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Assignment of the First Random Restriction Fragment Length Polymorphism (RFLP) Locus (*D14S1*) to a Region of Human Chromosome 14

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SUMMARY

A locus responsible for a restriction fragment length polymorphism (RFLP) has been identified by hybridization of Eco RI fragments to the random human DNA sequence in recombinant plasmid pAW101.

We have examined DNA extracted from 20 human × Chinese hamster somatic cell hybrids for the presence of sequences homologous to the human insert in pAW101. The hybrids were derived from six different human donors, five of whom were heterozygous, producing two bands on Southern transfers. The presence of homologous sequences in the hybrids correlated exclusively with the presence of human chromosome 14. Three hybrids contained chromosome 14 in a frequency of greater than one per cell and were positive for two alleles. Two hybrids contained only the distal half of the long arm of 14 as part of a translocation and were still positive. These results assign the first highly polymorphic random RFLP locus (*D14S1*) to region q21→qter of chromosome 14.

INTRODUCTION

Restriction fragment length polymorphisms (RFLPs) constitute a new class of genetic markers. Botstein et al. [1] suggested that RFLPs should be abundant

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throughout the human genome and could serve as marker loci for a complete human genetic map. The first random human sequence found to be homologous to a highly polymorphic locus was reported by Wyman and White [2]. The recombinant plasmid pAW101 contains a 5-kilobase (kb) insert of single-copy human DNA of unknown function and unknown chromosomal location. Wyman and White have shown that this cloned fragment, when used as a probe of Southern transfers of DNA from individuals, detects restriction fragments of at least eight different lengths, and that these RFLPs can be inherited as Mendelian alleles. Most individuals tested were heterozygous, having two different lengths of Eco RI fragments homologous to pAW101. Presumptive homozygotes, those with only a single Eco RI band, accounted for 20% of the population examined. The high degree of polymorphism observed makes this locus a very useful marker for linkage studies. A standard nomenclature for this new class of genetic markers has not yet been developed. In keeping with the recommendations of the Committee on Human Gene Nomenclature [3], we use the gene symbol *D14S1*, where D stands for DNA, S for segment, 14 for the chromosomal location, and 1 for the first unique restriction fragment assigned to this chromosome. Thus, the polymorphic DNA segment identified by hybridization to the human insert in plasmid pAW101 is designated *D14S1*.

Knowledge of the precise chromosomal location of RFLP loci will facilitate their use in linkage studies in two respects. First, the development of a complete human linkage map as visualized by Botstein et al. [1] requires that the RFLP marker loci are evenly distributed throughout the chromosome complement. Mapping of unique polymorphic DNA segments as they are discovered will assure that they are not clustered on a single chromosome or chromosome region. Second, once the chromosomal location of an RFLP site has been established, the search for closely linked genes can focus on other loci assigned to the same chromosome, as well as on unassigned genetic markers, while loci known to be on other chromosomes need not be considered.

In the experiments reported here, we have utilized a series of Chinese hamster × human somatic cell hybrids with partial human chromosome complements. DNA extracted from hybrid cell lines has been analyzed by the Southern transfer technique. By correlating these results with the human chromosome content of the hybrids, we have established localization of the polymorphic DNA segment homologous to pAW101 on chromosome 14. Furthermore, the analysis of hybrids made with human cells containing a t(X;14)(p22;q21) translocation has resulted in a more precise localization in the distal region of the long arm of chromosome 14 (region 14q21→qter).

MATERIALS AND METHODS

Somatic Cell Hybrids

Six different sets of Chinese hamster × human somatic cell hybrids were used in these studies. Hybrids from series X, XII, XIII, XV, and XVII were derived from 380-6 cells, HPRT-deficient derivatives of Chinese hamster lung fibroblast line V79. They were fused, as previously described, to the following different human cells: fibroblasts containing a

t(1;6)(p32;p21) translocation for series XV hybrids [4], a t(1;2)(q32;q13) translocation for series X [5], a t(15;22)(q14;q13.3) translocation for series XVII [6], and human leukocytes with a t(X;14)(p22;q21) translocation for series XII and XIII [7]. Hybrids of series XXI were obtained from a fusion between a23 cells (TK-deficient derivatives of the Chinese hamster Don lung fibroblast line) and human fibroblasts containing a t(11;15)(p11;p12) translocation [8, 9].

