Proc. Natl. Acad. Sci. USA
Vol. 79, pp. 7395–7399, December 1982
Genetics

Highly polymorphic DNA site *D14S1* maps to the region of Burkitt lymphoma translocation and is closely linked to the heavy chain γ1 immunoglobulin locus

(human genetics/DNA polymorphism/linkage analysis)

IVAN BALAZS*, MICHELE PURRELLO*, PABLO RUBINSTEIN†, BECKY ALHADEFF*, AND MARCELLO SINISCALCO*‡

*Sloan-Kettering Institute for Cancer Research and †New York Blood Center, New York, New York 10021

Communicated by Philip Levine, August 9, 1982

ABSTRACT **Using a phage λ Charon 4A recombinant DNA clone (λCH4A-rHs18) from a human genomic library, Wyman and White detected a multiallelic common polymorphism at an *Eco*RI site (*D14S1*) flanking the DNA region homologous to the probe [Wyman, A. R. & White, R. (1980) *Proc. Natl. Acad. Sci. USA* 77, 6754–6758]. Subsequent studies, carried out with the cell hybrid approach and the use of a subclonal derivative (pAW101) from λCH4A-rHs18 have assigned this locus to autosome 14 between 14q21 and 14qter [De Martinville, B., Wyman, A. R., White, R. & Franke, U. (1982) *Am. J. Hum. Gen.* 34, 216–226]. The data presented here permit the precise mapping of this locus to the subtelomeric region of autosome 14, below band 14q32, in close proximity to the heavy chain γ1 immunoglobulin locus. These conclusions are supported by three independent lines of evidence, including studies on gene dosage, somatic cell hybrids, and pedigree analysis. Our results are in agreement with the recent assignment of the heavy chain γ1 immunoglobulin locus to band 14q32 [Kirsch, I. R., Morton, C. C., Nakahara, K. & Leder, P. (1982) *Science* 216, 301–303] and are consistent with the generally held contention that one unit of meiotic recombination corresponds approximately to one million base pairs. It is to be expected that the location of the highly polymorphic *D14S1* site at a measurable distance from the cluster of the heavy chain genes will provide new opportunities for a genetic approach to the question of the specific gene order within the cluster, its possible individual variation, and its biological significance in normal development and disease. It is worthwhile to point out that such a highly polymorphic DNA sequence is located in the same chromosomal region where so much somatic rearrangement goes on normally (i.e., switch region between classes of heavy chain constant region genes) and which is involved with *de novo* translocations associated with malignancies.**

After the report of De Martinville *et al.* (1, 2) that the highly polymorphic *Eco*RI restriction site (*D14S1*) of Wyman and White (3) is located below band q21 of the autosome 14 long arm, we began a series of studies to investigate the relative position of this locus with respect to the band 14q32 which, from multiple evidence, seems to be highly prone to rearrangement in meiotic (4–6) and mitotic cell divisions (7–11). These studies were motivated by the already available knowledge that the type of genetic variation observed at the *D14S1* site appears itself to be "the result of DNA rearrangements rather than of base pair substitutions or modifications" (3).

We addressed the question with the use of three different complementary experimental approaches. The first involves gene dosage mapping studies performed on DNA preparations from individuals known to carry inborn balanced or unbalanced translocations at the chromosomal region under consideration. The second makes use of the well-known strategies of somatic cell genetics for the subregional mapping of human markers with rodent–human cell hybrids. The third is based on the classical pedigree analysis for the detection and estimation of human linkage. All three lines of evidence obtained concurrently indicate that the *D14S1* site is located in the long arm subterminal region of autosome 14 distal to the band 14q32. The finding of a close but not absolute linkage association with the γ heavy chain loci, assigned to the same region by *in situ* hybridization studies (12), confirms the expectation (13) that genetic markers located within a distance of a few million base pairs from one another should result in a measurable linkage and that, at least in the telomeric region of the autosome 14 long arm, one unit of meiotic recombination is approximately equivalent to one million base pairs.

