Proc. Natl. Acad. Sci. USA
Vol. 81, pp. 2816–2820, May 1984
Genetics

Orientation of loci within the human major histocompatibility complex by chromosomal *in situ* hybridization

(human leukocyte antigen/gene mapping)

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Contributed by Jack L. Strominger, December 29, 1983

ABSTRACT **We have determined the localization and orientation of two genetic probes within the human major histocompatibility complex by chromosomal *in situ* hybridization. Our data indicate that a cloned genomic probe cross-hybridizing to HLA-A, -B, and -C heavy chain loci is homologous to sequences located on chromosome 6 at band p21.3 while a subclone of the genomic HLA-DR α-chain gene corresponding to the nonpolymorphic p34 protein is homologous to sequences in band 6p21.1. Our data suggest that this technique may permit the estimation of map distances between linked gene loci, assuming a uniform frequency of map units in the human genome. The relative positions of these genes was confirmed in a mother and son carrying a chromosome rearrangement involving 6p and 14p in which the sequences hybridizing to a DR α-chain genomic clone were found at the distal end of the 6p− chromosome [der(6)] while the sequences hybridizing to the HLA-A, -B, -C α-chain probe were found in the 14p+ chromosome [der(14)].**

The major histocompatibility complex (MHC) in humans is a cluster of at least three gene families encoding cell surface glycoproteins—i.e., genes for class I (HLA-A, -B, and -C) and class II (HLA-DR) antigens in addition to class III genes (complement factors)—and spans 2–3 × 10^3 kilobase pairs (kb) of DNA (1, 2). Genetic mapping by somatic cell hybridization (3–7) and by the segregation of unique chromosomal rearrangements involving the MHC in families (8–19) has permitted localization of the serologically polymorphic 45,000-dalton glycoproteins of the class I genes (HLA-A, -B, and -C) to the short arm of human chromosome 6, with the shortest region of overlap being the 6p21 band (7, 17). Associated with the polymorphic glycoproteins of the HLA-A, -B, and -C antigens is a nonglycosylated 12,000-dalton invariant chain, β_2-microglobulin, that has been assigned to human chromosome 15 (20, 21). The class II genes, or the immune response genes, consist of heterodimers composed of a 34,000-dalton (HLA-DR α) glycoprotein for which no electrophoretic polymorphisms have been demonstrated and a polymorphic 29,000-dalton (HLA-DR β) glycopeptide (21–25). Polymorphism of the HLA-DR β gene has permitted a regional assignment of this locus to chromosome 6 proximal to 6p22.4 by a family study (15).

Recently, the HLA-DR α-chain locus has been mapped by using somatic cell hybrids (26) and, furthermore, this assignment has been refined to the region 6p2105–6p23 (27) by study of genomic blotting patterns in cell lines containing individual chromosomal deletions of the short arm of chromosome 6. Data on recombination within the HLA complex and with closely linked markers such as glyoxylase 1 (GLO 1) have permitted inferences to be drawn about the order and map distances between marker loci on the proximal portion of the short arm of chromosome 6, as follows: GLO 1—4 centimorgans (cM)—HLA-DR—0.3 cM—BF, C4B, C4A, C2—0.7 cM—HLA-B—0.1 cM—HLA-C—0.7 cM—HLA-A (16–18).

Improvements in chromosomal *in situ* hybridization have permitted the assignment of several gene loci and unspecified DNA segments (28–49). Application of this technique to cloned fragments of the human MHC may permit further elucidation of the chromosomal structure of that region and provide new insight into its protean effects on cell–cell interactions and disease susceptibilities. We have used this technology to determine the precise chromosomal localization and polarity of two gene clusters in the MHC (class I and class II genes) utilizing metaphase chromosomes from normal individuals and from two individuals in a family with a presumably balanced chromosomal rearrangement of 6p and 14p. In addition, we have investigated the usefulness of this procedure in obtaining estimates of map distances between genes albeit with the assumption that there is a uniform distribution of recombination in the human karyotype.

