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Assignment of the Human Dihydrofolate Reductase Gene to the q11→q22 Region of Chromosome 5

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Cells from a dihydrofolate reductase-deficient Chinese hamster ovary cell line were hybridized to human fetal skin fibroblast cells. Nineteen dihydrofolate reductase-positive hybrid clones were isolated and characterized. Cytogenetic and biochemical analyses of these clones have shown that the human dihydrofolate reductase (*DHFR*) gene is located on chromosome 5. Three of these hybrid cell lines contained different terminal deletions of chromosome 5. An analysis of the breakpoints of these deletions has demonstrated that the *DHFR* gene resides in the q11→q22 region.

The dihydrofolate reductase (DHFR) gene, *DHFR*, is one of the best-characterized "housekeeping" genes in mammalian cells because of the isolation and characterization of variants that have amplified this gene. Somatic cell variants resistant to methotrexate (MTX) have been isolated in mouse (2, 17, 40), hamster (20, 31, 34), and human (29, 43) cells. In general, these cell lines have a several hundredfold increase in DHFR activity and a corresponding increase in the number of copies of the *DHFR* gene. The increased copy number in conjunction with the overproduction of DHFR mRNA in these cells has allowed the cloning of both cDNA and genomic probes for the mouse (10, 36), hamster (9, 28, 32), and human (12, 35) genes.

The amplified *DHFR* genes have been shown to reside on either extrachromosomal elements called double minutes (25) or within homogeneously staining regions (HSRs), which appear frequently on specific chromosomes (17, 37, 39, 43). In MTX-resistant Chinese hamster ovary (CHO) cells, HSRs have been found most often on the short arm of chromosome 2, which carries the unamplified *DHFR* gene (19; M. Roberts, K. M. Huttner, R. T. Schimke, and F. H. Ruddle, J. Cell Biol. **87**:288, 1981). HSRs have been found also on specific chromosomes in MTX-resistant human cell lines (5, 39, 43). In particular, Wolman et al. (43) have associated amplification of the human *DHFR* gene with an HSR on the long arm of chromosome 10. These authors have suggested that a human *DHFR* gene is located on this chromosome. Srimatkandada et al. (39), however, have found HSRs containing amplified *DHFR* genes on chromosomes 5, 6, and 19 in MTX-resistant human leukemia cells. These studies raise doubts regarding whether the chromosomal location of an HSR marks the normal location of a nonamplified gene.

We have taken a direct approach to map the human *DHFR* gene by using somatic cell hybridization techniques. This approach became possible when Urlaub and Chasin (41) isolated mutants of CHO cells deficient in DHFR activity. These DHFR-negative ($DHFR^-$) cells have been shown to require thymidine, glycine, and a source of purines for growth (41). Urlaub and Chasin (41) have demonstrated that DHFR-positive ($DHFR^+$) cells can be obtained from the DHFR mutants by selecting for cells capable of growing solely in the absence of purines. Furthermore, the $DHFR^-$ phenotype has been shown to be recessive to the wild type in somatic cell hybrids (41).

Our approach was to hybridize cells from the DHFR mutant cell line DXB11 with $DHFR^+$ human skin fibroblast cells. Interspecific hybrid clones were selected in medium lacking purines and containing 1 μM ouabain (3). Nineteen hybrid clones were isolated and characterized. Cytogenetic and biochemical analyses of these clones have shown that the human *DHFR* gene is located on chromosome 5. Additionally, several hybrid clones have been shown to contain different terminal deletions of chromosome 5. An analysis of the breakpoints of these deletions has allowed us to make a regional assignment of the *DHFR* gene to the q11→q22 region.

MATERIALS AND METHODS

Cell lines and strains. CHO cell lines CHO-K1 ($DHFR^+$/$DHFR^+$) and DXB11 ($DHFR^-$/$DHFR^-$) were provided by L. Chasin, Columbia University (New York, N.Y.). Fetal skin fibroblasts from a normal male (strain CRL1475) were purchased from the American Type Culture Collection. Fibroblasts from a patient [46,XY,t(4;11)(q25;p13)] were donated by the Cytogenetics Unit, Alfred I. duPont Institute.

Growth of cells. CHO cells were grown routinely in Ham F12 medium (GIBCO Laboratories, Grand Island, N.Y.) supplemented with 10% (vol/vol) fetal bovine serum. This medium was designated as F12FBS10. Hybrid cells were maintained in F12 medium without hypoxanthine (K. C. Biological Inc., Lenexa, Kans.) and containing 10% (vol/vol) dialyzed fetal bovine serum. Dulbecco minimal essential medium (GIBCO) supplemented with 10% (vol/vol) fetal bovine serum was used for the growth of human fibroblast cells.

All cells were negative for mycoplasma contamination as determined by in situ staining of monolayer cultures with the DNA-binding dye Hoechst 33258 as described previously (13).

Somatic cell hybridization. Cells were hybridized by a modification of the procedure described by Davidson et al. (15). DXB11 cells (2×10^6) and human fibroblast cells (1×10^6) were plated together in 25-cm^2 tissue culture flasks containing 5 ml of F12FBS10 medium. After 24 h, the monolayers were washed once with 2 ml of Dulbecco minimal essential medium. Three ml of 50% (wt/wt) polyeth-

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ylene glycol (molecular weight, 1,000; J. T. Baker Chemical Co., Philipsburg, N.J.) dissolved in Dulbecco minimal essential medium was added to each flask. After 1 min at room temperature, the polyethylene glycol was removed, and the monolayers were rinsed rapidly five times with Hanks balanced salt solution. F12FBS10 medium (5 ml) was added to each flask, and the cells were incubated at 37°C for 24 h. The cells were harvested by trypsinization and seeded at densities of 1.2×10^5, 2.4×10^5, and 4.7×10^5 per 100-mm tissue culture dish. Each dish contained 10 ml of F12 medium without hypoxanthine supplemented with 1 μM ouabain (Aldrich Chemical Co., Milwaukee, Wis.) and 10% dialyzed fetal bovine serum (selective medium). After 14 days, colonies were isolated with cloning rings. Cells from each colony were removed by trypsinization and grown in selective medium without ouabain.