Cells were propagated in Eagle's minimum essential medium (MEM, Gibco, Grand Island, N.Y.) supplemented with 10% fetal calf serum (Sterile Systems, Logan, Utah), L-glutamine, and antibiotics (100 U/ml penicillin and 100 mcg/ml streptomycin) (Gibco). Hybrids were cloned in hypoxanthine-aminopterin-thymidine (HAT) selective medium [10]. Most of them were subcloned to reduce intraclonal heterogeneity. Some of the cell hybrids were counterselected in 10^{-5} M 8-azaguanine [11]. Both Chinese hamster cell lines and fibroblasts from the human donors used in the cell hybridizations were cultured under standard conditions and used for parental cell control DNA preparations.

Chromosome Analysis

All hybrids were characterized by karyotype analysis before propagation in culture. Both human and Chinese hamster chromosome contents were monitored during large-scale expansion of the hybrid cell populations. The relative frequency of each human chromosome was established on cells from the same subculture that was used for DNA extraction and for the preparation of cell pellets for electrophoretic analysis of enzyme markers. Mitotic cells were obtained by either standard harvests in suspension, or by an in situ method with cells grown on coverslips, and chromosomes were banded using a trypsin-Giemsa banding technique [12]. From 15 to 30 randomly selected metaphase spreads were photographed, and prints were analyzed in detail.

DNA Extraction

Nuclear DNA from parental cell lines and hybrids was prepared as described [13] with the following modifications: Briefly, cells were harvested by scraping them off Petri dishes. Crude nuclei were prepared by gentle homogenization in a buffer containing 0.32 M sucrose, 10 mM Tris, pH 7.6, 5 mM $MgCl_2$, and 1% Triton X-100. Nuclei were lysed by incubation at 37°C in 75 mM NaCl, 25 mM EDTA, pH 8.0, 1% SDS, and proteinase K (Boehringer-Mannheim, Indianapolis, Ind.). High molecular weight DNA was isolated by extraction of the aqueous phase with phenol and chloroform. Some of the samples were further purified by cesium chloride gradient centrifugation.

Southern Transfers

Endonuclease digestion, electrophoresis, transfer to diazo benzyloxymethyl (DBM) paper, hybridization, washing, and autoradiography were carried out as described [2]. The pAW101 probe was labeled by nick translation [2] with [^{32}P]α-dCTP (Amersham-Searle, Arlington Heights, Ill.) to a specific activity of approximately 10^8 cpm/μg. Hybridization of this probe to the DBM paper was allowed to proceed at 42°C for 18–22 hrs. The papers were washed at high stringency at 51°C in 0.1 SSC and 0.1% SDS.

Enzyme Electrophoresis

Harvesting of hybrid cells and preparation of cell lysates have been described [7]. Human gene products were detected after electrophoresis on cellulose acetate gel (Kalex, Chemeton, Milan, Italy) by activity staining procedures [14, 15] for the following enzyme markers: purine nucleoside phosphorylase (NP) (14q12-q20), glucose-6-phosphate dehydrogenase (G6PD) (Xq26-q28), adenosine deaminase (ADA) (20q13-qter), glucose phosphate isomerase (GPI) (19pter-q13), lactate dehydrogenase (LDHA) (11p12) and (LDHB) (12p12), and

cytoplasmic superoxide dismutase (SOD1) (21q22). Given in the second set of parentheses are the chromosome regions containing the respective enzyme loci [16].

RESULTS

For the first series of experiments designed to find the chromosomal location of the human sequence in pAW101, we selected a panel of Chinese hamster × human hybrids derived from five different human donors and two different Chinese hamster cell lines. To avoid possible difficulties in interpreting a negative result on the Southern blot, we chose hybrids having relatively high numbers of human chromosomes. Thus, for any given chromosome, most of the hybrids would give a positive signal that then can be correlated unambiguously with the presence of that chromosome. The frequency of representation (average copy number per cell) of each human chromosome present was usually on the order of .8 to .9 (table 1A). In addition to the 10 informative hybrids of the panel, we have used a positive control hybrid (X-7A) that has retained at least one copy of each human chromosome.