MATERIAL AND METHODS

Human Fibroblasts with the X/14 Inborn Translocation. The primary fibroblast strains GM73 and GM74 were obtained from the Human Genetic Mutant Cell Repository (Camden, NJ). The salient feature of these cell strains, derived from a mother (GM73) and her child (GM74), is the presence of an inborn reciprocal translocation between the subtelomeric region of the autosome 14 long arm and the distal two-thirds of the X chromosome long arm (14, 15). The mother has a balanced genotype, 46X,t(X,14) (Xpter→Xq13::14q32→14qter and 14pter→14q32::Xq13→Xqter), whereas the child has an unbalanced genotype with a total of 47 chromosomes, among which there are two doses of the translocation chromosome 14pter→14q32::Xq13→Xqter (referred to as t-14/X), one translocation chromosome Xpter→Xq13::14q32→14qter (referred to as t-X/14), one maternally derived normal X chromosome, and one paternally derived normal chromosome 14, in addition to the Y chromosome and a regular diploid dose of all other autosomes. Thus, the child is trisomic for the regions 14pter→14q32 and Xq13→Xqter, but disomic for the regions 14q32→14qter and Xpter→Xq13.

By using the high-resolution protocol of Yunis (16), it appears that the breakpoint on chromosome 14 is above the prophase band 14q32-12. The sketches in Fig. 1 *A* and *B* summarize the genetic constitution of these two cell strains with respect to the chromosomes X and 14.

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.

Abbreviations: Gm, gamma globulin; HAT, hypoxanthine/aminopterin/thymidine; HPRT, hypoxanthine phosphoribosyltransferase.

‡To whom reprint requests should be addressed.

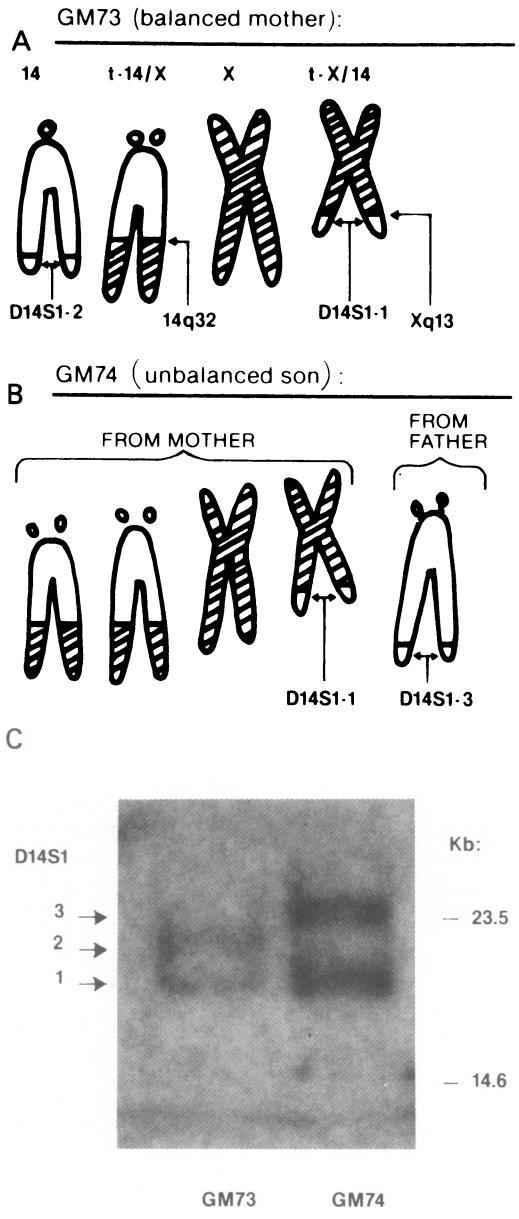

FIG. 1. Subregional mapping of *D14S1* site with the use of inborn reciprocal translocations. (*A*) Sketch of the reciprocal balanced translocation carried by GM73 (mother: 46,X,t(X,14) (14pter→14q32::Xq13→Xqter; Xpter→Xq13::14q32→14pter). Her D14S1 phenotype includes the fragments 1 and 2 as in *C*. The arrows at the sides of the chromosome indicate the translocation breakpoints on autosome 14 and on the X chromosome. The arrows between the chromatids indicate the most likely subregional location of the D14S1 site. (*B*) Sketch of the unbalanced genotype of GM74 (son): 47,XY,t(X,14) (14pter→14q32::Xq13→Xpter; Xpter→Xq13::14q32→14qter), der t(X,14) (14pter→14q32::Xq13→Xqter) mat. His D14S1 phenotype includes fragments 1 and 3. The maternal or paternal derivation of the relevant chromosomes is indicated. GM74 is clearly trisomic for the region 14pter→14q32 and disomic for the region 14q32→14qter. (*C*) DNA gel showing the *D14S1* pattern of GM73 and GM74. The equal relative intensity of the two fragments in each gel has been confirmed by densitometric tracing. This finding indicates that the *D14S1* site is not carried by the t-14/X chromosome and, therefore, must be located below the breakpoint 14q32.