Chromosome preparation and cytogenetic analyses. Chromosomes were prepared by standard techniques (45). Slides were stored at room temperature for at least 1 week before any staining procedures were used.

Alkaline Giemsa (G-11) staining was performed by a modification of the procedure described by Alhadeff et al. (1). Slides were immersed in freshly prepared 6% (vol/vol) Giemsa in 50 mM sodium phosphate buffer (pH 11.3) for 5 to 6 min at 37°C and rinsed in distilled water. If differential staining did not occur during this time interval, unstained slides were dehydrated first with two changes of 100% methanol and placed for 20 min in a drying oven maintained at 99°C. Slides were allowed to cool to room temperature before staining.

Chromosomes were banded by trypsin-Giemsa (GTG) staining. Slides were rinsed in Hanks balanced salt solution without calcium and magnesium, immersed in 0.2% (wt/vol) trypsin in the above salt solution for 30 to 60 s, stained in 4% (vol/vol) Giemsa in Gurrs buffer (pH 6.8) for 4 min, and rinsed in distilled water.

Normal Chinese hamster chromosomes were numbered according to the standard nomenclature proposed by Ray and Mohandas (38). The nomenclature proposed by Deaven and Petersen (16), Worton et al. (47), and Worton (44) was used for describing structurally abnormal Chinese hamster chromosomes.

Preparation of extracts. Cells were grown as monolayer cultures in 850-cm² roller bottles. Extracts were prepared by lysing monolayer cells in situ by a modification of the procedure described by Chang et al. (11). Cells were rinsed twice with Dulbecco phosphate-buffered saline and once with hypotonic solution (1 mM Tris-hydrochloride, 200 μM 2-mercaptoethanol, 20 μM EDTA) at 4°C. Hypotonic solution, warmed to room temperature, was then added, and the cells were swollen for 2 to 3 min at room temperature. The hypotonic solution was removed and replaced with 1 ml of buffer A (50 mM Tris-hydrochloride, 10 mM 2-mercaptoethanol, 1 mM EDTA, pH 7.4). Cells were lysed by scraping the swollen cells with a rubber policeman. Cellular debris was removed after centrifugation at 34,800 × *g* for 1 h at 4°C.

DHFR assay. DHFR was assayed by a modification of the method described by Frearson et al. (21). The reaction mixture (1 ml) contained 60 nmol of dihydrofolic acid (Sigma Chemical Co., St. Louis, Mo.), 150 nmol of NADPH (Sigma), 20 μmol of 2-mercaptoethanol, various amounts of KCl, and 100 mM potassium phosphate (pH 7.5), unless indicated otherwise. A $\Delta\varepsilon_{340}$ of 11,650 was used for the conversion of dihydrofolic acid and NADPH to tetrahydrofolic acid and $NADP^+$, respectively (24).

Protein was determined by using the Bio-Rad protein assay kit, which is based on the method reported by Bradford (7).

RESULTS

Cytogenetic characterization of the hamster and human cells used for somatic cell hybridization. The karyotype of the DXB11 cell line ($DHFR^-/DHFR^-$) has been reported to be different from other CHO sublines (33). In particular, the marker Z2 chromosome, which has been characteristic of most CHO cell lines examined previously (16, 44, 47), was absent in DXB11 cells. The Z2 and number 2 chromosomes have been shown to carry the CHO *DHFR* genes (19, 46; Roberts et al., J. Cell. Biol. **87**:288, 1981). Therefore, we felt it was important to confirm that DXB11 cells lacked the Z2 chromosome.

GTG-banded chromosomes from 50 metaphase DXB11 cells were examined for the presence of the Z2 and number 2 chromosomes. The Z2 chromosome and a chromosome designated as 2a were seen in 100% of the metaphase cells examined. The 2a chromosome contains an interstitial deletion of the short arm of a normal number 2 chromosome. This deletion can be seen when one compares the p arm of the 2a chromosome to the normal 2p arm of the Z2 chromosome (Fig. 1). The deletion in chromosome 2a seems to include bands p25 and p26, according to the standard nomenclature proposed by Ray and Mohandas (38). Since the *DHFR* gene has been mapped to the short arm (p) of chromosome 2 (19, 46), it was possible that deletion of region p25→p26 resulted in loss of the *DHFR* gene from chromosome 2a.

A representative karyotype from DXB11 (Fig. 1) shows 7 normal and 14 structurally altered hamster chromosomes. The banding pattern of 19 of the chromosomes from DXB11 seems to be identical to that illustrated by Milbrandt et al.