METHODS

Cloned DNA Fragments. Recombinant plasmids containing inserts of genomic DNA homologous to class I and class II HLA antigens were used as hybridization probes. Plasmid pHLA-7 contains a 5.8-kilobase (kb) insert in the *Eco*RI site of pBR322 on which a complete human class I heavy chain gene is located. This gene was isolated from a human B-cell lymphoma genomic library (6410, kindly supplied by P. Leder and M. Nau) constructed in phage Charon 4A from genomic DNA partially digested with *Hae* III and inserted into the cloning vector with *Eco*RI oligonucleotide linkers according to previously published techniques (50). Digestion of pHLA-7 with *Sac* I generates two fragments: a 2-kb *Sac* I fragment that hybridizes with pHLA-1, a 513-base-pair (bp) cDNA clone that corresponds to the COOH-terminal 46 amino acids of an HLA class I heavy chain plus a portion of the 3′-noncoding region of the mRNA (2), and a 900-bp *Sac* I fragment homologous to a cDNA clone encoding the HLA-B7 antigen (51). Plasmid pUR5 (3.2R1), hereafter referred to as pDRα (52), contains a 3.2-kb insert in the *Eco*RI insert site of pUR222 (53) on which a fragment of an HLA-DR heavy chain (p34) gene is located. This fragment was isolated from a human genomic library by cross-hybridization with an

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Abbreviations: MHC, major histocompatibility complex; kb, kilobase pair(s); cM, centimorgan(s); bp, base pair(s); der, derivative.

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HLA-DR heavy chain cDNA clone (54). Both pHLA-7 and pDRα inserts were free of reiterated sequences.

Midmetaphase Chromosome Preparations. Midmetaphase chromosome preparations were made from peripheral blood lymphocyte cultures established from normal human males according to a modification of the method of Moorhead *et al.* (55). Chromosome preparations were also made from a mother and son both of whom are carriers of a presumably balanced chromosome rearrangement involving the short arms of chromosomes 6 and 14. Karyotype analysis revealed a direct insertion of a portion of 6p into the short arm of chromosome 14, and this rearrangement may be described in the mother as 46,XX,dir ins(14;6)(14pter → 14p11::6p22 → 6p21.1::14p11 → 14qter; 6pter → 6p22::6p21.1 → 6qter) and in her son as 46,XY,dir ins(14;6)mat (37). Slides were prepared and aged in a dessicator at room temperature for at least 2 months prior to use in the mapping studies.

Chromosomal *in Situ* Hybridization and Gene Mapping. *In situ* hybridization was carried out as described (36) with recombinant plasmids nick-translated with tritium to a specific activity of $\approx 2.0 \times 10^7$ cpm/μg. After autoradiography, the slides were stained with quinacrine mustard dihydrochloride. Slides prepared from the lymphocytes from normal males were analyzed by an observer who was unaware of the identity of the DNA fragment used in the hybridization. Grain distribution was recorded with reference to a metaphase chromosome idiogram at the 400-band stage (56).

RESULTS

Chromosomal Localization of the pHLA-7 and pDRα Probes on Metaphase Chromosomes from Normal Human Males. Data on the overall distribution of silver grains were collected from 53 metaphases hybridized with pHLA-7 and 67 metaphases hybridized with pDRα. In the metaphase spreads hybridized with pHLA-7 (Fig. 1 *A* and *B*), 45% contained at least one chromosome 6 with a silver grain located at 6p21.3 and 24% of all the grains were located at that site (Fig. 2*A*). In the spreads hybridized with pDRα (Fig. 1 *C* and *D*), 58% contained at least one chromosome 6 with a grain located at 6p21.1 and 20% of all grains were found there (Fig. 2*B*). If the relative size of a single band in a 400-band chromosome idiogram (56) is used to estimate the expected number of grains at a single site, assuming a Poisson distribution (57), these data provide compelling evidence for an aggregation of grains at 6p21.3 for pHLA-7 and at 6p21.1 for pDRα.

The distance from the centromere to the center of silver grains lying on or adjacent to 6p was measured in photographic enlargements of two samples of 36 chromosomes hy-

