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Genetics

Human dihydrofolate reductase gene is located in chromosome 5 and is unlinked to the related pseudogenes

(human–mouse cell hybrids/DNA transfer hybridization/cDNA/diphtheria toxin sensitivity)

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ABSTRACT **The chromosomal location of the human dihydrofolate reductase (DHFR; EC 1.5.1.3) gene that is amplified in a methotrexate-resistant human cell line has been investigated by screening a large number of human–mouse cell hybrids containing overlapping subsets of human chromosomes. A correlation of genomic blotting data with the chromosome constitution of the individual cell hybrids has allowed the assignment of the human *DHFR* gene to chromosome 5. This chromosome assignment has been confirmed by the observation of a concomitant loss of the human *DHFR* gene and of sensitivity to diphtheria toxin, a marker associated with chromosome 5, in two human–mouse cell hybrids selected for resistance to the toxin. Six *Eco*RI fragments of human DNA containing *DHFR* pseudogenes or other *DHFR*-related sequences have been assigned to chromosomes other than chromosome 5.**

Recently, several cell variants resistant to high concentrations of the folate antagonist methotrexate (MTX) have been isolated from two different human cell lines, HeLa BU25 and VA_2-B (1). As described for other mammalian culture systems (2), these cell lines overproduce dihydrofolate reductase (DHFR; 5,6,7,8-tetrahydrofolate:$NADP^+$ oxidoreductase, EC 1.5.1.3), the target enzyme of MTX (1), as a consequence of selective amplification of the structural gene for DHFR (3). A striking feature of these MTX-resistant human cell variants is the pleomorphism of chromosomal alterations they exhibit. These include a highly variable number of double-minute chromosomes (DMs) (1), a homogeneously staining region (HSR) in one or more marker chromosomes, and a duplicated set of chromosomes in most cells of some variants (1). To understand the nature and origin of these chromosomal anomalies, the normal chromosomal location of the *DHFR* structural gene must be defined, since, in rodent cells (2) and human cells (unpublished data), both DMs and HSRs have been shown to be the sites of amplified *DHFR* genes. The recent cloning and characterization of human *DHFR* cDNAs (4) and the identification of the amplified *DHFR* structural gene fragments in *Eco*RI digests of genomic DNA (3) make it possible to study the segregation of the human *DHFR* gene in human–mouse hybrid cells containing overlapping subsets of human chromosomes and, by this approach, to identify the chromosome in normal human cells that is the site of the *DHFR* gene. In this study, the human *DHFR* gene is assigned to chromosome 5, and six *Eco*RI fragments containing *DHFR* pseudogenes or other *DHFR*-related sequences are shown to be located in human chromosomes other than 5.

MATERIALS AND METHODS

Materials. The restriction enzyme *Eco*RI was obtained from New England BioLabs or Bethesda Research Laboratories, *Escherichia coli* DNA polymerase I from New England Nuclear, agarose from Sigma, dextran sulfate from Pharmacia, nitrocellulose paper from Schleicher & Schuell, and [α-^{32}P]dCTP from Amersham.

Cell Lines and Conditions of Growth. The human cell lines VA_2-B and HeLa S3 were grown as described (1). The mouse cell line A9 was grown in Eagle's phosphate medium supplemented with 5% calf serum and azaguanine at 3 μg/ml. The parents of the hybrid cell lines of the AHA series were mouse A9 and human GM144; those of the 41pT series and of the FRY series, mouse A9 and human GM126; and those of the BDA series, mouse A9 and human GM589. The human cell lines GM144 and GM126 are two fibroblast lines with balanced chromosome translocations not involving chromosome 5, and the cell line GM589 is a fibroblast line with a balanced translocation of almost the entire chromosome 5 onto the long arm of chromosome 14 (Human Genetic Mutant Cell Repository, Camden, NJ, 1982). Nine independent hybrid clones, five subclones isolated from them by dilution plating, and two diphtheria toxin-resistant sublines derived from two independent clones were analyzed. The hybrids were grown as described (5).