Somatic Cell Hybrids Between GM73 Fibroblasts and Hypoxanthine Phosphoribosyltransferase (HPRT)-Deficient Cells. GM73 fibroblasts were the first example of cells with a balanced X autosomal translocation chromosome used for the subregional mapping of human gene markers (17, 18). As it is well known, the rationale (19, 20) of this experimental approach is based upon the circumstance that the normal X chromosome is the one preferentially inactivated in all somatic cells with balanced X autosomal translocation chromosomes. Thus, somatic cell hybrids between the HPRT-deficient murine cells and the HPRT-normal GM73 fibroblasts need to retain only the t-14/X chromosome (onto which the human HPRT gene has been transferred by the translocation) for their survival in the hypoxanthine/aminopterin/thymidine (HAT) selective medium. We had available from previous mapping studies (21) a large series of HAT-resistant hybrid clones derived from four independent fusions between GM73 fibroblasts and the HPRT-deficient L-A9 murine cells. Prior to their storage in liquid nitrogen, these hybrid clones had been fully characterized with respect to their content in human chromosomes with the use of cytogenetical and biochemical methods. The chromosomal characterization was performed on 25 metaphases with the sequential determination of Q-banding (22) and Giemsa-11 staining (23). The retention of the biochemical markers of the t-14/X chromosome was assessed by Cellogel electrophoresis, followed by chromogenic staining of the enzymatic bands (24).

The panel of hybrid cell lines chosen for the present study included six primary hybrid clones (F89:1, 3, 8, and 11, F103:2, and F130:1) and three secondary ones (F102:1-13, 1-16, and F102:2-2), which, after HAT selection, had all retained the t-14/X chromosome as proven directly from cytogenetical evidence and indirectly from the retention of the human enzymatic markers of this chromosome, namely nucleoside phosphorilase (NP), phosphoglycerate kinase (PGK), glucose-6-phosphate dehydrogenase (G6PD), and HPRT. The other translocation chromosome t-X/14 and the normal chromosome 14 were absent in all of these clones; in two of them (F89-1 and F103-2), the t-14/X chromosome, essential for survival in the HAT medium, was the only residual of the human genome. To this series of hybrids we added two HAT-selected primary hybrid clones, which, in addition to the t-14/X chromosome, had retained either both the t-X/14 and the normal 14 chromosomes (clone F102-2) or only the normal chromosome 14 (clone F89-20).

The genetic constitution of these hybrid clones with respect to the retention of the two translocation chromosomes and of autosome 14 is summarized in Table 1. An aliquot of 10^7 cells for each clone was taken from liquid nitrogen and used directly for DNA preparation.

Gamma Globulin (Gm)-Segregating Families. Peripheral blood lymphocytes from the critical members of 13 pedigrees segregating with phase known for Gm variants at the γ1 heavy chain Ig locus were recovered from liquid nitrogen and used directly for DNA extraction and determination of D14S1 phenotypes. These families had been typed through the last few years at the New York Blood Center to determine the frequencies of the Gm allotypes among North Americans of Anglo-Saxon extraction and to investigate their linkage relationship with other loci. The Gm typing had been carried out at the time of blood collection on plasmas from the same individuals with respect to the antigenic specificities Gm(a), Gm(x), and Gm(f), which are all encoded by alleles at the γ1 heavy chain Ig locus (25). For each pedigree found to segregate at both the *Gm* and the *D14S1* loci, lod scores (Z; log of odds) (26, 27) were calculated at values of recombination frequency between 0 and 0.3 with increments of 0.01, by the equation $Z_1 = \log_{10} 2^{s-1}(\psi^a\theta^b + \psi^b\theta^a)$, where θ is the chosen recombination value, $\psi = 1 - \theta$, a = the number of nonrecombinants in a given sibship, b = the number of recombinants, and $s = a + b$. The maximum likelihood estimate of the recombination fraction, the odds in favor of linkage, and the 90% upper limit of recombination were calculated (28) from the curve reported in Fig. 2.

