5′ ends and HLA-B-specific (CCCAAGCTTCCCGGCGACCTATAGGAGATG) and HLA-C-specific (CCCAAGCTTCCGGGAGATCTACTGGAGATG) primers at the 3′ ends. The 5′ primer introduces an *Eco*RI restriction site (underlined) and the 3′ primers introduce a *Hin*dIII restriction site (underlined) into the PCR products. PCR was performed using 1 μg of total DNA from YAC-containing clones as the template, with annealing at 65°C for 2 min and extension at 72°C for 3 min for 30 cycles. The PCR-amplified material was extracted with phenol and chloroform, ethanol-precipitated, and then digested with *Eco*RI and *Hin*dIII and subcloned into bacteriophage M13 mp18 and mp19. Single-stranded phage DNA was sequenced using the dideoxynucleotide chain-termination technique (28) and T7 DNA polymerase (United States Biochemical).

Isolation of YAC-Insert End Fragments. Subclones containing end fragments from the YAC inserts were prepared by double digestion of DNA from the YAC-containing strains with either *Cla* I or *Sal* I, which cut within the left or right arm of the pYAC4 vector, respectively, and an enzyme that cuts within the genomic insert, chosen by its ability to produce a conveniently sized restriction fragment. The digested DNA was fractionated by electrophoresis in an agarose gel and fragments in the appropriate size range were excised and purified using Gene Clean (Bio 101, La Jolla, CA). The gel-purified fragments were subcloned into pUC19, and transformants containing the end-fragments were identified by colony hybridization using probes corresponding to positions 375–656 or 657–895 of pBR322, identifying the left or right YAC vector arm, respectively. Restriction fragments devoid of repetitive sequences were recovered from these subclones for use as probes in Southern blotting experiments.

RESULTS AND DISCUSSION

To demonstrate the utility of YAC cloning for an analysis of the structure of the human MHC, we have isolated YAC clones containing the HLA-B and HLA-C genes and the intervening and flanking genomic DNA by screening the YAC library of the Center for Genetics in Medicine at Washington University (19, 20). Initial screening was by hybridization with a class I-crossreacting HLA-B cDNA probe. From among the HLA class I-positive clones, clones containing the HLA-B and HLA-C loci were identified by Southern blotting to detect characteristic HLA-B- and HLA-C-specific restriction fragments (7, 29). In this study, we have characterized three overlapping YAC clones from this region. A single YAC of 210 kb (designated B38D3) contains both the HLA-B and HLA-C genes. A YAC of 130 kb (B92H5) contains the HLA-B gene and the closely linked pseudogene, HLA-1.7p. The third YAC (B209D7) contains the HLA-C locus and 180 kb of DNA telomeric to the HLA-C locus.

Restriction Fragment Analysis of YAC Clones. To demonstrate the integrity of the class I sequences within these YAC clones, we performed Southern analysis comparing the isolated YACs to cosmid clones containing HLA-B, -C, and -1.7p and to genomic DNA from an Epstein–Barr virus-transformed cell line derived from the library donor (CGM1). A characteristic pattern of several cross-hybridizing class I sequences is seen in genomic DNA, with the HLA-B and HLA-C genes found on 6.5- and 8-kb *Eco*RI restriction fragments, respectively (29). YAC B38D3 contains both the HLA-B and HLA-C genes (Fig. 1*a*). YAC B209D7 contains only the HLA-C gene, and YAC B92H5 contains both the HLA-B gene and the HLA-1.7p pseudogene, which is found within the 27-kb *Eco*RI restriction fragment. Digestion of the YAC clones with *Hin*dIII (Fig. 1*b*) shows that the HLA-B and HLA-C genes are each present on ≈27-kb fragments (7). The HLA-B *Hin*dIII fragment in YAC B92H5 is ≈23 kb, smaller than the fragments in YACs B38D3 and B209D7. This smaller fragment is also seen in CGM1 and represents allelic variation of the HLA-B locus (see Fig. 2*a*). YAC B92H5 contains, in addition to the HLA-B-specific fragment, the expected 1.7-kb *Hin*dIII fragment containing the HLA-1.7p pseudogene. In all cases, the class I-hybridizing fragments in the YAC DNAs comigrate with fragments in the CGM1 DNA.