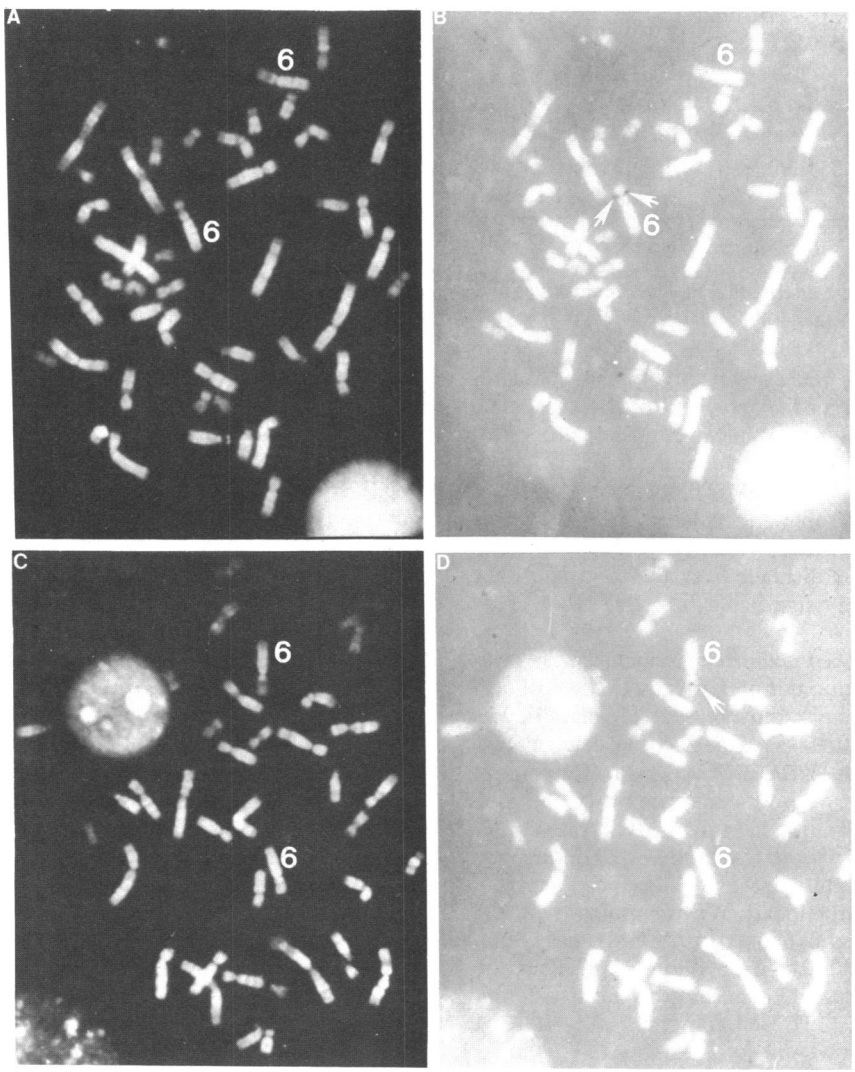

FIG. 1. Photographs illustrating the more distal localization of the pHLA-7 probe (*A* and *B*) and the more proximal localization of the pDRα probe (*C* and *D*) in band 6p21. (*A* and *C*) Metaphase spreads visualized with incident fluorescent light. (*B* and *D*) The corresponding spreads visualized under a combination of incident fluorescent and transmitted visible light. Two grains can be seen on chromatids of one chromosome 6 in *B* at 6p21.3 and one grain in *D* at 6p21.1.