Table 1. Evidence from L-A9/GM73 cell hybrids that *D14S1* is distal to 14q32 and that allele *D14S1* of GM73 is associated with the normal autosome 14

Fusion exp.	HAT-selected clones		t-14/X markers*	Retention of			*D14S1* fragment
	Primary	Secondary		t-14/X	t-X/14	14	
F89	1		+	+	−	−	ND
	3		+	+	−	−	ND
	8		+	+	−	−	ND
	11		+	+	−	−	ND
	20		+	+	−	+	2
F102		1-13	+	+	−	−	ND
		1-16	+	+	−	−	ND
	2		+	+	+	+	1, 2
		2-2	+	+	−	−	ND
F103	2		+	+	−	−	ND
F130	1		+	+	−	−	ND

The undetectability of the *D14S1* fragments in the eight independently derived hybrid clones, which have regularly retained only the t-14/X translocation chromosome and all of its markers, supports the location of the *D14S1* site below the translocation breakpoint 14q32. This conclusion is confirmed by the D14S1 phenotype of clone F102-2, which has the same D14S1 phenotype of the GM73 parental cells as expected from the presence in this clone of both translocation chromosomes and of the normal autosomal chromosome 14. From hybrid clone F89-20, it can be deduced that GM73 cells carry the *D14S1 2* allele of the normal chromosome 14. ND, not detectable.

* Markers were hypoxanthine phosphoribosyltransferase, nucleoside phosphorilase, and phosphoglycerate kinase.

Procedures for DNA Studies. For the determination of the D14S1 phenotype, we used the molecular probe pAW101 (kindly made available by R. White) which is a clonal derivative in pBR322 (3) of the phage λ Charon 4A recombinant clone λCH4A-rHs18 from the human genomic library of Maniatis *et al.* (29). The pAW101 probe was labeled by nick-translation with [^{32}P]dTTP (≈10^8 cpm/μg of DNA), as described by Rigby *et al.* (30).

Extraction of high molecular weight DNA from various cell types and *Eco*RI digestions were carried out by standard procedures (31). About 3 μg of *Eco*RI-digested DNA was used per gel slot to determine the D14S1 phenotype of the peripheral blood lymphocytes and the fibroblastic cells, whereas twice this amount was needed for the series of hybrid cells. This was necessary in view of the circumstance that, in the latter cells, the *D14S1* site was usually present with the ratio of 1 dose out of a total of 80 chromosomes, as opposed to the 2 doses out of 46 chromosomes in the normal lymphocytes and the GM73 cells and the 2 or 3 doses expected (depending upon the localization of the marker below or above the translocation breakpoint) out of 47 chromosomes in the GM74 fibroblasts.

*Eco*RI-digested DNA fragments were fractionated by electrophoresis in 0.6% agarose gels and directly hybridized to the ^{32}P-labeled DNA probe pAW101 as described by Purrello and Balazs (32). This hybridization protocol has been used in our laboratory to detect X chromosome-specific unique sequence probes on the basis of the relative amount of labeled probe hybridized to DNA preparations with different doses of the human X chromosome (unpublished data).

RESULTS

Gene Dosage Studies. *Eco*RI-digested DNA from the fibroblastic strains GM73 and GM74 was separated by agarose electrophoresis, and the DNA in the gels was directly hybridized with the ^{32}P-labeled pAW101 probe for the determination of the D14S1 phenotype. Both mother and child were heterozygous at the *D14S1* site (respectively 1,2 and 1,3) with only one allele in common (*D14S1 1*) (Fig. 1 *A*, *B*, and *C*), and two fragments of equal intensity were confirmed by densitometric tracing of the autoradiographic bands. Thus, it is clear that GM74 must have received the *D14S1 1* fragment with one of the two types of maternally derived translocation chromosomes and the *D14S1 3* fragments with the paternally derived normal chromosome 14. Moreover, the equal intensity of the two fragments in both mother and child suggests that the *D14S1* site is located in the subtelomeric region 14q32→14qter because this is the only region of autosome 14 that is present in equal dose in both genomes. Given the unbalanced genotype of GM74, the association of the allele *D14S1 1* with the translocation chromosome t-14/X should have resulted in a 2:1 dosage ratio of this fragment over the fragment *D14S1 3* of paternal origin. An alternative interpretation of the findings would require the postulation of a meiotic recombination event between the *D14S1* site and the translocation breakpoint in GM73. Such an unlikely event can be excluded on the basis of the cell hybrid data described below.