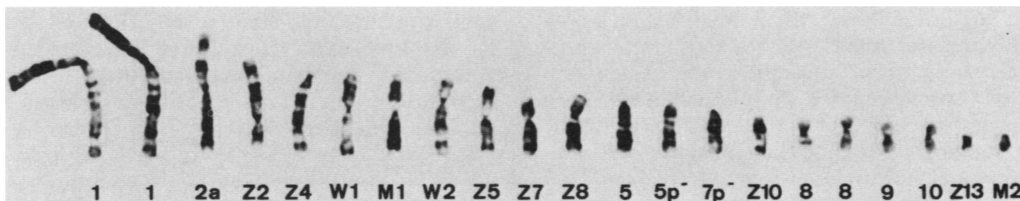

FIG. 1. Representative karyotype of the DXB11 cell line. The normal Chinese hamster chromosomes have been numbered according to the standard nomenclature proposed by Ray and Mohandas (38). Structurally abnormal hamster chromosomes have been designated according to the system used by Deaven and Petersen (16), Worton (44), and Worton et al. (47). The W1 and W2 chromosomes represent the t(Xp;Z3p) and $(Z3q)p^+$ chromosomes, respectively, which have been described by Worton (44). The chromosomes designated as 2a, M1, and M2 have not been described previously. Chromosome 2a contains an interstitial deletion (p25→p26) of the number 2 chromosome. The short arm of the M1 chromosome appears to be derived from the long arm of the hamster number 6 chromosome. The origin of the M2 chromosome is not clear.

(33). However, the classification of nine of these chromosomes differs between the karyotype represented here (Fig. 1) and that shown by Milbrandt et al. (33). We believe that the chromosome classified as an X by these workers is the Z2 chromosome and that the two Z2 derivative chromosomes are the t(Xp;Z3p) and (Z3q)p$^+$ chromosomes described previously by Worton (44). Furthermore, the two number 6 chromosomes identified by Milbrandt et al. (33) have been identified here as the Z8 and 5p$^-$ chromosomes, and the number 8, number 5, and Z5a chromosomes have been classified by us as the Z10, Z5, and Z7 chromosomes, respectively. It appears also that the number 2 chromosome shown by Milbrandt et al. (33) is analogous to the 2a chromosome represented here.

We have compared the DXB11 karyotype reported here (Fig. 1) to the described karyotypes of other CHO cell lines. Of the 14 marker chromosomes found in DXB11, 9 have been described in other CHO cell lines (16, 44, 47). Furthermore, two other DXB11 marker chromosomes, 5p$^-$ and 7p$^-$, lack the short arm of the normal number 5 and 7 chromosomes, respectively. Thus, the variation in the DXB11 karyotype is no more extensive than that found between other CHO sublines (44).

The CRL1475 cell strain had a 46,XY karyotype (data not shown) as reported by the American Type Culture Collection.

Chromosomal assignment of the human *DHFR* gene. Cells from DXB11 (DHFR$^-$/DHFR$^-$) were fused to either normal human fetal skin fibroblasts (46,XY) from strain CRL1475 (fusion 1) or to fibroblasts from a patient with a reciprocal translocation between the number 4 and 11 chromosomes [46,XY,t(4;11)(q25;p13)] (fusion 2) as described below. DHFR$^+$ colonies were obtained at an average frequency of 4.1×10^{-5}. No DHFR$^+$ colonies were obtained from 10^6 fused DXB11 cells. Nineteen hybrid clones (17 from fusion 1, 2 from fusion 2) were isolated and maintained in medium without purines for at least 2 months. This procedure selected for hybrid cells that retained the human chromosome carrying the *DHFR* gene while allowing the loss of other human chromosomes to occur.

Chromosomes were prepared from each hybrid clone as described in the text. The number of hamster and human chromosomes in each hybrid cell line was determined by the G-11 staining technique. This procedure has been shown to stain hamster chromosomes magenta and human chromosomes blue (8, 22). Each hybrid clone contained two to three genomic complements (2-3X) from DXB11 and various numbers of human chromosomes. The presence of a 2X complement of hamster chromosomes in human-hamster cell hybrids has been observed previously by Lai et al. (26). These workers have attributed this phenomenon to the increased stability of human chromosomes in a 2X CHO background.

Bobrow et al. (6) have shown that a red-staining area occurs on specific areas of some G-11 stained human chromosomes. These regions occur adjacent to the centromere on the long arm of chromosomes 1, 5, 9, and 20 and on the short arm of chromosomes 4, 7, 13, 14, 15, 17, 21, and 22. This differential staining of human chromosomes was preserved in the interspecific somatic cell hybrids analyzed here. Thus, we were able to identify many of the human chromosomes present in the hybrids by G-11 staining alone (Fig. 2A).

Each human chromosome was identified also after GTG staining (Fig. 2B). The results of the G-11 and GTG staining analyses for each hybrid clone (Fig. 3) show that chromosome 5 is the only human chromosome that complements the DHFR$^-$ phenotype of DXB11 cells.