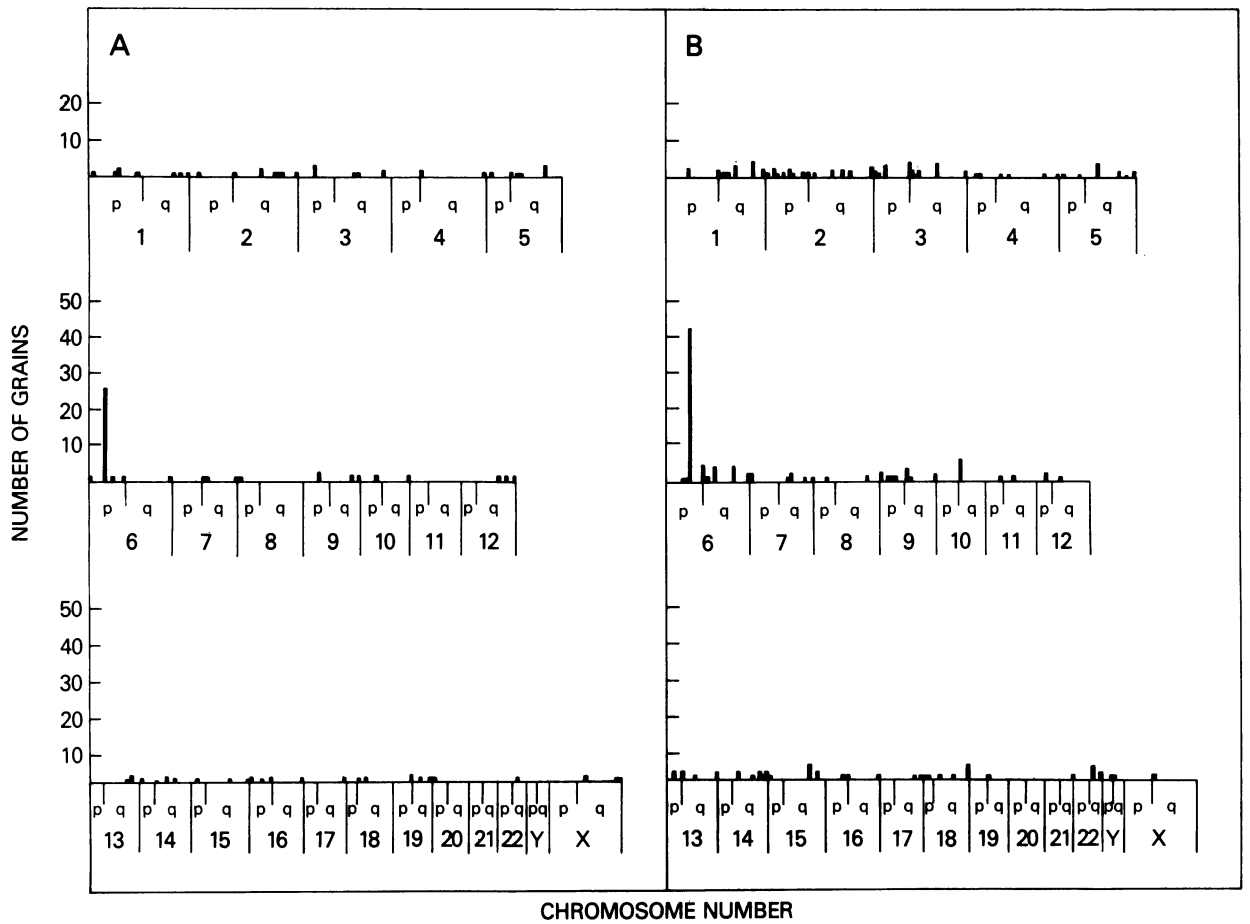

FIG. 2. Histograms of the distribution of silver grains over metaphase chromosomes for the pHLA-7 probe (*A*) and for pDRα (*B*). Plasmid pHLA-7 is localized to 6p21.3 and plasmid pDRα, to 6p21.1.

bridized with pHLA-7 or pDRα, respectively, and standardized to a total arm length for the short arm of chromosome 6 of 0.5 cm. The mean distance from the centromere of chromosome 6 to the silver grains corresponding to pHLA-7 was 0.254 cm with a standard error of 0.008 cm and a range of 0.15–0.38 cm. The comparable mean distance for pDRα was 0.216 ± 0.009 cm with a range of 0.09–0.30 cm. The observed difference in mean distance from the centromere for the two probes was highly significant (t = 3.11, 70 df, P = 0.0014).

To confirm this evidence that chromosomal *in situ* hybridization permits the resolution of pHLA-7 and pDRα probes, the photographs of the 72 chromosomes 6 were classified as to whether the silver grain was closer to the distal quinacrine-bright 6p22 band or to the proximal quinacrine-bright 6p12 band by an observer who was unaware of which probe had been used for hybridization in each cell that was scored. A nonparametric analysis of the resulting 2 × 2 contingency table (57) revealed a significant association between the probe used and the relative location of the grains, with the pDRα probe being scored as closer to the proximal quinacrine-bright 6p12 band in 19 of 36 photographs and the pHLA-7 probe as being classified as closer to the distal quinacrine-bright 6p22 band in 29 of 36 photographs (χ^2 = 8.67, 1 df, P = 0.0032).

Mapping of the HLA Probes in Two Individuals with a Chromosome Rearrangement of 6p. Data on the distribution of silver grains were collected from 100 metaphases hybridized with pHLA-7 and 100 metaphases hybridized with pDRα from a mother and son who are carriers of the same presumably balanced chromosome rearrangement involving 6p and 14p (56) (Fig. 3). A total of 217 grains were counted from the pHLA-7 hybridization to the mother's chromosome preparations. Forty-four or 20.3% of grains were localized at 6p21.3, 29 (13.4%) on the short arm of the derivative (der) chromosome 14, and only 8 (3.7%) on the short arm of the der(6). For the son 170 grains were recorded, of which 20 (11.8%) were localized at 6p21.3, 25 (14.7%) on the short arm of the der(14), and only 3 (1.8%) on the short arm of the der(6). Thus, two predominant sites of hybridization were present for the pHLA-7 probe: at 6p21.3 on the normal homolog and the short arm of the der(14) chromosome, which contained chromatin normally present between 6p22–6p21.1 on the short arm of chromosome 6 in karyotypically normal individuals.