Cell Hybrid Studies. To confirm the above evidence in favor of the localization of the *D14S1* site below the translocation breakpoint 14q32 of the GM73 cells, we adopted the classical methodology of gene assignment with translocation cell hybrids.

In order to ensure a meaningful comparison with the cytogenetical and biochemical studies performed at the time of the cell storage, the analysis of the cell hybrid DNAs was performed on DNA preparations obtained directly from the hybrid cells recovered from liquid nitrogen without further propagation in culture. We reasoned that if our conclusions on the localization of the *D14S1* site based on gene dosage were correct, the screening of the above described panel of hybrid clones with probe pAW101 should fail to show a regular association in the retention of the t-14/X chromosome with that of the *D14S1 1* fragment. The latter should instead be retained in association with the t-X/14 chromosome and, respectively, the fragment *D14S1 2* with the normal chromosome 14.

These expectations were fully confirmed by the data reported in Table 1. The absence of the *D14S1 1* fragment from all of the hybrid clones that only contain the t-14/X chromosome clearly indicates that the *D14S1* site resides in the region of autosome 14 below the breakpoint 14q32. An alternative interpretation such as the loss of the site from t-14/X through a small, undetectable *de novo* chromosomal rearrangement is unlikely in view of the separate origins of the individual clones from dif-

ferent fusion events and of the regular retention of all biochemical markers of the t-14/X translocation chromosome. The presence of the *D14S1 1* fragment in the hybrid clone F102-2, which is the only one to contain the other translocation chromosome, t-X/14, gives further support to the above conclusion. However, this clone and clone F89-20 were included in the hybrid panel to confirm the finding described in Fig. 1—namely, that in the genetically balanced mother (GM73 cells), the allele *D14S1 1* is associated with the translocation chromosome t-X/14, and the allele *D14S1 2*, with the normal chromosome 14.

Linkage Studies. It could be argued that the data reported so far justify the assignment of the *D14S1* site to the subtelomeric region of the autosome 14 long arm involved in the reciprocal translocation in GM73 cells but not necessarily to the same region of the normal 14 autosome. To evaluate this issue, we decided to adopt an independent approach based on the search for measurable linkage between the *D14S1* polymorphism and the serum Gm allotypes, the well-known polymorphisms of the heavy chain immunoglobulin region that *in situ* hybridization studies (12) had recently established to be in close proximity to band 14q32. We have examined 13 pedigrees already known to be segregating for Gm variants at the γ1 *Gm* locus. *Eco*RI-restricted DNA from the critical members of these families were classified with respect to the D14S1 phenotype, and nine sibships were found to give information. In six of these, the double heterozygous parent is the mother. The two loci were found to segregate in close association, as only 1 recombinant was found among 31 scorable sibs. The family with the recombinant child (Table 2) happens to be one of three with a double heterozygous father. The alternative interpretation of illegitimacy rather than recombination is made very unlikely by the circumstance that the father and the recombinant child share the uncommon HLA haplotype *A2, B6, BR3* (whose population frequency is 2×10^{-3}) and are homozygous for the rarest of the alleles of the C3 complement system (C3F). Given these findings, the scores from all sibships have been pooled together for calculating the maximum likelihood estimate of the recombination fraction. This was found to be 3.1% with 90% fiducial limits for the upper recombination value of 11.5% (Fig. 2). The finding of such a close linkage indicates that the two loci must be located within a short interval from one another. Unpublished data of W. McBride and colleagues (personal communication) are in agreement with this conclusion as they found that, in rodent–human (GM73) hybrid cells, the regions of homology with all heavy chain immunoglobulin probes are consistently located below the breakpoint of the GM73 reciprocal translocation. In addition, our unpublished *in situ* hybridization data (to be reported separately) localize the pAW101 probe not only to the telomere of the translocation chromosome t-X/14 but also to that of the normal autosome 14 present in GM73.