Nucleotide Sequence Analysis of YAC-Encoded Class I Genes. To confirm the identity of the HLA-B and HLA-C loci and to determine which alleles were present and thus from which donor chromosome each YAC clone was derived, DNA fragments spanning exons 2 and 3 of the genes (encoding the polymorphic α1 and α2 domains of the class I heavy chain) were amplified by PCR from each of the three YACs, subcloned into M13 mp18 and mp19, and sequenced. The nucleotide sequence of this portion of the HLA-B gene (Fig. 2*a*) from YAC B38D3 is identical to the reported sequence for

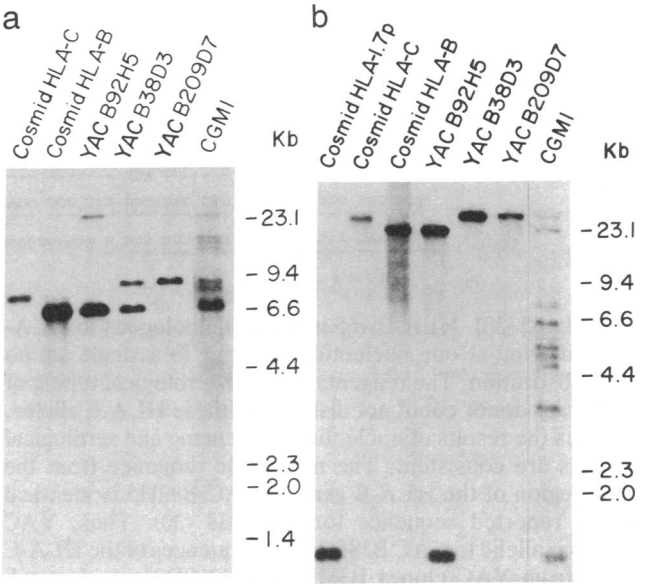

FIG. 1. Southern blot analysis of YAC and cosmid clones. Cosmid (0.2 ng), YAC (0.2 μg), or CGM1 (15 μg) DNA was digested with *Eco*RI (*a*) or *Hin*dIII (*b*), fractionated by electrophoresis in 1% agarose gels, and analyzed by Southern blotting using a 350-base-pair *Eco*RI–*Pst* I fragment from an HLA-B cDNA (22, 27). Washing was in 0.2× SSC/0.1% $NaDodSO_4$ at 65°C.