The results of the pDRα hybridization in the mother in which a total of 159 grains were scored revealed 6 (3.8%) to be present at 6p21.1 on the normal homolog, 7 (4.4%) at the short arm of the der(6), and only 2 (1.3%) to be localized on the short arm of the der(14) chromosome. From the analysis of the son, a total of 158 grains were counted. Eight grains (5.1%) were located to 6p21.1, 5 (3.2%) were found on the short arm of the der(6), and only 1 grain (0.06%) was present on the short arm of the der(14) chromosome. In summary, two sites of hybridization were noted for the pDRα probe: at 6p21.1 on the short arm of both the der(6) chromosome and its normal homolog. These data then localize the breakpoint in the chromosomal rearrangement to a point between 6p21.1 and 6p21.3 that separates the sequences hybridizing to the DR α-chain genomic clone in 6p21.1 from the se-

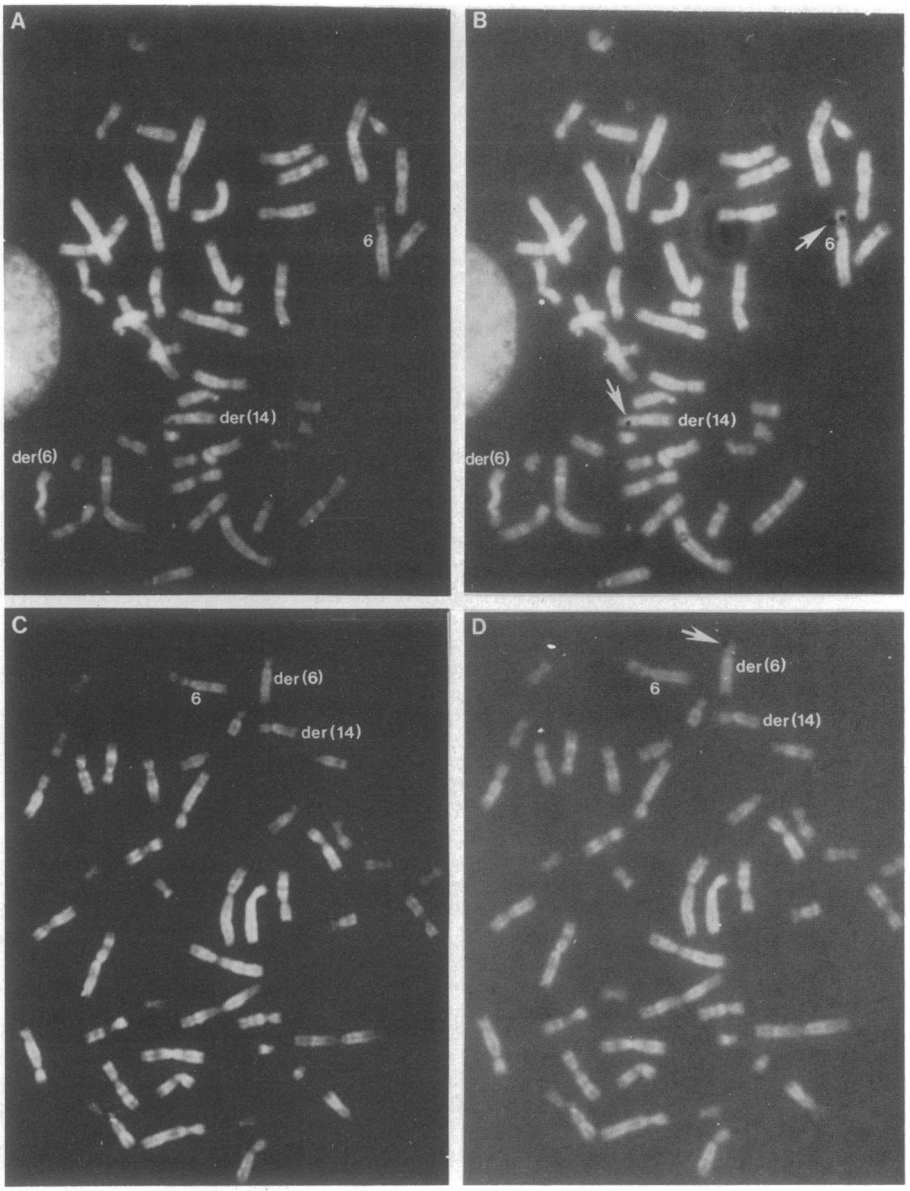

FIG. 3. Photographs showing hybridization of the pHLA-7 probe to the der(14) chromosome and chromosome 6 (*B*) and of the pDRα probe to the der(6) chromosome (*D*) in the karyotype of a carrier of a chromosomal rearrangement involving the short arms of chromosomes 6 and 14. (*A* and *C*) Metaphase spreads visualized with incident fluorescent light. (*B* and *D*) The corresponding spreads visualized under a combination of incident fluorescent and transmitted visible light. Silver grains can be seen at 6p21.3 and on the short arm of the der(14) chromosome in *B* and on the short arm of the der(6) chromosome in *D*.

quences hybridizing to the HLA-A, -B, -C α-chain probe in 6p21.3.[||]

DISCUSSION

Our studies of two genes in the human MHC by chromosomal *in situ* hybridization show that the HLA-DR α-chain gene is located closer to the centromere than the HLA-A, -B, and -C gene cluster and have permitted rather precise estimates of the chromosomal assignments of these genes to be made to 6p21.1 and 6p21.3, respectively. To our knowledge, the localization of the HLA-DR α-chain gene to a chromosomal region linked to the polymorphic HLA-DR light chain loci represents the first example of which the genetic determinants of a multimeric protein have been found to be syntenic in man.

Chromosomal rearrangements have been used extensively in previous gene localizations for deletion mapping and for the construction of somatic cell hybrids in which the segregation of rearranged chromosomes can be correlated with a particular gene. Our analysis has shown that these rearrangements are also of value for gene mapping by *in situ* hybridization. Our assignment of pHLA-7 to 6p21.3 and pDRα to 6p21.1 is confirmed by the hybridization of the class I probe to the der(14) chromosome, which contains gene sequences from 6p21.3 $\rightarrow$ 6p21.1, and the finding of pDRα homology to the der(6) chromosome, which contains chromatin from 6p21.1 $\rightarrow$ 6qter.