Table 2. A family with a child (no. 3) carrying a probable recombination between the loci *Gm* (γ1) and *D14S1*

Family relationship	Gm-specificities a(1)	x(2)	f(4)	*D14S1* fragments	Probable combined genotype
Father	+	−	+	3,5	3,+−−/5,−−+
Mother	−	−	+	3,5	3,−−+/5,−−+
Child:					
1	−	−	+	5	5,−−+/5,−−+
2	−	−	+	3,5	3,−−+/5,−−+
3	+	−	+	5	5,+−−/5,−−+
4	−	−	+	5	5,−−+/5,−−+

The Gm types were determined with respect to the antigenic specificities Gm(a), Gm(x), and Gm(f). The denomination of the *D14S1* fragments is according to their size, which varies approximately from 15 kb (fragment 1) to 30 kb (fragment 5). The most probable genotypes at the *Gm* locus were induced from the independent knowledge that only certain haplotypes (+−−, −−+, and ++−) occur with appreciable frequencies in the populations (North American Caucasians) from which the family is derived. The phase given to the parental genotypes was deduced from the children.

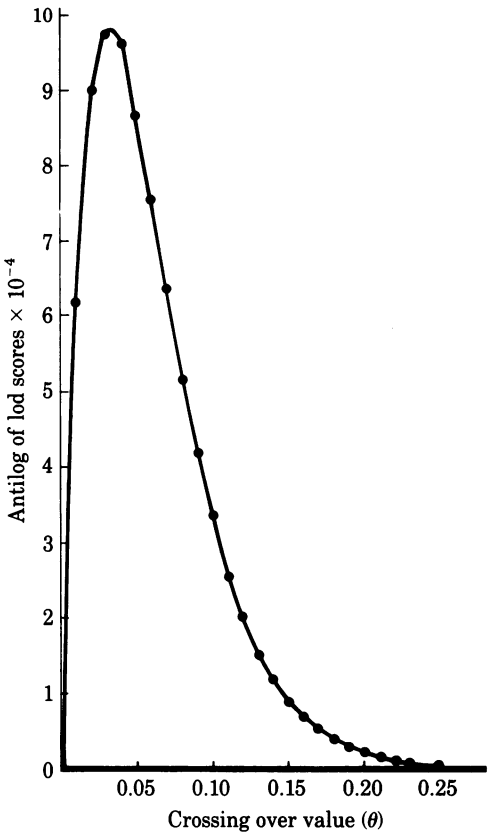

FIG. 2. Linkage between *Gm* locus and DNA polymorphic site *D14S1*. The average height of the curve (H) = 69,576, the odds in favor of linkage (H/20) = 3,479:1, the maximum likelihood estimate of θ = 0.031, and the 90% upper limit of θ = 0.115.

DISCUSSION

Our data evaluated in conjunction with *in situ* hybridization of Kirsch *et al.* (12) indicate that both the *D14S1* site and the *Gm* locus are included in the subtelomeric region of the autosome 14 long arm distal to band 14q32. Because this region is about 9.23% of chromosome 14 and this is in turn 3.72% of the total length of the human genome (33), the subtelomeric region in question should include about 10 million base pairs, assuming the total genome to consist of 3×10^9 base pairs. Accordingly, one can conclude that within an interval of 10 million base pairs, genetic recombination can occur with a frequency between 3% and 10%. At this time, it is unknown whether this relationship between the frequency of meiotic recombination and the number of base pairs holds true for the human genome in general or whether it is limited to the telomeric region under consideration. To answer this interesting question, it would be necessary to have available several additional examples of common DNA polymorphism (hopefully multiallelic as in *D14S1*) within the subtelomeric region under discussion and at an appreciable distance from it. These circumstances are about to be fulfilled because it is very likely that the other molecular probes already available for the heavy chain Ig genes (34–36) and the recently isolated human gene for α1-antitrypsin (37)—another genetic marker of autosome 14 estimated to be at about 30 crossing-over

units away from the *Gm* cluster (38)—will all lead to the discovery of common genetic variation within the DNA regions homologous to these probes or in those flanking them.

One question of particular significance would then be to determine whether the recombination event reported in Table 2 has occurred at a site nearer to the *Gm* cluster than to the *D14S1* locus. It is clear that families like the one reported in Table 2 may provide information for mapping the order of the different heavy chain Ig loci in genetical terms and for comparing this map to those obtained from the fine structure analysis of the gene end products (39) or, more recently, from the molecular mapping of genomic clones derived from a single total human DNA library (40, 41).