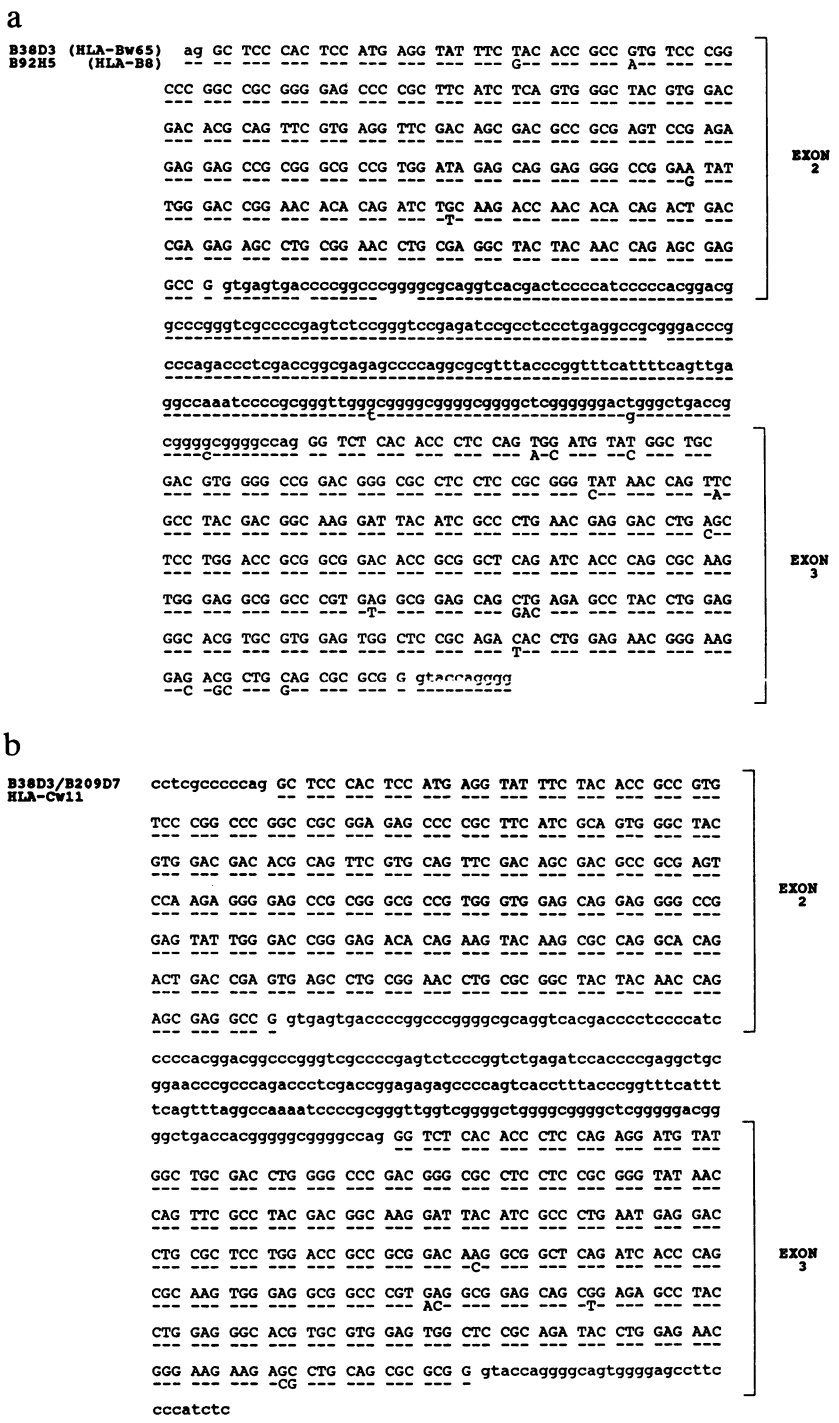

FIG. 2. Nucleotide sequences of YAC-derived HLA-B and HLA-C genes. The α1 (exon 2) and α2 (exon 3) domains of the HLA-B and HLA-C genes were amplified from the YAC clones by PCR. Exon sequences are designated by uppercase and intron sequences by lowercase letters. (*a*) DNA sequence of the HLA-B gene amplified from YAC B38D3 is identical to that of HLA-Bw65 (30) and is compared with the DNA sequence of the HLA-B gene amplified from the HLA-B8 YAC B92H5. Dashes indicate identity between the two sequences. (*b*) DNA sequences of the HLA-C genes amplified from YACs B38D3 and B209D7 are identical and are 98.9% identical to the nucleotide sequence of HLA-Cw11 (30).

HLA-Bw65 (30). HLA-Bw65 is highly homologous to HLA-B14, differing at one nucleotide resulting in a single amino acid substitution. The reagents used for serological typing of the library donor could not distinguish these HLA-B alleles, and thus the results of nucleotide sequencing and serological analysis are consistent. The nucleotide sequence from the same region of the HLA-B gene in YAC B92H5 is identical to the reported sequence for HLA-B8 (30). Thus, YAC B92H5 is allelic to YAC B38D3. The sequences of the HLA-C genes from YAC clones B38D3 and B209D7 are identical, consistent with these two YACs having been derived from the same donor chromosome (Fig. 2*b*). They are highly homologous to the sequence for HLA-Cw11 (30). These data confirm that the class I gene sequences in these three YAC clones represent the HLA-B and HLA-C loci and support the results of serological typing of the loci. Additionally, they show that both donor chromosomes are represented in the YAC clones spanning this region and identify the donor chromosome from which each YAC is derived.