DNA Extraction. High molecular weight DNA from VA_2-B, HeLa S3, and A9 cells was prepared by the method of Gross-Bellard *et al.* (6). Cell hybrid DNAs were prepared as reported (5), additionally digested with RNase and Pronase, extracted with phenol, precipitated with ethanol, and suspended in 10 mM Tris·HCl/1 mM EDTA, pH 8.0.

Preparation of Radioactive Probes. Nick-translation of the insert fragment of pHD84 (4), of the "probe 1.7," a derivative of pAT153 containing a 1.14-kilobase (kb) chromosome 5-specific single-copy DNA fragment (provided by P. Pearson), was carried out as described (4), using DNase I at 25 pg/ml, 4 units of *E. coli* DNA polymerase I, and 75–125 μCi (1 Ci = 37 GBq) of [α-^{32}P]dCTP (2000–3000 Ci/mmol) per 100 ng of DNA, with the other unlabeled dNTPs at 20 μM, to give a specific activity of 4–8 $\times$ 10^8 cpm/μg. The *Bam*HI fragments *e* and *f* of the c-*fms* human oncogene (7), which is located on human chromosome 5 (7, 8), were nick-translated by a similar procedure.

DNA Transfer and Hybridization Techniques. DNA samples (amounts specified in figure legends) were digested to completion with an excess of *Eco*RI restriction enzyme, electrophoresed through a 0.7% agarose slab gel, and transferred to nitrocellulose filters by the method of Southern (9).

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Abbreviations: MTX, methotrexate; DHFR, dihydrofolate reductase; DM, double-minute chromosome; HSR, homogeneously staining region; kb, kilobase(s).

Hybridization with the ^{32}P-labeled probe was carried out as described (3), except for the absence of poly(cytidylic acid), using $1.5–2.0 \times 10^7$ cpm of probe per filter in 5 ml of hybridization buffer. The filters were washed at 68°C once with 6× NaCl/Cit (1× NaCl/Cit = 0.15 M NaCl/0.015 M sodium citrate)/10× Denhardt's solution (10)/0.1% sodium pyrophosphate/0.1% $NaDodSO_4$ for 30 min, three times with 2× NaCl/Cit/0.1% $NaDodSO_4$, and three times with 0.5× NaCl/Cit/0.1% $NaDodSO_4$, 20 min each. The filters were then dried and exposed for autoradiography (4).

Isozyme and Karyotype Analysis. All clones and subclones were subjected to isozyme analysis and most were karyotyped; furthermore, the DNA from the majority of the hybrids was also tested with chromosome 5-specific probes (*Bam*HI fragments *e* and *f* of the c-*fms* human oncogene and "probe 1.7") and with a chromosome 12-specific probe (5). The isozyme tests were previously described (5). The chromosome constitution of the hybrids was determined on 25–50 Giemsa-banded metaphases per hybrid. The following criteria were used for characterization of the cell hybrid lines: A cell line was scored positive (+) for a human chromosome when its presence was revealed by appropriate isozyme expression and by karyotypic identification in >20% of metaphases; in some cases, a clear karyotypic identification alone or isozyme expression in the absence of karyotype data was used for positive chromosome scoring. When no isozyme markers were expressed and when, in the karyotyped hybrids, <5% of cells exhibited the specific human chromosome, the hybrid was scored negative (−). In the case of chromosome 5, the results of the specific probe hybridization assays were also used as criteria, and they were always found to be in agreement with the other scoring criteria, so that all scorings for chromosome 5 were based on at least two criteria. A hybrid was scored as weakly positive (+/−) when the chromosome was present in 5–20% of metaphases and the isozyme analysis gave inconsistent results in multiple assays. In cases in which the isozyme and karyotype data were insufficient to clearly characterize a hybrid, this was scored 0 and the corresponding data were not included in the mapping summary. In the hybrids of the series BDA deriving from A9 and the human fibroblast line GM589 that were positive for chromosome 5 by karyotypic analysis, both translocated and nontranslocated chromosomes 5 were observed and scored. In three of these hybrids (10a3-1, 10a3-4, and 10a3-6), the translocated chromosomes 5 were predominant (83–100%), in two other hybrids (17b17 and $10a4aFa_{9-1}$), mostly nontranslocated chromosomes 5 were observed (79% and 63%, respectively).