It is noteworthy that one of the most polymorphic DNA sequences thus far known maps to the same chromosomal region which (*i*) has evidently been the theater of a long series of DNA duplication events in the evolutionary time, (*ii*) is the site of the molecular switch mechanisms underlying the phenomenon of allelic exclusion in the immunoglobulin producing cells, and (*iii*) is the same region frequently involved in molecular rearrangements associated with Burkitt lymphoma and other malignancies (8–11, 42, 43). There can be little doubt that the restriction enzyme analysis of the DNA from the malignant type of cell with the use of all the molecular probes discussed above may soon lead to a detailed understanding of the molecular events associated with such malignant transformations. In turn, the *de novo* chromosomal rearrangements in malignant cells of monoclonal origin may serve—like the inborn chromosomal aberrations—as biological tools for the subregional mapping of specific genes. For instance, if it is true that one of the autosomes 14 is constantly translocated with a breakpoint at band 14q325 (43) in all the Burkitt lymphoma tumors, it soon should be possible to establish with precision which of the loci already localized to the subtelomeric region of the chromosome 14 long arm is removed by the translocation. However, it should be borne in mind that, a tumor clonal cell population remains essentially the expansion of one abnormal somatic cell from a single individual; therefore, the molecular organization found in a tumor tissue can hardly be taken as true representative of the normal human genome.

Ultimately, the critical answer to the problem of whether or not the rearrangement of the chromosomal region(s) in question is the "cause" or the "effect" of the malignancies associated with it, will have to come from classical population and linkage studies. When a detailed map will be available for this region, it will be of obvious interest to investigate the possible existence of genetic variation between normal individuals (classical gene restriction-fragment-length polymorphisms, but also inversions, deletions, and the like) and within individuals (comparison of germ-line constitution of a given individual with that of clonal derivatives of his normal or diseased somatic tissues). From a genetic point of view, this remains the only efficient way to define the relative role of nature and nurture in the control of normal development and disease, malignancies included.

We are grateful to Dr. Ray White for making available to us probe pAW101, to Renata Klatt and Iraida Pagan-Charry for expert technical assistance, and to Dr. Janet Stavnezer for critical reading of the manuscript. These studies were supported by National Institutes of Health Grants HD16782 to M.S. and HL09011 to P.R.