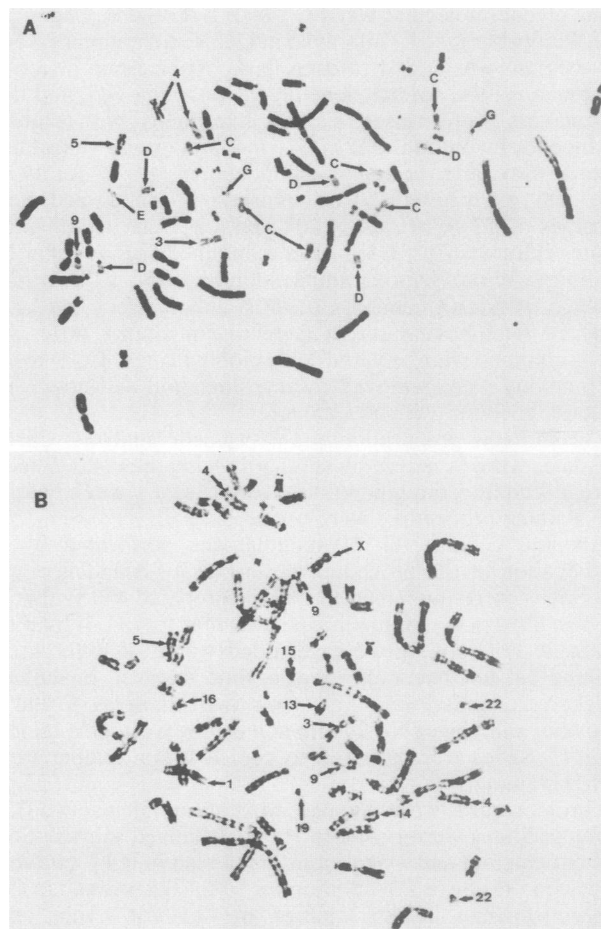

FIG. 2. Representative metaphase spreads from hybrid B6. (A) Chromosomes were G-11 stained as described in the text. Human chromosomes (→), which stain blue, appear lighter in black and white photographs than the hamster chromosomes, which stain magenta. Human chromosomes that could not be identified by G-11 staining alone were classified as either C, D, E, or G group chromosomes. (B) Chromosomes were banded with trypsin-Giemsa as described in the text. Human chromosomes are indicated by the arrows.

Hybrid clones A5, A10, and B3 contained different terminal deletions of chromosome 5. These deleted chromosomes are shown in Fig. 4 along with a normal number 5 chromosome from hybrid B6 for comparative purposes. As described earlier, the G-11 stained normal number 5 chromosome contains a red-staining area adjacent to the centromere on the long arm. This red-staining area was useful for determining the breakpoints of the structurally abnormal chromosomes in the hybrid cells. Hybrid A5 contained only the long arm of chromosome 5 as shown by the G-11- and GTG-stained chromosomes in Fig. 4. Hybrid A10 contained a translocation chromosome derived from part of the number 8 chromosome (pter→q11) and part of the long arm of the number 5 chromosome (q11→q31). A dicentric chromosome derived from a different part of the number 5 chromosome (pter→q22) was seen in hybrid B3. This derivative chromosome was classified as dicentric based on both its banding pattern and the presence of two red-staining areas on the representative G-11-stained chromosome (Fig. 4). The short-

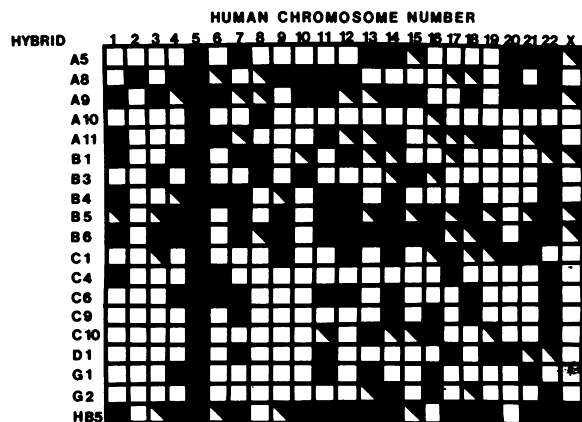

FIG. 3. Human chromosome distribution in CHO-human hybrid clones. Hybrids G1 and G2 were obtained from fusion 2, and the rest of the hybrids were obtained from fusion 1 as described in the text. G-11- or GTG-stained chromosomes from at least 20 metaphase spreads were analyzed for each hybrid clone. The average frequency per spread (X) for each human chromosome was recorded as follows: ■, $X \geqq 0.30$; ◪, $0 < X < 0.30$; □, $X = 0$.

est region of overlap between the deleted chromosomes in hybrids A5, A10, and B3 was q11→q22 (Fig. 5). These results suggest that the *DHFR* gene resides within this segment of chromosome 5.

Characterization of the DHFR produced by the hybrid cells. The results above show that the q11→q22 region of human chromosome 5 complements the DHFR$^-$ phenotype of DXB11 cells. Although this suggests that the q11→q22

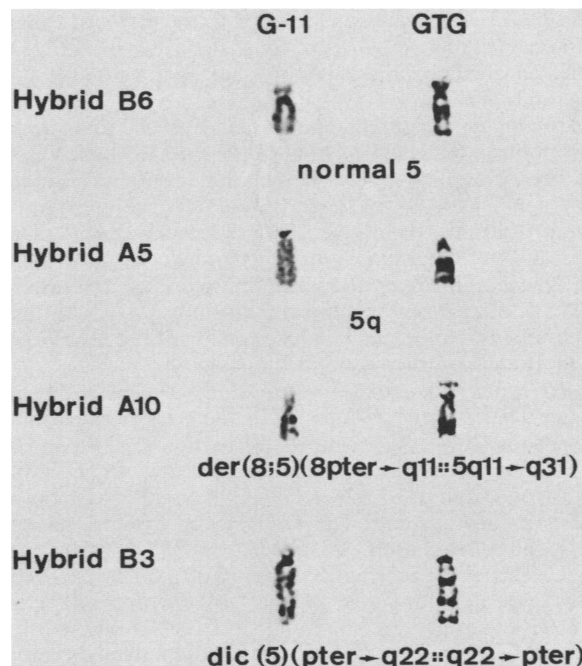

FIG. 4. Representative G-11-stained and GTG-banded derivatives of human chromosome 5. Chromosomes were stained with either G-11 or GTG as described in the text. Human chromosomes stain blue in G-11 and appear gray in black and white photographs. The red-staining area that occurs adjacent to the centromere on the long arm of a normal chromosome 5 appears darker than the rest of the chromosome in black and white photographs.