RESULTS

Organization of *DHFR*-Specific Sequences in Genomic DNAs from Human and Mouse Cells. The plasmid pHD84 is a human *DHFR* cDNA clone derived from the MTX-resistant VA_2-B derivative 6A3 and contains the complete human DHFR-encoding sequence (3, 4). The coding sequence of human *DHFR* shows an 89% nucleotide sequence homology to that of the mouse (11). The plasmid pHD84 was used to screen human–mouse somatic cell hybrid DNAs for human *DHFR*-specific sequences. Southern blots of *Eco*RI-digested human genomic DNA probed with pHD84 show a complex pattern consisting of fragments of the human *DHFR* gene that is amplified in VA_2-B 6A3 cells and of fragments containing other *DHFR*-specific sequences (3). Two of the latter fragments have been shown to contain portions of an intronless pseudogene (3), while the nature of the other *DHFR*-related sequences is uncertain. In this paper, fragments that hybridize to the *DHFR* pHD84 probe, but that do not belong to the structural gene amplified in 6A3 cells, will be referred to as *DHFR*-related sequences.

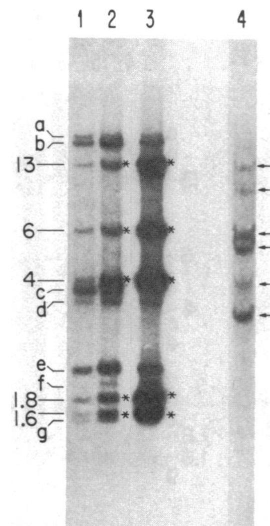

FIG. 1. Patterns of hybridization with a human *DHFR* probe of *Eco*RI-digested DNA from human and mouse cell lines. DNA samples (10 μg) were digested to completion with *Eco*RI, electrophoresed through agarose slab gels, transferred to nitrocellulose paper, and hybridized with the ^{32}P-labeled insert of pHD84. The numbers on the left indicate the normal human *DHFR* gene fragments (also marked by asterisks) and represent their sizes in kb, and the letters refer to DNA fragments containing human *DHFR*-related sequences. The long arrows on the right of lane 4 indicate the mouse *DHFR* gene fragments; the short arrows refer to DNA fragments containing mouse *DHFR*-related sequences. The band corresponding to the upper fragment containing mouse *DHFR*-related sequences appears with variable intensity in different blots and may represent a fragment particularly resistant to *Eco*RI. Lane 1, GM589; lane 2, HeLa S3; lane 3, VA_2-B; lane 4, A9.

Fig. 1 illustrates the distribution of *DHFR*-specific sequences that hybridize to the pHD84 probe in *Eco*RI digests of genomic DNA from the normal human fibroblast line GM589 (lane 1), HeLa S3 (lane 2), and VA_2-B, a human cell line containing a moderate amplification of the human *DHFR* gene (3) (lane 3). The fragments of the structural gene amplified in VA_2-B DNA, which correspond to those amplified in the DNA from the MTX-resistant VA_2-B 6A3 cell line (3), are 13, 6, 4, 1.8, and 1.6 kb in size. These fragments, which span the entire reading frame for human *DHFR*, have recently been shown to be a part of the human structural gene for DHFR by molecular cloning and DNA sequence analysis (12). The 1.6-, 4-, and 13-kb *Eco*RI fragments have been cloned, and the organization of this portion of the gene has been determined (13). Fragments not associated with the structural gene are designated in Fig. 1 by letters *a–g* and have molecular sizes of 18, 17, 3.8, 3.5, 2.3, 2, and ≈1.6 kb, respectively (ref. 4 and present work). The two fragments *d* and *e* contain the recently isolated intronless pseudogene (3) mentioned above.