1. De Martinville, B., Wyman, A. R., White, R. & Franke, U. (1981) *Pediatr. Res.* **15**, 560.
2. De Martinville, B., Wyman, A. R., White, R. & Franke, U. (1982) *Am. J. Hum. Genet.* **34**, 216–226.
3. Wyman, A. R. & White, R. (1980) *Proc. Natl. Acad. Sci. USA* **77**, 6754–6758.
4. Allderdice, P. W., Miller, O. J., Klinger, H. P., Opitz, J. M. & Pallister, P. D. (1971) *Proc. Int. Congr. Hum. Genet.* **4**, 14–15.
5. Buckton, K. E., Jacobs, P. A., Rae, L. A., Newton, M. S. & Sanger, R. (1971) *Ann. Hum. Genet.* **35**, 171–178.
6. Seckhon, G. S., Taysi, K. & Rath, R. (1978) *Hum. Genet.* **44**, 99–103.
7. Mattei, M. G., Ayme, S., Mattei, J. F., Avram, Y. & Giraud, F. (1979) *Cytogenet. Cell Genet.* **23**, 95–102.
8. Manolov, G. & Manolova, Y. (1972) *Nature (London)* **237**, 33–34.
9. Zech, I., Haglund, U., Nilsson, K. & Klein, G. (1976) *Int. J. Cancer* **17**, 47–56.
10. Hecht, F., McCaw, B. K. & Koler, R. D. (1973) *N. Engl. J. Med.* **289**, 286–291.
11. McCaw, B. K., Hecht, F., Harnden, D. G. & Teplitz, R. L. (1975) *Proc. Natl. Acad. Sci. USA* **72**, 2071–2075.
12. Kirsch, I. R., Morton, C. C., Nakahara, K. & Leder, P. (1982) *Science* **216**, 301–303.
13. Botstein, D., White, R., Skolnick, M. & Davies, R. (1980) *Am. J. Hum. Genet.* **32**, 314–331.
14. Opitz, J. M., Ruddle, F. H. & Miller, R. C. (1973) *Cytogenet. Cell Genet.* **12**, 289–290.
15. Opitz, J. M., Ruddle, F. H. & Miller, R. C. (1973) *Cytogenet. Cell Genet.* **12**, 291–292.
16. Yunis, J. J. (1976) *Science* **191**, 1268–1270.
17. Grzeschick, K. H., Allderdice, P. W., Grzeschick, A., Opitz, J. M., Miller, O. J. & Siniscalco, M. (1972) *Proc. Natl. Acad. Sci. USA* **69**, 69–73.
18. Ricciutti, F. C. & Ruddle, F. H. (1973) *Genetics* **74**, 661–678.
19. Siniscalco, M. (1970) in *Symposia of the International Society for Cell Biology*, ed. Padykula, M. A. (Academic, New York), Vol. 9, pp. 205–231.
20. Siniscalco, M. (1979) in *Progress in Medical Genetics, New Series*, eds. Steinberg, A. G., Bearn, A. G., Motulsky, A. G. & Childs, B. (Saunders, Philadelphia), Vol. 3, pp. 221–307.
21. Seravalli, E., DiBona, P., Velivasakis, M., Pagan-Charry, I., Hershberg, A. & Siniscalco, M. (1976) in *Human Gene Mapping 3, Birth Defects*, Original Article Series, The National Foundation March of Dimes, ed. Bergsma, D. (Karger, New York), Vol. 12, pp. 219–222.
22. Caspersson, T., Zech, L., Johansson, C. & Modest, E. J. (1974) *Chromosoma* **30**, 215–227.
23. Bobrow, M. & Cross, J. (1974) *Nature (London)* **251**, 77–79.
24. Meera Khan, P. (1971) *Arch. Biochem. Biophys.* **145**, 470–483.
25. World Health Organization (1968) *W. H. O. Tech. Rep. Ser.*, Karger, New York, 402.
26. Morton, N. E. (1955) *Am. J. Hum. Genet.* **7**, 277–318.
27. Morton, N. E. (1957) *Am. J. Hum. Genet.* **9**, 55–75.
28. Smith, C. A. B. (1968) *Ann. Hum. Genet.* **32**, 127–150.
29. Maniatis, T., Hardison, R. C., Lacy, E., Lauer, J., O'Connell, C., Quon, D., Sim, G. K. & Efstratiadis, A. (1978) *Cell* **15**, 687–701.
30. Rigby, P. W. J., Dieckmann, M., Rhodes, C. & Berg, P. (1977) *J. Mol. Biol.* **113**, 237–251.
31. Steffen, D. & Weinberg, R. A. (1978) *Cell* **15**, 1003–1010.
32. Purrello, M. & Balazs, I. (1982) *Anal. Biochem.*, in press.
33. Hulten, M. (1973) *Hereditas* **76**, 55–78.
34. Shimizu, A., Takahashi, N., Yamawaki-Kataoka, Y., Nishida, Y., Kataoka, T. & Honjo, T. (1981) *Nature (London)* **289**, 149–153.
35. Hieter, P. A., Hollis, G. F., Korsmeyer, S. J., Waldmann, T. A. & Leder, P. (1981) *Nature (London)* **294**, 536–540.
36. Ellison, J., Buxbaum, J. & Hood, L. (1982) *DNA* **1**, 11–18.
37. Chandra, T., Stackhouse, R., Leight, M., Long, G., Kurachi, K., Davie, E. W. & Woo, S. L. C. (1982) *DNA* **1**, 210.
38. Gedde-Dahl, T., Frants, R. R., Olaisen, B., Eriksson, A. W., Van Loghem, E. & Lamm, L. (1981) *Ann. Hum. Genet.* **45**, 143–153.
39. Natvig, J. B. & Kunkel, H. G. (1968) *Ser. Haematol.* **1**, 66–86.
40. Honjo, T., Kataoka, T., Yaoita, Y., Shimizu, A., Takahashi, N., Yamawaki-Kataoka, Y., Nikaido, T., Obata, N. M., Kawakami, T. & Nishida, Y. (1981) *Cold Spring Harbor Symp. Quant. Biol.* **45**, 913–923.
41. Ellison, J. & Hood, L. (1982) *Proc. Natl. Acad. Sci. USA* **79**, 1984–1988.
42. Fukuhara, S., Shirakawa, S. & Uchino, H. (1976) *Nature (London)* **259**, 210–211.
43. Manolova, Y., Manolov, G., Kieler, J., Levan, A. & Klein, G. (1979) *Hereditas* **90**, 5–10.