DNA samples from the parental lines and hybrids were digested with Eco RI, subjected to electrophoresis, transferred to DBM paper, and hybridized with radioactive pAW101. Nine of the 10 panel hybrids in table 1A (lanes 10–15 in fig. 1 and lanes 9, 11, and 13 in fig. 2) produced a positive signal, as did the positive control hybrid (X-7A, lane 6, fig. 1). None of the DNAs from the two Chinese hamster parental cell lines (lanes 7 and 8, fig. 1) cross-hybridized with the human probe under the stringent conditions used. Hybridization with pAW101 in the nine hybrids and its absence in XXI-23A-2d (lane 16, fig. 1) correlates only with human chromosome 14, as shown in table 1A.

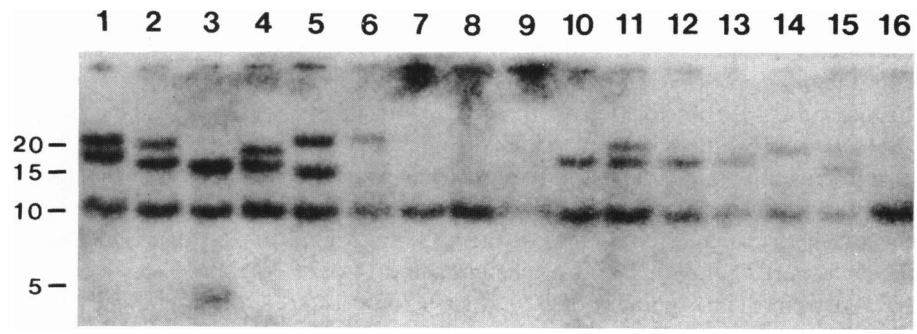

FIG. 1.—Autoradiogram of DBM filter hybridized with α-^{32}P-labeled recombinant plasmid pAW101. Ten μg of DNA cut with Eco RI was applied to each slot from the following sources: *lane 1*, human fibroblast TH-5, parent of hybrids XV-18A-8b (*lane 10*), XV-18A-10b aza (*lane 11*), XV-18B-7a (*lane 12*), and XV-15A-4a (*lane 13*); *lane 2*, human fibroblast KG-7, parent of hybrids XXI-51B (*lane 14*), XXI-33A-b (*lane 15*), and XXI-23A-2d (*lane 16*); *lane 3*, human fibroblast MN-4; *lane 4*, human fibroblast MN-3; *lane 5*, human fibroblast PA-2, parent of hybrid X-7A (*lane 6*); *lane 7*, Chinese hamster cell line V79/380-6; *lane 8*, Chinese hamster cell line Don/a23; *lane 9*, mouse cell line 3T3 TK$^-$. The single 10-kb band present in *all lanes* is due to a contaminant that has homology to pBR322 (data not shown).

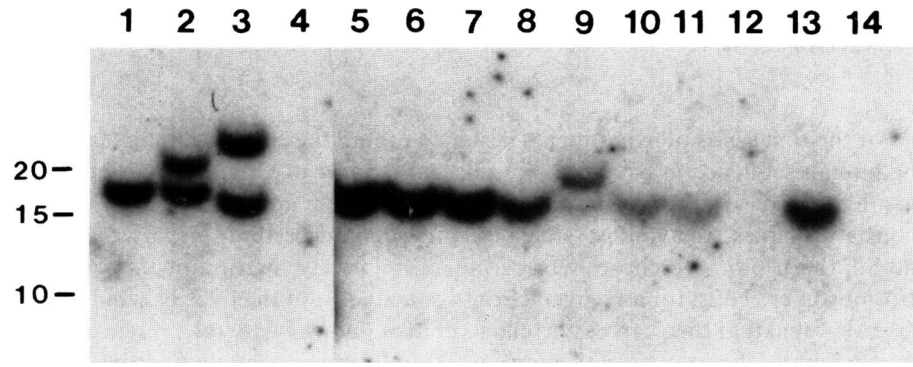

FIG. 2.—Hybridization of α-^{32}P-labeled pAW101 to Eco RI fragments of DNA from the following sources: *lane 1*, MN-4, human donor of hybrids XIII-3C (*lane 5*), XIII-3D (*lane 6*), XIII-3A (*lane 7*), XIII-3A aza (*lane 8*), XIII-1B (*lane 10*), XIII-7A (*lane 11*), XIII-7A aza (*lane 12*), and XIII-5B (*lane 13*); *lane 2*, MN-3, human donor of hybrid XII-12B aza (*lane 9*); *lane 3*, human fibroblast PA-2; *lane 4*, Chinese hamster V79/380-6; *lane 14*, hybrid XVII-10A-12a. Ten μg of DNA from the parental cell lines was applied to *lanes 1 through 4*; 15 μg of hybrid DNA was applied to the others.