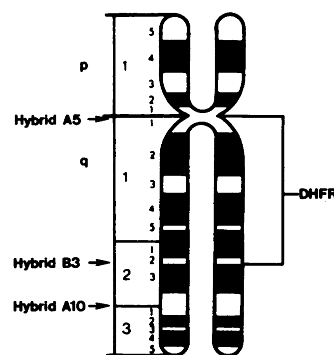

FIG. 5. Idiogram of human chromosome 5 showing regional assignment of the *DHFR* gene. Arrows indicate the locations of breakpoints that occurred in human chromosome 5 from hybrids A5, A10, and B3.

region contains the *DHFR* structural gene, it was possible also that a gene or genes in this region activated the expression of a hamster (DXB11) *DHFR* gene. To distinguish between these possibilities, we determined whether the hybrids produced a DHFR enzyme of human or hamster origin.

DHFR activities in crude cellular extracts were assayed as described above. The DHFR$^+$ cell line CHO-K1 was used as a source of hamster DHFR since DXB11 cells have been shown to lack DHFR activity (41). The data in Table 1 show that the CHO-K1 DHFR enzyme was activated by KCl, as has been reported for other mammalian DHFR enzymes (4, 18, 23). Maximal stimulation of DHFR activity in the CHO-K1 extracts occurred at about 400 mM KCl (Fig. 6). Jarabak and Bachur (23) have shown that the DHFR enzyme from other rodent cells responds similarly to KCl.

The DXB11 extract did not contain detectable DHFR activity at pH 7.5, either with or without the addition of KCl (Table 1). However, significant DHFR activity was seen at pH 7.0. This activity was due to DHFR, since it was sensitive to inhibition by 10 μM MTX (data not shown). However, this activity was inhibited by KCl (Table 1), in contrast to what was observed for the CHO-K1 DHFR and for other mammalian DHFR enzymes (4, 18, 23). Thus, DXB11 cells appear to produce an altered DHFR enzyme. This enzyme apparently does not function well enough in vivo to supply the cell with adequate tetrahydrofolate for growth.

The specific activity of the human (CRL1475) DHFR enzyme was about 30-fold lower than that of CHO-K1 (Table 1). The results in Table 1 and Fig. 6 show that the human DHFR enzyme was stimulated also by KCl; however, maxi-

TABLE 1. DHFR activities in hamster, human, and hybrid cells

Source	DHFR activity[a]			
	pH 7.5	pH 7.5, 150 mM KCl	pH 7.0	pH 7.0, 150 mM KCl
CHO-K1	4.03	5.89	3.60	5.34
DXB11	<0.01	<0.01	1.23	0.13
CRL1475	0.14	0.30	0.19	ND
Hybrid A8	0.20	0.49	0.57	0.43
Hybrid A10	0.21	0.52	1.55	0.42

[a] Nanomoles of dihydrofolate reduced per minute per milligram of protein under the conditions shown. ND, Not determined.

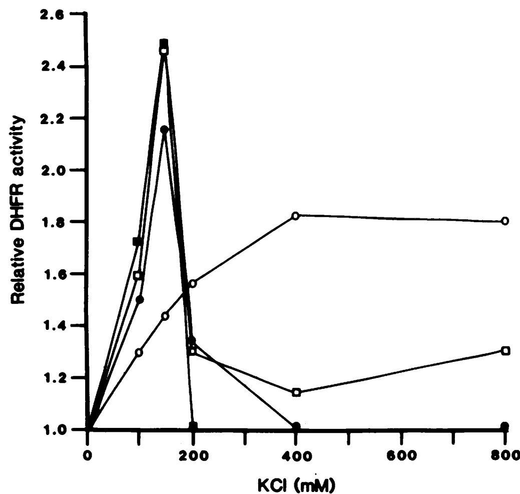

FIG. 6. Effect of KCl on DHFR activity in hamster, human, and hybrid cells. DHFR activity was assayed at pH 7.5 in the presence of the indicated concentrations of KCl as described in the text. Relative DHFR activity is represented as the percentages of control values (nanomoles of dihydrofolate reduced per minute per milligram of protein), which were 4.03 for CHO-K1 (○), 0.14 for CRL1475 (●), 0.20 for hybrid A8 (□), and 0.21 for hybrid A10 (■).

mal stimulation of DHFR activity occurred at 150 mM. A salt optimum of 150 mM has been observed for a purified DHFR enzyme from human KB cells (18). Therefore, the human (CRL1475) and hamster (CHO-K1) DHFR enzymes differ significantly in their reponse to KCl. This allowed us to distinguish between the production of human or hamster DHFR in the hybrid cells. Furthermore, we were able to determine whether the hybrids continued to express the DXB11 *DHFR* gene by analyzing the salt sensitivity of DHFR activity at pH 7.0.

Extracts from hybrids A8 and A10 contained significant DHFR activity at pH 7.0, which was not stimulated by KCl (Table 1). Thus, most of this activity seems to be representative of that of DXB11. In contrast to the DXB11 cells, however, the hybrid cells also contained DHFR activity at pH 7.5. The specific activity of this enzyme was similar to that of the human CRL1475 enzyme (Table 1) and showed a response to KCl similar to that of the human enzyme (Fig. 6). Thus, extracts from hybrids A8 and A10 seem to contain two distinct DHFR activities, one characteristic of that of DXB11 (DHFR activity at pH 7.0 which was not stimulated by KCl) and the other representative of the human CRL1475 enzyme (DHFR activity at pH 7.5 which was stimulated by 150 mM KCl). These results rule out the possibility that a gene or genes on chromosome 5 has activated the expression of a DXB11 *DHFR* gene.

DISCUSSION

Using a somatic cell hybridization approach, we have demonstrated that human chromosome 5 complements the $DHFR^-$ phenotype of DXB11 cells. Extracts from two of the interspecific hybrids characterized in this study were shown to contain two distinct DHFR activities. One activity was characteristic of the altered DHFR enzyme found in the DXB11 cells; the other activity showed properties similar to those of the human (CRL1475) DHFR enzyme. These results demonstrate that the human *DHFR* gene is located on chromosome 5.

