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Genetics

Regional assignment of genes for human α-globin and phosphoglycollate phosphatase to the short arm of chromosome 16

(somatic hybrid/gene mapping)

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ABSTRACT **The human α-globin and phosphoglycollate phosphatase (EC 3.1.3.18) genes have been regionally localized to the short arm of human chromosome 16 (HC16). This was accomplished by fusing mouse fibroblasts (A9) to human fibroblasts that contain a reciprocal translocation between the long arms of chromosomes 16 and 11. The murine A9 cells are deficient in adenine phosphoribosyltransferase (APRT), an enzyme present on the long arm of HC16 (HC16q). Hybrid cells were grown in selection culture medium that required the cells to retain human APRT. Therefore, the hybrids exhibited stable retention of the entire HC16 or the rearranged chromosome containing HC16q. We isolated five independent primary and secondary hybrid cell lines which retained either HC16 or HC16q at a high frequency. The presence of human α-globin genes in the various clones was established directly by DNA extraction and hybridization to a cDNA probe for human α-globin genes. Autoradiographs showed that hybrid cells containing the long arm, but not the short arm, of HC16 showed only the background mouse bands. Hybrid cells that retained the entire HC16 demonstrated the band(s) containing the human α-globin genes. Hybrid cells that contained HC16 with its α-globin genes were then placed in culture medium that contained diaminopurine, which is lethal for cells containing APRT. These counter-selected hybrid cells had lost HC16 and also lost the human α-globin genes as determined by blot hybridization. The presence of α-globin gene sequences in the hybrid clones was concordant with HC16 only and not with any other human chromosome. These results confirm the assignment of α-globin genes to HC16 and localize the genes to the short arm. We also assign the locus for phosphoglycollate phosphatase to the short arm of HC16.**

The technique of somatic cell hybridization has allowed a large number of human genes to be assigned to specific chromosomes and chromosome regions (1). This is based on the phenomenon that rodent–human hybrid cells preferentially lose human chromosomes. Methods to identify a specific human gene product in the hybrid cells allow gene assignments to be made by correlating the expression of the specific human gene product with the presence of a particular human chromosome. However, this is limited to those human genes that are expressed in the rodent–human cell hybrids in culture. Recently, the techniques of restriction endonuclease digestion, nucleic acid hybridization, and cloning of specific human DNA gene sequences by using recombinant DNA methods have become available. These molecular techniques in concert with somatic cell genetics allow precise chromosomal mapping of human genes in the absence of synthesis of specific human mRNA or protein products in the somatic cell hybrids.

The assignment of the human α-globin genes to chromosome 16 has been made by liquid molecular hybridization of a complementary α-globin DNA (cDNA) from partially purified α-globin mRNA with DNA from various mouse–human hybrids (2).

We report here that the human α-globin genes are located on the short arm of human chromosome 16 (HC16) as determined by the techniques of restriction endonuclease digestion of DNA from mouse–human hybrids, nitrocellulose blot transfer, and nucleic acid hybridization using the human α-globin gene as a probe. We simultaneously demonstrate that the gene for human phosphoglycollate phosphatase (PGPase; EC 3.1.3.18), recently assigned to chromosome 16 (3), is syntenic with the α-globin genes on the short arm of 16.

MATERIALS AND METHODS

Cell Hybrids. Mouse line A9 (GM 0346, Institute for Medical Research, Camden, NJ) is deficient in adenosine phosphoribosyltransferase (APRT) enzyme (4). The human fibroblasts containing the 16/11 translocation (GM 3005) [46,XX,t(11;16)(q13;p11)] were obtained from the Institute for Medical Research.

A total of 4×10^6 A9 cells were mixed with an equal number of human fibroblasts and plated into a 100-mm culture dish. Twenty-four hours later the mixed monolayer was treated with 2000 hemagglutination units of inactivated Sendai virus (Connaught Laboratories, Toronto, Canada) as described (5). The following day the monolayer was trypsinized and distributed equally among 25 small (60 mm) culture dishes, each containing 3 ml of medium that selects for APRT (minimal essential medium supplemented with nonessential amino acids, 10% dialyzed fetal calf serum, 0.05 mM adenine, and 0.01 mM azaserine) (6). The selection medium also contained 0.05 mM ouabain to eliminate unfused human fibroblasts (7). Hybrid colonies were visible in 3 weeks and were isolated from the dishes by using cloning cylinders. Only one clone was isolated from a dish to ensure independent origin of the primary hybrid clones.