The hybridization pattern of an *Eco*RI digest of mouse A9 cell DNA probed with the human DHFR probe pHD84 is shown in lane 4 of Fig. 1. The four expected *Eco*RI fragments of the mouse *DHFR* gene can be seen (14), as indicated by long arrows. In addition, two previously unreported mouse DNA fragments containing *DHFR*-related sequences are detected by the human *DHFR* probe, as shown by short arrows. The fragments of the human *DHFR* gene with sizes of 13, 6, and 4 kb migrate to positions in the gel that correspond closely to those of mouse DNA fragments hybridizing with the *DHFR* probe. In contrast, the 1.8- and 1.6-kb gene fragments are found in regions of the blot free of mouse-specific bands. Thus, it has been possible to score the human–mouse hybrid DNAs for the human *DHFR* gene by using the 1.8- and 1.6-kb bands as diagnostic of the entire gene. How-

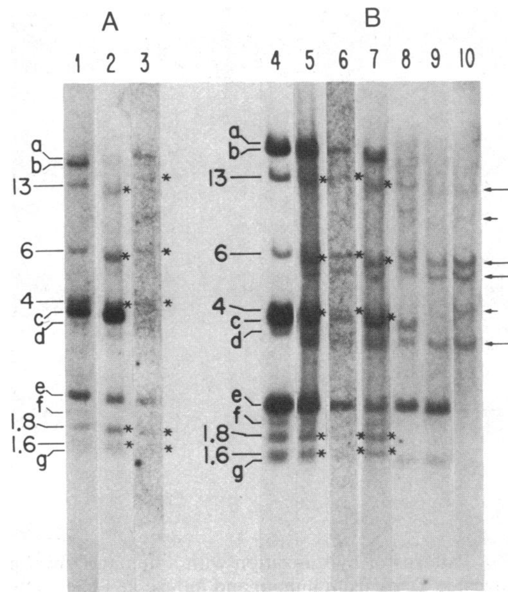

FIG. 2. Patterns of hybridization with a human *DHFR* probe of *Eco*RI digested DNA from human–mouse cell hybrid clones. DNA samples [10 μg, except BDA 10a3-4 and BDA 10a3-6 (≈2 μg)] digested with *Eco*RI were treated as described for Fig. 1. (*A*) Lane 1, GM589, human parental line; lane 2, BDA 17b17; lane 3, BDA 10a3-6. (*B*) Lane 4, HeLa S3; lane 5, BDA 10a3-1; lane 6, BDA 10a3-4; lane 7, BDA 10a4aFa$_{9\text{-}1}$; lane 8, BDA 14b25; lane 9, BDA 14b25-2; lane 10, A9, mouse parental line. For an explanation of numbers, letters, and arrows, see legend to Fig. 1. The asterisks indicate the human *DHFR* gene fragments in the cell hybrid DNAs.

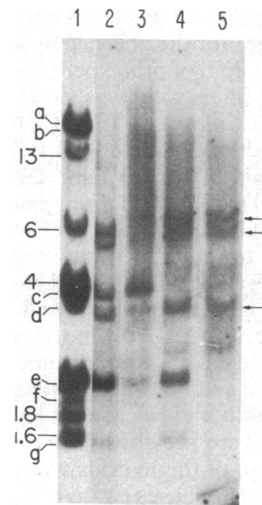

FIG. 3. Patterns of hybridization with a human *DHFR* probe of *Eco*RI-digested DNA from human–mouse hybrid cell DNAs. DNA samples (10 μg) digested with *Eco*RI were treated as described for Fig. 1. Lane 1, HeLa S3; lane 2, AHA 16e; lane 3, AHA 3d-2; lane 4, FRY-1; lane 5, A9, mouse parental line. For an explanation of numbers, letters, and arrows, see legend to Fig. 1.

ever, bands corresponding to the other expected human *DHFR* gene fragments were always observed whenever the 1.8- and 1.6-kb bands were present. Furthermore, in several cases, from the intensity of the signal given by these other bands (in particular the 13- and 6-kb bands) and/or the absence of expected mouse gene fragments, the association of these bands with human gene fragments could be inferred. One also sees in Fig. 1 that the human DNA fragments containing *DHFR*-related sequences *a*, *b*, *c*, *e*, *f*, and *g* are not obscured by cross-hybridizing mouse DNA fragments.