Maurer et al. (30), using a different approach, have reported also that the human *DHFR* gene resides on chromosome 5. These authors used a human DHFR cDNA probe to analyze genomic DNA from a panel of human-rodent somatic cell hybrids. Human *DHFR* gene sequences were concordant only with the presence of human chromosome 5 in these hybrid cells. These results, together with those reported here, confirm the assignment of the human *DHFR* gene to chromosome 5.

This assignment is interesting when one considers the evolutionary relationship that has been observed between human chromosome 5 and the long arm of hamster chromosome 2. Dana and Wasmuth (14) have shown that the order of three genes (*LARS*, *CHR*, and *EMT*) has been conserved on human chromosome 5 and the long arm of hamster chromosome 2. As shown here, the human *DHFR* gene has been assigned also to chromosome 5; however, the hamster *DHFR* gene has been mapped previously to the short arm of chromosome 2 (19, 46). Thus, our results, taken together with those cited above, provide evidence for homology between both the short and long arms of hamster chromosome 2 and human chromosome 5.

Three of the somatic cell hybrids described in this study were shown to contain terminal deletions of human chromosome 5 (Fig. 4). An analysis of the breakpoints of these deletions has allowed us to make a regional assignment of the *DHFR* gene to the q11→q22 region. The human *EMT* and *CHR* genes have been assigned to region q23→q35 and band q35, respectively (14). This implies that the gene order on the long arm of chromosome 5 is centromere-*DHFR*-*EMT*-*CHR*. The inferred order of these genes on the hamster number 2 chromosome is *DHFR*-centromere-*EMT*-*CHR*. This suggests that a pericentric inversion involving the *DHFR* gene occurred during the evolution of the human number 5 and hamster number 2 chromosomes.

The *LARS* gene has been assigned to the pter→q11 region of human chromosome 5 (14). Thus, the order of *LARS* and *DHFR* on chromosome 5 is not clear at the present time. Dana and Wasmuth (14) have characterized a series of human-hamster tertiary somatic cell hybrids that contain different terminal deletions of chromosome 5. These hybrids have been classified as to whether they contain the human *LARS* gene. DNA from these hybrids can be analyzed by Southern blotting techniques with a human DHFR cDNA probe (35) to determine which hybrids carry the human *DHFR* gene. This may allow one to determine the order of *LARS* and *DHFR* on the number 5 chromosome. Additionally, this analysis may lead to a localization of the *DHFR* gene to a more defined region of chromosome 5.

Other work presented in this study suggests that the hamster *DHFR* gene is located in the p25→p26 region of chromosome 2. A cytogenetic analysis (Fig. 1) of the DXB11 ($DHFR^-/DHFR^-$) cell line has shown that an interstitial deletion of region p25→p26 occurred in an otherwise normal number 2 chromosome. The DXB11 cell line was isolated after gamma irradiation of UKB25 ($DHFR^+/DHFR^-$) cells (41). Urlaub et al. (42) have shown that gamma irradiation causes large deletions that span at least the entire length of the *DHFR* gene (~26 kb) in most DHFR mutants. It is conceivable, therefore, that gamma irradiation led directly to deletion of region p25→p26 and to subsequent loss of the $DHFR^+$ gene in UKB25 cells. This would imply that the *DHFR* gene is located in the p25→p26 region of chromosome 2. Two independent studies support this proposal. First, abnormally banding regions containing amplified *DHFR* genes have been found adjacent to band 2p25 in CHO cell lines moderately resistant to MTX (27). Second, Flintoff

et al. (19) have shown that breakpoints, all of which are at or next to band p25, occur during the amplification and translocation of *DHFR* genes to chromosome 5 in class III MTX-resistant CHO cells. Thus, all of these studies show an association between region 2p25→p26 and *DHFR* gene amplification or loss. If complete loss of a *DHFR* gene from chromosome 2a occurred in DXB11 cells, the other *DHFR* gene on chromosome Z2 should code for the altered DHFR protein seen in DXB11 cell extracts (Table 1).

Urlaub et al. (42) have isolated several DHFR mutants in which all *DHFR* gene sequences are deleted. It will be of interest to determine whether deletions involving region 2p25→p26 occur in other DHFR$^-$ cell lines. Additionally, interspecific cell hybrids that segregate DXB11 chromosomes can be used to determine whether hamster *DHFR* gene sequences are absent in hybrids that have segregated the Z2 chromosome, but retained chromosome 2a.

The well-characterized deletions of human chromosome 5 and hamster chromosome 2 described in this study should be useful for regionally mapping other genes assigned to these chromosomes. A more complete map of both hamster chromosome 2 and human chromosome 5 is needed before an understanding of the exact evolutionary relationship of these two chromosomes emerges.