Restriction Mapping of the HLA-B/C YACs. Partial restriction maps of these class I-containing YAC clones are shown in Fig. 3. The restriction endonucleases chosen for this analysis represent a group whose recognition sequences are C+G-rich and contain the dinucleotide CpG. In addition these enzymes are sensitive to 5-methylcytosine in the CpG sequence. In vertebrate DNA, unmethylated recognition sequences for these endonucleases are relatively rare and, when clustered, often indicate the presence of constitutively expressed genes (31, 32). In yeast, methylation of cytosines is not observed (33). Consequently, these enzymes typically cleave human YACs more frequently than the corresponding region of genomic DNA. This may complicate comparisons

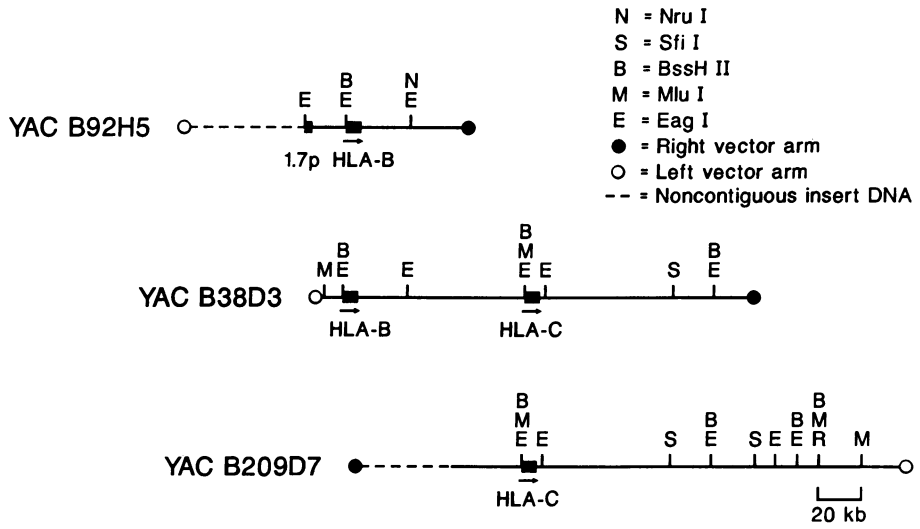

FIG. 3. Partial restriction map of HLA-B and HLA-C YACs. Data were obtained by hybridizing Southern blots of yeast DNA digested with infrequently cutting restriction endonucleases to probes for HLA-B, a locus-specific HLA-C-flanking sequence, Blur11 (Alu), the 350-base-pair *Eco*RI–*Bam*HI fragment of pBR322 (left vector arm), the 1.1-kb *Bam*HI–*Ava* I fragment of pBR322 (right vector arm), and end-fragment probes from the YAC B92H5, B38D3, and B209D7 inserts.

of the restriction maps of YAC clones with preexisting pulsed-field maps prepared using uncloned genomic DNA. It may, however, facilitate the recognition of CpG islands and permit accurate determination of fragment sizes. Our studies show that the HLA-B and HLA-C loci are separated by 85 kb (Fig. 3), a distance consistent with that proposed by previous pulsed-field mapping experiments (1–3, 15, 16). As observed in studies using isolated cosmid clones, both loci are associated with CpG islands, with the islands marking the 5′ end of each class I gene (15, 16). Together with our data this indicates that each gene is located telomeric of these islands and transcribed in a centromeric-to-telomeric orientation.

Although the restriction maps of YAC clones derived from the same donor chromosome are internally consistent, apparently allelic differences in restriction maps can be found. For example, YAC B38D3 (HLA-Bw65) and YAC B92H5 (HLA-B8) each have a site that is not present in the other clone. YAC B92H5 contains an *Nru* I site 40 kb telomeric of the HLA-B gene that is not found in YAC B38D3. YAC B38D3, but not YAC B92H5, contains an *Mlu* I site 5 kb centromeric of the HLA-B gene. Given the high level of polymorphism associated with this region of the genome and in particular with the 5′ regions of the class I genes, it is likely that these CpG-rich restriction sites are polymorphic.