Analysis of the Human *DHFR* Gene and Related Sequences in Human–Mouse Cell Hybrid DNAs. Southern blots of *Eco*RI-digested DNA from several human–mouse cell hybrids probed with pHD84 are shown in Figs. 2 and 3. In almost all cell hybrid DNAs shown, the characteristic bands of the mouse *DHFR* gene can be seen. By contrast, the fragments of the human structural gene for *DHFR* (asterisks) are present in only 5 of the 10 human-mouse DNA samples shown. Two hybrids, BDA10a3-4 and BDA10a3-6, gave weak hybridization signals, due to the lower amounts of DNA available. However, the 1.8- and 1.6-kb bands were clearly discernible (especially on the original autoradiogram); furthermore, the 13- and 6-kb bands, because of their intensity, could be assigned with reasonable confidence to human *DHFR* gene fragments. In all blots, one or more fragments with human *DHFR*-related sequences are also observed. The results of genomic blotting and chromosome

Table 1. Human chromosome distribution in human–mouse cell hybrids and summary of genomic blotting results

	Reaction of genomic *Eco*RI fragments with pHD84*							Presence of human chromosome, as determined by human isozyme expression and by cytogenetic analysis†																						
Hybrid cell line	*DHFR*	*a*	*b*	*c*	*d*/*e*	*f*	*g*	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	X
BDA14b25	−	0	−	+	0/+	−	+	+	−	+	+	−	−	+	0	−	−	−	−	−	+	−	−	−	−	−	−	−	−	+
BDA14b25-2	−	0	−	−	0/+	−	+	+	−	+	+	−	−	−	+	−	−	−	−	+	−	−	−	+	−	−	−	−	−	+
BDA10a3-1	+	+	+	+	+/+	+	0	−	+	+	−	+	+	−	+	−	+	+	+	−	−	−	+	+	−	−	+	−	−	+
BDA17b17	+	+	+	+	0/+	0	0	+	−	+	+	+	−	−	−	+	−	−	+	−	+	+	−	−	+	+	+	+	+	+
FRY-1	−	−	−	−	0/+	−	+	−	−	−	−	−	−	−	−	−	−	+	−	−	−	−	−	−	−	−	−	−	−	−
41pT2a‡	−	+	+	+	+/+	0	+	−	+	+	+	−	+	−	+	+	+	−	+	−	+	+	+	−	+	+	−	+	+	+
BDA10a3-4	+	0	+	+	+/+	0	0	−	+	+	0	+	+	−	0	−	+	−	+	0	−	−	0	0	−	−	+	−	0	+
BDA10a3-6	+	0	0	0	0/+	0	0	−	+	+	0	+	+	−	0	−	+	−	+	0	−	−	0	0	−	−	+	−	0	+
AHA3d2	−	−	−	+	0/+	0	+	−	−	+	+	−	−	0	0	−	+	+	+	−	−	−	−	−	+	+	−	−	0	+
AHA16e	−	−	−	+	0/+	−	+	−	−	0	−	+/−	−	−	−	0	+	+	+	+	+	−	−	−	+	+	+	+	+	+
AHA11a‡	−	−	−	−	0/−	0	0	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	+
BDA10a4aFa$_{9\text{-}1}$	+	0	+	+	+/+	+	−	−	+	+	+	+	+	−	−	−	+	+	+	+	+	−	−	+	−	−	−	−	−	+
BDA10a3	+	+	+	+	0/+	+	+	−	+	+	+	+	+	−	+	−	+	+	+	−	−	−	−	+	+	+	−	−	−	+
BDA10a3DT	−	+	+	+	0/+	+	+	−	+	0	−	−	+	−	+	0	+	+	+	+	−	−	+	+	+	−	+	−	+	+
BDA17b17-1	+	0	0	+	0/+	−	+	+/−	+/−	0	+	+	−	−	+	0	−	−	+/−	+	−	−	+	−	−	−	+	+	+	+
BDA17b17-1DT	−	0	0	+	0/+	−	+	+/−	−	0	+	−	−	−	−	0	−	−	−	+	−	−	0	−	−	−	−	+	+	+

*+, Fragment(s) present; −, fragment(s) absent; 0, undetermined.
†See *Materials and Methods* and ref. 5 for the techniques of isozyme and karyotype analysis.
‡Genome blots not shown.