Four of the five human donors (lanes 1–5, fig. 1) were heterozygous, as indicated by the presence of two bands in lanes 1, 2, 4, and 5. Thus, it was possible to determine whether one or both alleles (or homologs of chromosome 14) were present in each hybrid. In two hybrids (XV-18A-10b aza, lane 11, fig. 1) and (XXI-33A-b, lane 15, fig. 1), both fragment lengths ("alleles") were detected. This correlated well with frequencies of chromosomes 14 of 1.5 and 1.3, respectively. In the other positive clones, the frequencies of chromosome 14 were lower than 1.0, which correlated with the presence of only one of the two possible restriction fragments. This experiment was repeated two more times, using either DNA samples obtained from different preparations or samples that were further purified by cesium chloride gradient centrifugation. The results were consistent.

In one of the positive hybrids (XIII-7A), only the distal half of the long arm of chromosome 14, translocated to the X chromosome, was present. This der(X), t(X;14) chromosome had been retained under HAT selective pressure, while the normal chromosome 14 and the reciprocal translocation product der(14),t(X;14) had been lost (for illustration of the rearrangement, see [7]). The positive result with this hybrid suggested that the sequence homologous to pAW101 is located on the distal part of the long arm of chromosome 14 (q21→qter).

To confirm the assignment of *D14S1* to chromosome 14 and specifically to the distal 14q, we prepared a second set of DNA samples from hybrids selected for one of the following characteristics: (1) hybrids that had retained almost exclusively human chromosome 14 and hybrids that had retained a large number of the human chromosomes except chromosome 14; and (2) hybrids from series XII and XIII in which the der(X),t(X;14) translocation chromosome was first retained by growth in HAT medium, and subsequently selected against by growth in medium containing 8-azaguanine (8AG). When the DNAs from this second panel of hybrid clones were analyzed, we obtained the following results (fig. 2 and table 1B):

Hybrids XIII-3C, XIII-3D, and XIII-3A (lanes 5–7) that had retained both the normal chromosome 14 and the der(X),t(X;14) translocation chromosome produced an intense positive signal. MN-4 (lane 1), the human donor for these hybrid clones, was apparently not heterozygous. After counterselection of hybrid XIII-3A in 8AG, a decrease in the intensity of the signal was observed in hybrid XIII-3A aza (lane 8), which is consistent with the absence of the der(X),t(X;14) chromosome and retention of chromosome 14. Similar intensity bands were observed in the hybrids that possessed only the der(X),t(X;14) chromosome (lanes 10, 11, and 13). The 8AG-resistant derivative of hybrid XIII-7A (clone XIII-7A aza) produced no signal with pAW101 (lane 13), which is consistent with the absence of both the normal chromosome 14 and the der(X),t(X;14) chromosome. Hybrid XVII-10A-12a (lane 14) gave no signal and contained no chromosome 14, while 12 other human chromosomes, including chromosome 22, were present at high frequencies (table 1B). Since the human parental cells of series XVII hybrids were not available, we analyzed a hybrid from this series (clone XVII-18B-2a) that contained chromosome 14 at a frequency of 1.5. Two bands of hybridization, corresponding to fragments of 15 kb and 17 kb, indicated that the human donor of series XVII hybrids was heterozygous for *D14S1* (data not shown).