For counterselecting human chromosome 16, hybrid cells were first grown in normal growth medium (minimal essential medium supplemented with nonessential amino acids and 10% fetal calf serum) for 3 weeks and subsequently in normal growth medium containing 2,6-diaminopurine at 10 μg/ml.

Cytogenetic Analysis. Standard air-dried preparations were Q banded as described (8). Chromosome analysis was done on photographed metaphases, and at least 25 spreads were ana-

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Abbreviations: PGPase, phosphoglycollate phosphatase; APRT, adenosine phosphoribosyltransferase; NaCl/Cit, standard saline citrate (0.15 M NaCl/0.015 M sodium citrate, pH 7.0); kb, kilobase(s).

lyzed per hybrid clone. Cell hybrids were harvested for cytogenetic analysis at the same passage level as the one at which they were developed for isozyme analysis and extraction of DNA.

Extraction of DNA. Approximately 1×10^8 cells were pelleted and then suspended in 50 mM EDTA/0.5% $NaDodSO_4$ containing 100 μg of proteinase K (Beckman) per ml for 16 hr at 37°C. An equal volume of Tris-saturated phenol was added to the cell suspension and the aqueous phase was removed. The phenol extraction was repeated and the aqueous phase was extracted with an equal volume of chloroform/phenol, 1:1 (vol/vol). The aqueous phase was separated and an equal volume of chloroform/isoamyl alcohol, 24:1, was added. The aqueous phase was separated and two ether extractions were performed, and 2 vol of 100% ethanol was added to the aqueous phase. The DNA was immediately spooled on a wooden stick and resuspended in deionized water. An 18-μg sample of DNA was digested with *Eco*RI or *Hin*dIII (Bethesda Research Laboratories, Rockville, MD) at 4 units of restriction endonuclease per μg of DNA in buffers recommended by the manufacturer at 37°C for 5 hr. A side reaction containing 1 μg of genomic DNA and 0.4 μg of wild-type λ DNA was assayed for complete digestion by gel electrophoresis. The digestions were terminated by extraction with phenol and ether.

The digested DNA was electrophoresed in 1% agarose. DNA was transferred from the gels to nitrocellulose filters (Schleicher & Schuell BA 85, 0.45-μm pores) in 10-fold concentrated standard saline citrate (NaCl/Cit) as described (9). The blots were prehybridized and hybridized as described (10).

The cDNA probe was the plasmid JW101 (11). The DNA was labeled *in vitro* by nick-translation as described (12). The resulting probe (specific activity, approximately 4×10^8 cpm/μg of DNA) was boiled for 10 min and added to the nitrocellulose filter and hybridization solution in a Scotchpak bag at 42°C for 12 hr. After hybridization, the filters were washed twice in 20× NaCl/Cit at 26°C, twice in 2× NaCl/Cit at 55°C, twice in 0.2× NaCl/Cit at 55°C, and twice in 0.1× NaCl/Cit at 65°C. Each wash was for 15 min. The washed filters were exposed to x-ray film (Kodak XR-5) with an intensifying screen (Dupont Lightning Plus) at −80°C.

Isozyme Analysis. Electrophoresis for PGPase was by a modification (13) of the method described by Barker and Hopkinson (14): 12% Electrostarch; electrophoresis at 5 V/cm for 17 hr; and pH 7.0 Tris buffer in the stain.