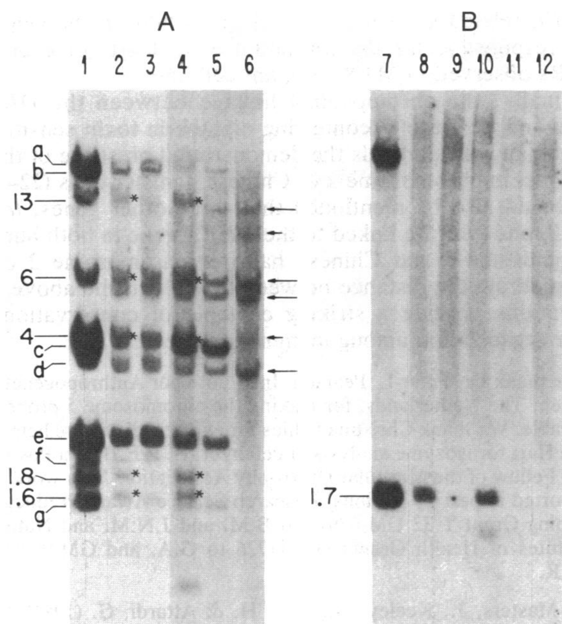

FIG. 4. Correlation of resistance to diphtheria toxin with the loss of the human *DHFR* gene (*A*) and the loss of DNA sequences complementary to a human chromosome 5-specific probe (*B*) in two human–mouse cell hybrids. Two human–mouse cell hybrid clones were grown in the presence of diphtheria toxin and a toxin-resistant subline was derived from each (see text). (*A*) DNA samples (10 μg) digested with *Eco*RI were treated as described for Fig. 1. Lane 1, HeLa S3; lane 2, BDA 10a3; lane 3, BDA 10a3DT, toxin-resistant; lane 4, BDA 17b17-1; lane 5, BDA 17b17-1DT, toxin-resistant; lane 6, A9. Normal *DHFR* gene fragments in lanes 2 and 4 are indicated by asterisks. (*B*) DNA samples (10 μg) digested with *Eco*RI and transferred to nitrocellulose paper were hybridized with ^{32}P-labeled probe 1.7 specific for human chromosome 5 (see text). Lane 7, HeLa S3; lane 8, BDA 10a3; lane 9, BDA 10a3DT, toxin-resistant; lane 10, BDA 17b17-1; lane 11, BDA 17b17-1DT, toxin-resistant; lane 12, A9.

analysis for these and other cell hybrids described below are detailed in Table 1. The data in Table 1 strongly suggest that chromosome 5 contains the *DHFR* gene (see *Discussion*). The results of Table 1 also indicate that none of the DNA fragments containing human *DHFR*-related sequences segregate with chromosome 5.