Hybrid XII-12B aza (lane 9, fig. 2) is of interest in that it allowed us to define the sensitivity of our detection method. The human donor was a male carrier of the balanced reciprocal t(X;14) translocation, inherited from his mother who was the human donor of series XIII hybrids. Fibroblasts from the male (MN-3, lane 2) were heterozygous for D14S1 fragment lengths, while fibroblasts from his mother (MN-4, lane 1) were apparently homozygous. The restriction fragment that they have in common must be the one derived from the der(X),t(X;14) chromosome. Hybrid XII-12B aza (lane 9) had retained a normal chromosome 14 that produced the positive signal in the position of the upper band of the MN-3 control (lane 2). However, selection in 8AG has apparently not been complete. Two out of 25 metaphase cells analyzed still contained the der(X),t(X;14) translocation chromosome, which explains the presence of the faint lower band in lane 9.

The results of the enzyme marker studies were generally consistent with the chromosome analyses (table 1). In particular, the expression of human purine nucleoside phosphorylase (NP-assigned to region 14q12→q20) was used as a marker for the presence of human chromosome 14 and/or the der(14),t(X;14) translocation chromosome. Human G6PD expression served as a marker for the der(X),t(X;14) chromosome in series XII hybrids, as well as in series XIII hybrids derived from the mother, since we have previously shown that in this reciprocal X/autosome translocation, the normal X chromosome is always the inactive one [7, 11].

Hybrid XXI-23A-2d was the only exceptional clone. It produced a very weak positive signal for pAW101 upon longer exposure of the autoradiograms (data not shown), as well as a weak band for NP, while chromosome analysis failed to detect human chromosome 14 in the 15 cells analyzed at this passage and 20 cells studied earlier. This hybrid was also positive for human LDHB and G6PD in the absence of the respective chromosomes 12 and X. Mosaicism with the presence of the

TABLE 1

CHROMOSOME CONTENT, MARKER ENZYME EXPRESSION, AND HYBRIDIZATION WITH pAW101 IN 20 CHINESE HAMSTER × HUMAN HYBRIDS

HYBRID	NO.*	HUMAN CHROMOSOMES (AVERAGE FREQUENCY PER CELL)														
		1	2	3	4	5	6	7	8	9	10	11†	12†	13	14†	14q21-qter
A																
XV-18A-8b	26	...	1.	.8	1.7	.8	...	...	.9	...	...	1.	1.	...	1.	...
XV-18A-10ab aza	20	a) 1.	.9	1.7	2.	1.	a) 1.	...	.8	.9	...	.9	1.	1.7	1.5	...
XV-18B-7a	24	1.	.9	1.1	...	.9	b) 1.	...	1.	.9	...	c) .4	.9	...	.8	...
XV-15A-4a	13	a) .2	.6	.8	.8	...	a) .2	.7	.9	...	.4	1.2	1.	.5	1.	...
XXI-51B	18	...	...	.9	.7	.6	...	1.3	.8	.2	.6	.7	...	...	.7	...
XXI-33A-b	16	...	...	.8	...	...	d) .4	e) .9	f) .7	g) .2	h) .7	...	.7	.8	1.3	...
XXI-23A-2d	15	...	...	...	...	.5	.6	...	...	...	...	j) 1.	...	.8	...	...
XIII-7A	24	...	...	...	...	.2	...	.1	1.1	.1	...	...	...	1.1	...	1.
XIII-5B	22	.2	...	k) .5	...	...	...	...	...	...	...	.8	.4	...	m) .6	.7
XII-12B aza	25	.9	...	.9	1.	...	.6	...	.7	...	...	...	.7	...	1.	0.08
B																
XIII-3C	15	...	...	...	...	...	...	...	...	...	...	...	...	...	.5	.7
XIII-3D	12	...	...	...	...	...	...	...	...	...	...	...	...	...	.7	.6
XIII-3A	17	...	...	...	...	...	...	...	...	...	...	...	...	...	1.	.8
XIII-3A aza	23	...	...	...	...	...	...	...	...	...	...	...	...	...	.9	...
XIII-1B	30	...	...	...	...	...	...	...	...	...	...	...	...	.8	...	.4
XIII-7A aza	20	...	...	...	...	.5	...	.4	.6	...	...	...	...	1.5	...	...
XVII-10A-12a	30	...	...	.9	p) 1.	...	1.7	1.	...	...	.9	1.	.9	...	...	...
XVII-18B-2a	25	...	.2	.8	...	...	.9	...	...	...	...	.8	.8	.9	1.5	...
XXI-22A-gq-1a	10	...	...	...	...	.8	.8	...	.8	...	...	1.	...	.8	...	...
XIII-4D-1c aza	20	...	...	.5	...	...	.6	...	...	...	...	...	...	...	...	...
% Discordant clones§		59	58	44	56	63	80	71	50	59	72	56	42	58	0	