LITERATURE CITED

1. **Alhadeff, B., M. Velivasakis, and M. Siniscalco.** 1977. Simultaneous identification of chromatid replication and of human chromosomes in metaphases of man-mouse somatic cell hybrids. Cytogenet. Cell Genet. **19**:236–239.
2. **Alt, F. W., R. E. Kellems, J. R. Bertino, and R. T. Schimke.** 1978. Selective multiplication of dihydrofolate reductase genes in methotrexate-resistant variants of cultured murine cells. J. Biol. Chem. **253**:1357–1370.
3. **Baker, R. M., D. M. Brunette, R. Mankovitz, L. H. Thompson, G. F. Whitmore, L. Siminovitch, and J. E. Till.** 1974. Ouabain-resistant mutants of mouse and hamster cells in culture. Cell **1**:9–21.
4. **Bertino, J. R.** 1962. Activation of tetrahydrofolate dehydrogenase by certain cations. Biochim. Biophys. Acta **58**:377–380.
5. **Bertino, J. R., S. Srimatkandada, D. Engel, W. D. Medina, D. I. Scheer, B. A. Moroson, and I. Dube.** 1982. Gene amplification in a methotrexate-resistant human leukemia line, K-562, p. 23–27. *In* R. T. Schimke (ed.), Gene amplification. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
6. **Bobrow, M., K. Madan, and P. L. Pearson.** 1972. Staining of some specific regions of human chromosomes, particularly the secondary constriction of no. 9. Nature (London) New Biol. **238**:122–124.
7. **Bradford, M. M.** 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein dye binding. Anal. Biochem. **72**:248–254.
8. **Burgerhout, W.** 1975. Identification of interspecific translocation chromosomes in human-Chinese hamster hybrid cells. Humangenetik **29**:229–231.
9. **Carothers, A. M., G. Urlaub, N. Ellis, and L. A. Chasin.** 1983. Structure of the dihydrofolate reductase gene in Chinese hamster ovary cells. Nucleic Acids Res. **11**:1997–2012.
10. **Chang, A. C. Y., J. H. Nunberg, R. J. Kaufman, H. A. Erlich, R. T. Schmike, and S. N. Cohen.** 1978. Phenotypic expression in *E. coli* of a DNA sequence coding for mouse dihydrofolate reductase. Nature (London) **275**:617–624.
11. **Chang, T., J. S. Limanek, and C. C. Y. Chang.** 1981. A simple and efficient procedure for the rapid homogenization of cultured animal cells grown in monolayer. Anal. Biochem. **116**:298–302.
12. **Chen, M., T. Shimada, A. D. Moulton, A. Cline, R. K. Humphries, J. Maizel, and A. W. Nienhuis.** 1984. The functional human dihydrofolate reductase gene. J. Biol. Chem. **259**:3933–3943.
13. **Chen, T. R.** 1977. *In situ* detection of mycoplasma contamination in cell cultures by fluorescent Hoechst 33258 stain. Exp. Cell. Res. **104**:255–262.
14. **Dana, S., and J. J. Wasmuth.** 1982. Selective linkage disruption in human-Chinese hamster cell hybrids: deletion mapping of the *leuS*, *hexB*, *emtB*, and *chr* genes on human chromosome 5. Mol. Cell. Biol. **2**:1220–1228.
15. **Davidson, R. L., K. A. O'Malley, and T. B. Wheeler.** 1976. Polyethylene glycol-induced mammalian cell hybridization: effect of polyethylene glycol molecular weight and concentration. Somat. Cell. Genet. **2**:271–280.
16. **Deaven, L. L., and D. F. Petersen.** 1973. The chromosomes of CHO, an aneuploid Chinese hamster cell line: G-band, C-band, and autoradiographic analyses. Chromosoma **41**:129–144.
17. **Dolnick, B. J., R. J. Berensen, J. R. Bertino, R. J. Kaufman, J. H. Nunberg, and R. T. Schimke.** 1979. Correlation of dihydrofolate reductase elevation with gene amplification in a homogeneously staining chromosomal region in L5178Y cells. J. Cell Biol. **83**:394–402.
18. **Domin, B. A., Y. Cheng, and M. T. Hakala.** 1982. Properties of dihydrofolate reductase from a methotrexate-resistant subline of human KB cells and comparison with enzyme from KB parent cells and mouse S180 AT/3000 cells. Mol. Pharmacol. **21**:231–238.
19. **Flintoff, W. F., E. Livingston, C. Duff, and R. G. Worton.** 1984. Moderate-level gene amplification in methotrexate-resistant Chinese hamster ovary cells is accompanied by chromosomal translocation at or near the site of the amplified *DHFR* gene. Mol. Cell. Biol. **4**:69–76.
20. **Flintoff, W. F., M. K. Weber, C. R. Nagainis, A. K. Essani, D. Robertson, and W. Salser.** 1982. Overproduction of dihydrofolate reductase and gene amplification in methotrexate-resistant Chinese hamster ovary cells. Mol. Cell. Biol. **2**:275–285.
21. **Frearson, P. M., S. Kit, and D. R. Dubbs.** 1966. Induction of dihydrofolate reductase activity by SV40 and polyoma virus. Cancer Res. **26**:1653–1660.
22. **Friend, K. K., S. Chen, and F. H. Ruddle.** 1976. Differential staining of interspecific chromosomes in somatic cell hybrids by alkaline Giemsa stain. Somat. Cell Genet. **2**:183–188.
23. **Jarabak, J., and N. R. Bachur.** 1971. A soluble dihydrofolate reductase from human placenta: purification and properties. Arch. Biochem. Biophys. **142**:417–425.
24. **Kaufman, B. T., and R. C. Gardiner.** 1966. Studies on dihydrofolate reductase. I. Purification and properties of dihydrofolic reductase from chicken liver. J. Biol. Chem. **241**:1319–1328.
25. **Kaufman, R. J., P. C. Brown, and R. T. Schimke.** 1979. Amplified dihydrofolate reductase genes in unstably methotrexate-resistant cells are associated with double minute chromosomes. Proc. Natl. Acad. Sci. U.S.A. **76**:5669–5673.
26. **Lai, E. C., F. Kao, M. L. Law, and S. L. C. Woo.** 1983. Assignment of the α_1-anti-trypsin gene and a sequence-related gene to human chromosome 14 by molecular hybridization. Am. J. Hum. Genet. **35**:385–392.
27. **Lewis, J. A., J. L. Biedler, and P. W. Melera.** 1982. Gene amplification accompanies low level increases in the activity of dihydrofolate reductase in antifolate-resistant Chinese hamster lung cells containing abnormally banding chromosomes. J. Cell. Biol. **94**:418–424.
28. **Lewis, J. A., D. T. Kurtz, and P. W. Melera.** 1981. Molecular cloning of Chinese hamster dihydrofolate reductase-specific cDNA and the identification of multiple dihydrofolate reductase mRNAs in antifolate-resistant Chinese hamster lung fibroblasts. Nucleic Acids Res. **9**:1311–1322.
29. **Masters, J., B. Keeley, H. Gay, and G. Attardi.** 1982. Variable content of double minute chromosomes is not correlated with degree of phenotype instability in methotrexate-resistant human cell lines. Mol. Cell. Biol. **2**:498–507.
30. **Maurer, B. J., P. E. Barker, J. N. Masters, F. H. Ruddle, and G. Attardi.** 1984. Human dihydrofolate reductase gene is located in chromosome 5 and is unlinked to the related pseudogenes. Proc. Natl. Acad. Sci. U.S.A. **81**:1484–1488.
31. **Melera, P. W., D. Wolgemuth, J. L. Biedler, and C. Hession.** 1980. Antifolate-resistant Chinese hamster cells: evidence from independently derived sublines for the overproduction of two