Correlation of Human *DHFR* Structural Gene, Chromosome 5 Markers, and Diphtheria Toxin Sensitivity in Human–Mouse Cell Hybrids. The assignment of the human *DHFR* gene to chromosome 5 indicated by the mapping experiments discussed above suggested a confirmatory experiment based upon the selectable loss of a marker previously mapped in human chromosome 5. It has been shown that sensitivity to diphtheria toxin in human–mouse cell hybrids is associated with a gene or genes located in human chromosome 5 (15). Therefore, human–mouse cell hybrids can be selected for the loss of human chromosome 5 by growing them in the presence of diphtheria toxin. Two human–mouse cell hybrids containing the human *DHFR* gene were exposed to Connaught diphtheria toxin at 2×10^{-5} L_f units/ml (15), and a toxin-resistant subline was isolated from each. The four lines were subjected to isozyme and karyotype analysis (Table 1); in particular, they were screened for expression of hexosaminidase B, a human chromosome 5 marker (16), and further tested for the presence of chromosome 5 by probing genomic blots with a human chromosome 5 unique sequence DNA marker (probe 1.7). By both criteria, the two cell lines had retained human chromosome 5 while the two diphtheria toxin-resistant derivatives had lost this chromosome. It should be noticed that chromosome 5 was the only chromosome lost by *both* toxin-resistant sublines. The DNAs from the four lines were then screened for the human *DHFR* structural gene. Fig. 4 shows the results of the blotting experiment utilizing the human *DHFR* probe (Fig. 4*A*, lanes 2–4) or the chromosome 5-specific probe 1.7 (Fig. 4*B*, lanes 8–11). The absence of the 1.8- and 1.6-kb human *DHFR* bands from the DNA blots of the diphtheria toxin-resistant lines indicates that the human *DHFR* gene has been lost. Thus, the loss of chromosome 5 in the diphtheria toxin-resistant lines is accompanied by the loss of the human *DHFR* gene. This result strongly supports the assignment of the human *DHFR* gene to chromosome 5.

Polymorphism of the probe 1.7 in human populations has been reported (17). This is reflected in the appearance of additional probe 1.7-related bands in HeLa cell DNA (Fig. 4*B*, lane 7) and in the DNA from the cell hybrid BDA 17b17-1 (Fig. 4*B*, lane 10).

DISCUSSION

The distribution of the human *DHFR* gene in all the hybrid cell lines analyzed, including the diphtheria toxin-resistant derivatives, is summarized in Table 2. It is clear that chromosome 5 is the only chromosome showing a complete concordance of segregation with the *DHFR* gene. Other genomic blotting data (not shown) have revealed the absence of the *DHFR* gene in six additional human–mouse cell hybrids lacking chromosome 5. We conclude that the *DHFR* structural gene that has been amplified in the human cell lines VA_2-B and VA_2-B 6A3 resides in human chromosome 5.

The present analysis has also provided information concerning the chromosomal location of several fragments containing human *DHFR*-related sequences. Table 3 summarizes the most probable chromosome assignment of each of the *Eco*RI fragments *b*–*g*. Although the available data do not

Table 2. Assignment of the structural gene for DHFR to human chromosome 5

Segregation*	Number of hybrids showing concordant or discordant segregation of human *DHFR* gene with each chromosome																						
	1	2	3	4	5†	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	X
Concordant																							
+/+	1	5	6	4	7	5	0	3	1	5	3	6	2	2	1	2	3	2	2	5	2	2	7
−/−	6	7	2	4	8	7	7	4	5	5	5	5	5	6	8	6	7	5	6	7	6	4	1
Discordant																							
+/−	5	1	0	1	0	2	7	2	5	2	4	0	3	5	6	3	2	5	5	2	5	3	0
−/+	2	2	4	5	0	2	1	3	1	4	4	4	4	3	1	2	2	4	3	2	3	4	8
Total	7	3	4	6	0	4	8	5	6	6	8	4	7	8	7	5	4	9	8	4	8	7	8

Only the unambiguous data of Table 1 (+ or −) were used in the compilation of this table.

*In each case the first plus or minus refers to the presence or absence, respectively, of the human *DHFR* gene, as determined by genomic blotting experiments. The second plus or minus refers to the presence or absence of the chromosome numbered on the top of the table, as determined by isozyme and karyotype data.