Hybrid	No.*	Human chromosomes (average frequency per cell.)										Human enzyme		Hybridization with pAW101
		15	16	17	18	19†	20†	21†	22	X†	Y	NP	G6PD	
A														
XV-18A-8b	26	1.	1.9	.9	···	1.	1.	1.	1.	1.	0.9	+	+	+
XV-18A-10ab aza	20	.9	.8	···	···	.9	1.2	.8	1.4	···	0.9	+	−	+
XV-18B-7a	24	.9	.7	.9	.7	.7	1.1	.9	1.2	.8	0.9	+	+	+
XV-15A-4a	13	···	1.2	.5	.5	···	.8	.3	.8	.9	0.5	+	+	+
XXI-51B	18	.8	.8	.2	.7	.7	.6	···	.9	···	1.4	+	−	+
XXI-33A-b	16	.3	···	···	···	.6	···	i) .4	.6	···	···	+	−	+
XXI-23A-2d	15	···	···	.1	···	.4	.6	1.	.9	···	···	±	±	±
XIII-7A	24	.5	···	···	1.	···	.6	···	1.1	o) 1.	···	−	+	+
XIII-5B	22	···	···	···	···	···	···	···	n) .7	o) .7	···	+	+	+
XII-12B aza	25	.7	1.	···	.8	.8	···	.9	.8	o) .08	···	+	+	+
B														
XIII-3C	15	···	.8	···	···	···	···	···	.6	o) .7	···	+	+	+
XIII-3D	12	···	.2	···	···	···	···	···	.5	o) .6	···	+	+	+
XIII-3A	17	···	.3	···	···	···	···	···	.2	o) .8	···	+	+	+
XIII-3A aza	23	···	···	···	···	···	···	···	.7	···	···	+	−	+
XIII-1B	30	···	···	···	···	···	.6	···	···	o) .9	···	−	+	+
XIII-7A aza	20	.2	···	···	.8	···	···	···	.8	···	···	−	−	−
XVII-10A-12a	30	.8	···	···	···	···	.3	.9	.9	.9	···	−	+	−
XVII-18B-2a	25	r) .8	···	···	···	.8	.6	.9	s) .8	···	···	n.s.‡	n.s.	+
XXI-22A-gq-1a	10	1.0	···	.7	.8	···	.4	.8	.7	···	···	n.s.	n.s.	−
XIII-4D-1c aza	20	···	···	···	···	···	···	···	···	···	···	n.s.	n.s.	−
% Discordant clones§		56	32	63	63	42	47	56	24	50	25	···	···	···

NOTE: a) 1qter→1p32; 6p21→6pter; b) 6p21→6qter; c) i (11p); d) 6pter→cen; e) i (7p); f) 8cen→qter; g) 9p22→qter; h) 10cen→qter; i) 21cen→qter; j) 11p11→qter; k) 3cen→qter; m) 14pter→q21; n) 22q+; o) Xp22→Xqter; p) 4cen→qter; r) 15q14→qter; s) 22pter→q13.31.

* No. = no. metaphase spreads analyzed.

† Confirmed by enzyme marker studies.

‡ n.s. = not studied.

§ Rates of discordant segregation between each chromosome, and hybridization to pAW101, were calculated after exclusion of clone XXI-23A-2d and of all data of chromosomes involved in rearrangements or present at a frequency less than 0.1.

respective chromosomes at very low frequencies could account for the observed discrepancies. However, hybrid XXI-22A-g-1a derived from the same fusion and having similar human chromosome content was unambiguously negative (data not shown). Furthermore, the results obtained with the remaining 19 hybrids were unequivocal, showing concordant segregation. The rates of discordant segregation of *D14S1* with any other chromosome ranged from 24% to 80% (table 1).