Analysis Using YAC-Insert End Fragments. A critical step in the analysis of each YAC clone is the isolation of unique-sequence probes from the ends of the genomic insert. These end probes are necessary for analysis of overlapping clones and for "chromosome walking." In addition, they are valuable reagents for detecting the presence of noncontiguous DNA in the YAC insert. We have identified end-fragment probes from both the right and the left end of each of the YACs described here. An example of the use of these probes to demonstrate overlaps between YAC clones is shown in Fig. 4*a*, by hybridization of *Eco*RI-digested YAC and genomic DNA with a probe derived from the end of the YAC B38D3 insert adjacent to the right arm of the YAC vector. A single hybridizing fragment was detected in YAC B38D3, YAC B209D7, CGM1, and a microcell hybrid cell line containing only human chromosome 6 in a mouse background. No hybridization was seen in DNA from YAC B92H5. This, then, is the telomeric end of the B38D3 insert. When the DNA samples were digested with *Hin*dIII, this probe hybridized to two allelic restriction fragments of 15 and 9 kb in CGM1 DNA (data not shown). The 9-kb allelic fragment is derived from the HLA-Bw65 haplotype, and the 15-kb fragment is from the HLA-B8 haplotype.

Hybridization using restriction fragments derived from the left end of the YAC B38D3 insert failed to identify any unique-sequence probes; however, given that the 5′ end of the HLA-B gene is within 10 kb of the left vector arm in YAC B38D3, failure to isolate a probe from the exact end of the left portion of the insert is of little consequence in assembling the map of this region.

Analysis of end clones from YAC B92H5 shows that the fragment from the right end of YAC B92H5 is telomeric and

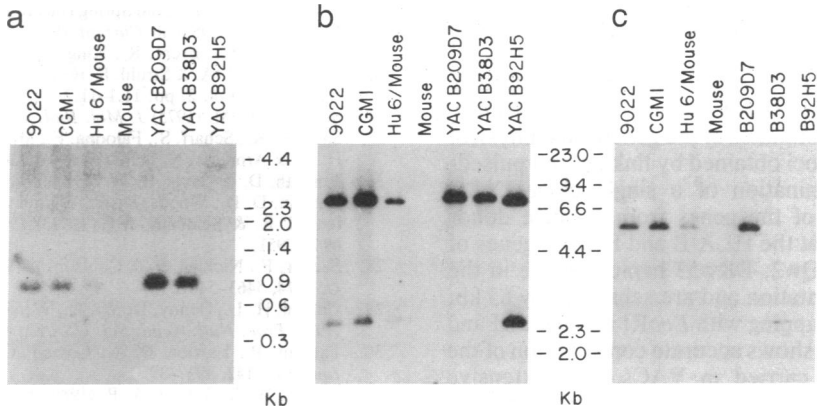

FIG. 4. Demonstration of overlap between YAC clones by hybridization with end fragments from YACs B38D3, B92H5, and B209D7. DNA samples from the indicated YAC clones (0.2 μg) or from mammalian cell lines (15 μg) were digested with *Eco*RI and analyzed by Southern blot hybridization with a right end probe from YAC B38D3 (0.8-kb *Pst* I fragment) (*a*), right end probe from YAC B92H5 (0.7-kb *Eco*RI–*Pst* I fragment) (*b*), and a left end probe from YAC B209D7 (0.5-kb *Pst* I fragment) (*c*).

identifies an *Eco*RI polymorphism (Fig. 4*b*). One allele is represented by an ≈8.5-kb fragment, as seen with YACs B209D7 and B38D3. The other allele is represented by two *Eco*RI fragments, ≈8 kb and ≈2.5 kb. The two-fragment pattern can be seen in YAC B92H5 and the chromosome 6-specific microcell hybrid, as well as in the A1, B8, DR3 homozygous cell line 9022. Both patterns are visible in CGM1 DNA. This agrees with the sequence from the HLA-B gene in YAC B92H5, which was identical to the published sequence for HLA-B8 (Fig. 2*a*). This probe also identifies polymorphic fragments with several other restriction enzymes (data not shown). This may be a valuable probe for analysis of HLA restriction fragment length polymorphism in normal and disease populations. It is in close physical linkage with both the HLA-B and HLA-C loci but is located far enough away from the genes themselves to recognize single-copy sequences when used for hybridization at moderate stringency.

In contrast to this right end-fragment probe, fragments from the left end of the B92H5 insert do not hybridize to human chromosome 6 DNA (data not shown). Thus, YAC B92H5 is composed of noncontiguous genomic DNA fragments joined within the insert. The junction between the chromosome 6 sequences and the non-chromosome 6 sequences is undefined, but it lies between the HLA-1.7p locus and the left vector arm.