†Complete concordance of segregation between the *DHFR* structural gene and human chromosome 5 indicates that the structural gene resides on this chromosome.

allow an unambiguous chromosome assignment for these fragments, they do exclude most chromosomes, including chromosome 5, as their sites. Two of these fragments, *d* and *e* (Fig. 1), have recently been shown to contain an intronless *DHFR* pseudogene, *ψHD1* (3). On the basis of the data reported here, an assignment of the pseudogene *ψHD1* to chromosome 3 or X has been made, with the evidence strongly favoring chromosome 3. Two other fragments, *b* and *c* (Fig. 1), because of their size and the intensity of the hybridization signal, appear to be good candidates for containing two additional intronless *DHFR* pseudogenes that have recently been isolated from human DNA libraries (13). In particular, fragment *b* has a size (≈17 kb) that is compatible with the >15-kb *Eco*RI subfragment carrying the *hDHFR-ψ2* pseudogene isolated from a fetal DNA library, and fragment *c* has a size (≈3.8 kb) very close to that of the fragment containing the *hDHFR-ψ1* pseudogene isolated from an adult DNA library. The present analysis indicates that fragment *b* is located in chromosome 2 or 6, while fragment *c* is located in chromosome 3 or X.

The nature of the other fragments producing faint bands in the *Eco*RI restriction pattern of *DHFR*-specific sequences in human genomic DNA (fragments *a*, *f*, and *g*, Fig. 1) is unknown. The low intensity of the signals given by these fragments with a *DHFR* cDNA probe and the chromosome segregation pattern would exclude the possibility that they represent *DHFR* genes with different *Eco*RI restriction patterns.

The present chromosome assignment of the human *DHFR* gene is relevant to the origin of the chromosomal anomalies, DMs and HSRs, observed in several MTX-resistant cell lines (1, 18, 19). The human MTX-resistant cell line VA_2-B 6A3 exhibits an HSR containing amplified *DHFR* genes in the long arm of a chromosome clearly distinct from chromosome 5 (unpublished data). HSRs observed in other human MTX-resistant cell lines have been reported to be located in chromosomes 6, 19 (ref. 18), and 10 (ref. 19). The observation in human MTX-resistant cell lines of HSRs containing amplified *DHFR* genes in chromosomes other than chromosome 5 strongly suggests that a translocation event from chromosome 5 to other chromosomes has occurred in these cell lines. A role for DMs in the amplification and translocation of the *DHFR* gene has been suggested (2, 20), but very little evidence has been reported about their possible involvement. Recently, an apparent translocation and amplification of the c-*myc* gene from its normal position in human chromosome 8 to HSRs in the X chromosome has been described in malignant neuroendocrine cells derived from a human colon carcinoma (21). In the present case, an interesting possibility is suggested by the observation that the human genome contains, besides the *DHFR* structural gene that is amplified in 6A3 cells, up to as many as six other loci showing homology to the human *DHFR* probe. It is conceivable that translocation events involving homologous recombination between the *DHFR* structural gene in chromosome 5 and other *DHFR*-related sequences located elsewhere in the genome are responsible for the formation of at least some of the HSRs observed in MTX-resistant cell lines.

Finally, the chromosomal linkage between the *DHFR* gene and the gene(s) conferring diphtheria toxin sensitivity to human cells parallels the demonstrated presence of these two loci in chromosome 2 of Chinese hamster cells (22–24). It should also be mentioned that three other genes, ***leuS***, ***emtB***, and ***chr***, are linked to the *DHFR* gene in both human chromosome 5 and Chinese hamster chromosome 2 (25). Considering the distance between these loci, the above observations provide a striking example of conservation of gene organization among mammalian species.

Table 3. Mapping of *DHFR* structural gene, *DHFR* pseudogenes, and other *DHFR*-related sequences in the human genome

*Eco*RI band* (ref.)	Molecular size, kb	Chromosome
DHFR fragment	13, 6, 4, 1.8, 1.6	5
b (*hDHFR-ψ2* ?) (13)	17	2 or 6
c (*hDHFR-ψ1* ?) (13)	3.8	3 or X
d/*e* (*ψHD1*) (4)	3.5/2.3	3 or X†
f	2	2 or 6
g	≈1.6	3 or X

*See Fig. 1.
†Since band *e* is present in equal quantities in human male and female placental DNAs (data not shown), the most probable chromosome is no. 3.

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