DISCUSSION

In these studies, we have assigned the highly polymorphic locus *D14S1*, defined by the human DNA fragment inserted in the recombinant plasmid pAW101, to the distal part of chromosome 14 (region q21→qter). This is the first RFLP identified and mapped in the human species.

The uniqueness of the human pAW101 sequence is documented by its sole hybridization to a part of human chromosome 14. The restriction fragment patterns seen were the same in a hybrid containing at least one copy of each human chromosome, a hybrid containing just chromosomes 14 and 22, and in hybrids with only region q21→qter of chromosome 14 (in addition to variable numbers of other chromosomes). Furthermore, the intensity of positive signals correlated roughly with the copy number of chromosome 14 [and/or region 14q21→qter contained in the der(X),t(X;14) chromosome] irrespective of the total number of chromosomes present. Counterselection against the der(X)t(X;14) chromosome in a hybrid (XIII-7A) without the normal chromosome 14 resulted in complete absence of a signal. Apparently, no other human chromosome and no other region of chromosome 14 contains sequences homologous to the pAW101 insert.

The fact that five of our six human donors were heterozygous at this locus allowed us to identify each homolog of chromosome pair 14 in somatic cell hybrids. Thus, in three hybrids that had retained both chromosomes 14 (frequency greater than 1.0), both restriction fragment lengths were detected. On the other hand, only one *D14S1* allele was present in hybrids with chromosome 14 frequencies less than 1.0. This is explained by the clonal nature of these hybrids which may have lost the second chromosome 14 early after heterokaryon formation.

Hybridization with pAW101 is sensitive enough to detect a specific chromosome 14, even at low frequency. From the study of mother (MN-4) and son (MN-3) with the t(X;14) translocation, we could determine which restriction fragment was located on the translocation chromosome. This band was faintly present in a hybrid (XII-12B aza, fig. 2, lane 9) that contained the respective chromosome in only two of 25 metaphase spreads. The lower limit of detection of sequences homologous to pAW101 must, therefore, be at a chromosome frequency of less than 0.1.

The high degree of polymorphism at this locus has been reported using DNA extracted from leukocytes [2]. We found that of five human fibroblast strains each produced a unique pattern of Eco RI fragments hybridizing to pAW101. Furthermore, in two series of hybrids (XII and XIII) made with human leukocytes, the restriction fragment lengths were the same as in fibroblast strains derived from the donor individuals (MN-3 and MN-4). These results confirm the presence of this

DNA polymorphism in tissues other than leukocytes. They further support the notion that the polymorphism is present in germ line DNA, consistent with Mendelian inheritance, and has not been generated by DNA splicing events in leukocyte precursors.

What can be learned from the localization of *D14S1* in region 14q21→qter with respect to its usefulness in family linkage studies? The only other locus assigned to the same chromosome region by somatic cell genetic studies is the gene for tryptophanyl-tRNA synthetase (TRPRS or WARS) [11]. It is unknown whether this locus is polymorphic in human populations. The locus for NP, more proximal on 14q, is polymorphic with electrophoretic variants, null alleles, and homozygous deficiency in rare patients with a T-cell defect [17, 18]. Several other loci have been provisionally assigned to chromosome 14 without regional mapping information: creatine kinase BB, the isozyme predominantly expressed in the brain [19]; and two enymes catalyzing subsequent steps in the purine biosynthesis pathway, phosphoribosylglycineamide formyltransferase (GART) [20] and phosphoribosyl formylglycinamidine synthetase (PFGS) [21]. It is not known whether these loci are polymorphic and would be suitable for family linkage studies. The immunoglobulin heavy chain (GM) gene cluster has an inconsistent assignment to 14 [22], which has been confirmed indirectly by our recent assignment of *P1* (the locus for α_1-antitrypsin) to chromosome 14 [23]. *GM* and *P1* are linked with a lod score of 7.55 at $\theta = .23$ [24]. *GM* and *P1* are highly polymorphic and constitute excellent markers for linkage studies with *D14S1*. Conversely, loci for genetic disorders that have been assigned to chromosomes other than 14, for example, by linkage to HLA loci or to already mapped blood group markers, would not be suitable candidates for such studies. With the vast majority of known Mendelian traits as yet unassigned, there are many opportunities to fill the map of distal 14q, around *D14S1*, with clinically relevant or otherwise important human genes.

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