dihydrofolate reductases encoded by different mRNAs. J. Biol. Chem. **255**:319–322.

32. **Milbrandt, J. D., J. C. Azizhan, K. S. Greisen, and J. L. Hamlin.** 1983. Organization of a Chinese hamster ovary dihydrofolate reductase gene identified by phenotypic rescue. Mol. Cell. Biol. **3**:1266–1273.
33. **Milbrandt, J. D., J. C. Azizhan, and J. L. Hamlin.** 1983. Amplification of a cloned Chinese hamster dihydrofolate reductase gene after transfer into a dihydrofolate reductase-deficient cell line. Mol. Cell. Biol. **3**:1274–1282.
34. **Milbrandt, J. D., N. H. Heintz, W. C. White, S. M. Rothman, and J. L. Hamlin.** 1981. Methotrexate-resistant Chinese hamster ovary cells have amplified a 135 kilobase-pair region that includes the dihydrofolate reducatase gene. Proc. Natl. Acad. Sci. U.S.A. **78**:6043–6047.
35. **Morandi, C., J. H. Masters, M. Mottes, and G. Attardi.** 1982. Multiple forms of human DHFR mRNA: cloning and expression in *E. coli* of their DNA coding sequence. J. Mol. Biol. **156**:583–607.
36. **Nunberg, J. H., R. J. Kaufman, A. C. Y. Chang, S. N. Cohen, and R. T. Schimke.** 1980. Structure and genomic organization of the mouse dihydrofolate reductase gene. Cell **19**:355–364.
37. **Nunberg, J. H., R. J. Kaufman, R. T. Schimke, G. Urlaub, and L. A. Chasin.** 1978. Amplified dihydrofolate reductase genes are localized to a homogenously staining region of a single chromosome in a methotrexate-resistant Chinese hamster ovary cell line. Proc. Natl. Acad. Sci. U.S.A. **75**:5553–5556.
38. **Ray, M., and T. Mohandas.** 1976. Proposed banding nomenclature for the Chinese hamster chromosomes (*Cricetulus griseus*). Cytogenet. Cell Genet. **16**:83–91.
39. **Srimatkandada, S., W. D. Medina, A. R. Cashmore, W. Wtyhe, D. Engel, B. A. Moroson, C. T. Franco, S. K. Dube, and J. R. Bertino.** 1983. Amplification and organization of dihydrofolate reductase genes in a human leukemic cell line, K-562, resistant to methotrexate. Biochemistry **22**:5774–5781.
40. **Tyler-Smith, C., and T. Alderson.** 1981. Gene amplification in methotrexate-resistant mouse cells. I. DNA rearrangement accompanies dihydrofolate reductase gene amplification in a T-cell lymphoma. J. Mol. Biol. **153**:203–218.
41. **Urlaub, G., and L. A. Chasin.** 1980. Isolation of Chinese hamster cell mutants deficient in dihydrofolate reductase activity. Proc. Natl. Acad. Sci. U.S.A. **77**:4216–4220.
42. **Urlaub, G., E. Kas, A. M. Carothers, and L. A. Chasin.** 1983. Deletion of the diploid dihydrofolate reductase locus from cultured mammalian cells. Cell **33**:405–412.
43. **Wolman, S. R., M. L. Craven, S. P. Grill, B. A. Domin, and Y. Cheng.** 1983. Quantitative correlation of homogeneously stained regions on chromosome 10 with dihydrofolate reductase enzyme in human cells. Proc. Natl. Acad. Sci. U.S.A. **80**:807–809.
44. **Worton, R. G.** 1978. Karyotypic heterogeneity in CHO cell lines. Cytogenet. Cell Genet. **21**:105–110.
45. **Worton, R. G., and C. Duff.** 1979. Karyotyping. Methods Enzymol. **58**:322–344.
46. **Worton, R. G., C. Duff, and W. Flintoff.** 1981. Microcell-mediated co-transfer of genes specifying methotrexate resistance, emetine sensitivity, and chromate sensitivity with Chinese hamster chromosome 2. Mol. Cell. Biol. **1**:330–335.
47. **Worton, R. G., C. C. Ho, and C. Duff.** 1977. Chromosome stability in Chinese hamster ovary cells. Somatic Cell Genet. **3**:27–45.