A hybridization probe generated from the left end of YAC B209D7 hybridizes to an ≈6-kb *Eco*RI fragment in YAC B209D7, CGM1, and the chromosome 6-specific microcell hybrid DNA (Fig. 4*c*). This telomeric end clone has been sequenced and a PCR assay has been developed in order to rescreen the YAC library for clones that will link the HLA-B and HLA-C loci with the other cloned class I sequences. While this fragment from the left end of YAC B209D7 hybridizes to chromosome 6-specific DNA samples, a fragment from the right end of this clone is not derived from chromosome 6, again indicating the presence of noncontiguous genomic fragments within the insert of this clone (data not shown). The discontinuity in the YAC B209D7 insert can also be detected by comparison of the restriction map of its insert with that of the overlapping clone, YAC B38D3 (Fig. 3). Between the telomeric end of clone B38D3 and the HLA-C locus, YAC B38D3 and YAC B209D7 show no detectable differences in structure as assessed with infrequently cutting restriction enzymes; however, centromeric of HLA-C, their respective restriction maps diverge. Because the right end fragment of YAC B92H5 hybridizes to YAC B209D7, the discontinuity in YAC B209D7 must lie centromeric to this sequence. Our data underscore the need to analyze YAC clones carefully for the presence of noncontiguous insert fragments. In this regard, during analysis of additional YACs from other portions of the MHC, we have observed a frequency of clones with noncontiguous inserts of 60–65% (data not shown).

The YACs analyzed in these studies provide a molecular linkage of the HLA-B and HLA-C genes. These molecular clones confirm previous estimates of the genetic and physical distances between these loci obtained by linkage and pulsed-field gel analyses. Examination of a single clone, YAC B38D3, containing both of the genes from a single donor chromosome indicates that the HLA-B and HLA-C genes of the A29, Bw65, DR7, DQw2, DRw53 haplotype are in the same transcriptional orientation and are separated by 85 kb. Analysis by restriction mapping with *Eco*RI and *Hin*dIII and by nucleotide sequencing shows accurate conservation of the structures of the genes carried in YACs. The extensive overlap of YACs B92H5 and B209D7 with YAC B38D3 confirms the structure of B38D3 and supports the fidelity of the YAC cloning technology. These clones provide ready access to the HLA-B and HLA-C genes themselves, to the intergenic DNA, and to 180 kb of DNA telomeric of the HLA-C locus. Finally, end clones isolated from these YACs are valuable mapping and walking tools in the further analysis of this large multigene family in the human MHC.