Our investigation also provides a useful indication of the resolving power of chromosomal *in situ* hybridization technology and its potential value in determining the polarity of gene clusters. Available linkage data suggest that the HLA-A, -B, and -C loci are separated from the polymorphic HLA-DR light chain locus by 1–1.8 cM. Although the exact map distance between the HLA-A, -B, -C cluster and the nonpolymorphic HLA-DR heavy chain locus identified by the pDRα probe is not known with certainty, our data indicate that the interval between them corresponds to 7.6% of the total length of 6p. Whereas 6p represents approximately 2% of the total autosomal genome, if one assumes a total autosomal map length of 33 Morgans and a uniform distribution of

[||]Since submission of this manuscript, we have analyzed metaphases prepared from peripheral blood lymphocytes from a normal male and from two family members who carry the chromosome rearrangement involving 6p and 14p after hybridization with a cDNA clone for the human complement protein factor Bf (58), a class III gene. Preliminary results of this study, which was performed in collaboration with Derek E. Woods, indicate hybridization of pBfA7 to 6p21 on normal male chromosomes and to 6p21 and the der(14) chromosome on metaphases from the two individuals with the rearrangement.

map distances, these observations suggest that an estimate of 5 cM can be made for the distance between these probes. Application of this technique to earlier metaphase or pro phase mitotic spreads would doubtlessly increase the precision of the method.

Our analysis has shown only one predominant site of hybridization for each of the two HLA probes. Thus, we have no evidence for dispersion of either of these genes to other regions of the genome outside of the MHC, as has been observed in other gene systems, particularly the immunoglobulin loci (59). We anticipate that the technique of chromosomal *in situ* hybridization and the methods of analysis used in this study will be increasingly utilized for the high-resolution mapping of structural genes and pseudogenes, for the determination of the polarity of gene clusters, and for the localization of breakpoints in chromosomal rearrangements.

We gratefully acknowledge the expert assistance of T. Mayo and T. Broderick in preparing the manuscript. This work was supported in part by National Institutes of Health Grants AM-30241, HD-18012, GM-17088, and GM-07492. This is paper no. 198 from the Department of Human Genetics of the Medical College of Virginia.

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