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1. Carroll, M. C., Katzman, P., Alicot, E. M., Koller, B. H., Geraghty, D. E., Orr, H. T., Strominger, J. L. & Spies, T. (1987) *Proc. Natl. Acad. Sci. USA* **84**, 8535–8539.
2. Dunham, I., Sargent, C. A., Trowsdale, J. & Campbell, R. D. (1987) *Proc. Natl. Acad. Sci. USA* **84**, 7237–7241.
3. Lawrance, S. K., Smith, C. L., Srivastava, R., Cantor, C. R. & Weissman, S. M. (1987) *Science* **235**, 1387–1390.
4. Zinkernagel, R. M. & Doherty, P. C. (1974) *Nature (London)* **248**, 701–702.
5. Nathenson, S. G., Geliebter, J., Pfaffenbach, G. M. & Zeff, R. A. (1986) *Annu. Rev. Immunol.* **4**, 471–502.
6. Morrison, L. A., Braciale, V. L. & Braciale, T. J. (1986) *Immunol. Res.* **5**, 294–304.
7. Koller, B. H., Geraghty, D., Orr, H. T., Shimizu, Y. & DeMars, R. (1987) *Immunol. Res.* **6**, 1–10.
8. Koller, B. H., Geraghty, D. E., Shimizu, Y., DeMars, R. & Orr, H. T. (1988) *J. Immunol.* **141**, 897–904.
9. Geraghty, D. E., Wei, X. H., Orr, H. T. & Koller, B. H. (1990) *J. Exp. Med.* **171**, 1–18.
10. Geraghty, D. E., Koller, B. H. & Orr, H. T. (1987) *Proc. Natl. Acad. Sci. USA* **84**, 9145–9149.
11. Srivastava, R., Chorney, M. J., Lawrance, S. K., Pan, J., Smith, Z., Smith, C. L. & Weissman, S. M. (1987) *Proc. Natl. Acad. Sci. USA* **84**, 4224–4228.
12. Kovats, S., Main, E. K., Librach, C., Stubblebine, M., Fisher, S. J. & DeMars, R. (1990) *Science* **248**, 220–223.
13. Orr, H. T. & DeMars, R. (1983) *Immunogenetics* **18**, 489–502.
14. Koller, B. H., Geraghty, D. E., DeMars, R., Duvick, L., Rich, S. S. & Orr, H. T. (1989) *J. Exp. Med.* **169**, 469–480.
15. Chimini, G., Pontarotti, P., Nguyen, C., Toubert, A., Boretto, J. & Jordan, B. R. (1988) *EMBO J.* **7**, 395–400.
16. Pontarotti, P., Chimini, G., Nguyen, C., Boretto, J. & Jordan, B. R. (1988) *Nucleic Acids Res.* **16**, 6767–6778.
17. Burke, D. T., Carle, G. F. & Olson, M. V. (1987) *Science* **236**, 806–812.
18. Trent, J. M., Stanbridge, E. J., McBride, H. L., Meese, E. U., Casey, G., Araujo, D. E., Witkowski, C. M. & Nagle, R. B. (1990) *Science* **247**, 568–571.
19. Little, R. D., Porta, G., Carle, G. F., Schlessinger, D. & D'Urso, M. (1989) *Proc. Natl. Acad. Sci. USA* **86**, 1598–1602.
20. Brownstein, B. H., Silverman, G. A., Little, R. D., Burke, D. T., Korsmeyer, S. J., Schlessinger, D. & Olson, M. V. (1989) *Science* **244**, 1348–1351.
21. Green, E. D. & Olson, M. V. (1990) *Proc. Natl. Acad. Sci. USA* **87**, 1213–1217.
22. Maniatis, T., Fritsch, E. F. & Sambrook, J. (1982) *Molecular Cloning: A Laboratory Manual* (Cold Spring Harbor Lab., Cold Spring Harbor, NY).
23. Treco, D. A. (1989) in *Current Protocols in Molecular Biology*, eds. Ausubel, F. M., Brent, R., Kingston, R. E., Moore, D. D., Seidman, J. G., Smith, J. A. & Struhl, K. (Greene, New York; Wiley-Interscience, New York), Vol. 2, pp. 11.1–11.2.
24. Southern, E. M. (1975) *J. Mol. Biol.* **98**, 503–517.
25. Saiki, R. K., Scharf, S., Faloona, F., Mullis, K. B., Horn, G. T., Erlich, H. A. & Arnheim, N. (1985) *Science* **230**, 1350–1354.
26. Vollrath, D. & Davis, R. W. (1987) *Nucleic Acids Res.* **15**, 7865–7876.
27. Chaplin, D. D., Woods, D. E., Whitehead, A. S., Goldberger, G., Colten, H. R. & Seidman, J. G. (1983) *Proc. Natl. Acad. Sci. USA* **80**, 6947–6951.
28. Sanger, F., Nicklen, S. & Coulson, A. R. (1977) *Proc. Natl. Acad. Sci. USA* **74**, 5463–5467.
29. Coppin, H. L., Denny, D. W., Jr., Weissman, S. M. & McDevitt, H. O. (1985) *Proc. Natl. Acad. Sci. USA* **82**, 8614–8618.
30. Parham, P., Lawlor, D. A., Lomen, C. E. & Ennis, P. D. (1989) *J. Immunol.* **142**, 3937–3950.
31. Brown, W. R. & Bird, A. P. (1986) *Nature (London)* **322**, 477–481.
32. Lindsay, S. & Bird, A. P. (1987) *Nature (London)* **327**, 336–338.
33. Proffitt, J. H., Davie, J. R., Swinton, D. & Hattman, S. (1984) *Mol. Cell Biol.* **4**, 985–988.