RESULTS

Chromosomal and Enzyme Analysis of Mouse–Human Hybrid Cells. We fused mouse fibroblasts (A9) to human fibroblasts which contained a reciprocal translocation between chromosomes 16 and 11 {46,XX,t(11;16) (q13;p11)}. As a result of this chromosome rearrangement, the human cells had two marker chromosomes in addition to structurally normal chromosomes 16 and 11. One of these marker chromosomes [der(16)] included the entire long arm and centromere of chromosome 16 and most of the long arm of chromosome 11 (11qter→11q13::16p11→16qter). The second marker chromosome [der(11)] consisted of the short arm and centromere of chromosome 11 and most of the short arm of chromosome 16 (11pter→11q13::16p11→16pter). The murine A9 cells are deficient in APRT, a locus present on the long arm of human chromosome 16 (4, 15). Hybrid cells were grown in selection culture medium that required the cells to retain the human APRT. Therefore, the mouse–human hybrids exhibited stable retention of the entire HC16 or the rearranged chromosome der(16) containing the long arm of HC16 (HC16q). We isolated several independent primary and secondary hybrid cell lines that retained, at a high frequency, either HC16 or der(16) or both.

Karyotypes of the hybrid cells were analyzed with Q banding. Two independent primary clones containing HC16 (52-58 and 52-30) and a third one containing only the HC16q (52-56) were used in the studies reported here (Table 1; Fig. 1). A fourth independent primary hybrid clone (52-46) containing HC16 and der(16) was subcloned and two secondary clones, 56-46-6 and 52-46-5, were also analyzed; the former retained only the der(16) and the latter retained both HC16 and der(16).

The gene for PGPase has been localized to HC16 (3). We found that all hybrid clones that contained HC16 expressed human PGPase (Fig. 2; Table 1). The hybrids that contained only the long arm of HC16 did not express human PGPase. We therefore assign the gene locus for PGPase to the short arm of HC16.

Restriction Endonuclease Analysis of Human α-Globin Gene in Mouse–Human Hybrid Cells. The mouse–human hybrid cell lines and clones were expanded to 2×10^8 cells. The cells were collected for karyotype, PGPase analysis, and DNA extraction. The presence of human α-globin gene sequences in the cells was established directly by digestion of the DNA with *Eco*RI or *Hin*dIII, electrophoresis in 1% agarose, transfer to nitrocellulose filters, and hybridization to a radiolabeled probe containing human α-globin gene sequences. A 23-kilobase (kb) band was seen in the autoradiograph of the human leukocyte control DNA digested with *Eco*RI (Fig. 3*A*). This is the expected *Eco*RI pattern for human α-globin genes (16). When the same analysis was applied to the mouse A9 control DNA, several bands of hybridization were present which represented cross-reactivity of the human α-globin cDNA probe with mouse sequences. Autoradiographs of similarly treated *Eco*RI-digested DNA from two clones (52-26, 52-46-6) of mouse–human hybrid cells that contained the long arm but not the short arm of HC16 showed only the background mouse bands. Autoradiographs of *Eco*RI-digested DNA from three mouse–human hybrid clones (52-28, 52-30, and 52-46-5) that retained the entire HC16 showed the 23-kb band containing the human α-globin genes as well as the background mouse bands.

The DNA from the mouse–human hybrids was also digested with *Hin*dIII. Human leukocyte DNA digested with *Hin*dIII and hybridized to a human α-globin cDNA probe produced the expected three restriction fragments, 19, 4.5, and 3.7 kb (16) (Fig. 3*B*). When the same analysis was applied to the mouse A9 control DNA, several bands of hybridization were present which represent crossreactivity of the human α-globin cDNA probe with mouse DNA sequences. Autoradiographs of simi-

Table 1. Biochemical and cytogenetic analyses of hybrid clones

Mouse–human hybrid clone	Frequency of human chromosomes in cells analyzed			Human markers	
	16	der(16)	der(11)	PGPase	α-Globin gene
52-26	−	26/28	−	−	−
52-28	27/30	−	−	+	+
52-30	29/30	−	−	+	+
52-46-6	−	27/28	−	−	−
52-46-5	22/26	2/26	−	+	+
After diaminopurine selection:					
52-28R	0/26	−	−	−	−
52-30R	0/24	−	−	−	−

der(16), rearranged chromosome containing 16p11→16qter (long arm); der(11), rearranged chromosome containing 16p11→16qter (short arm).

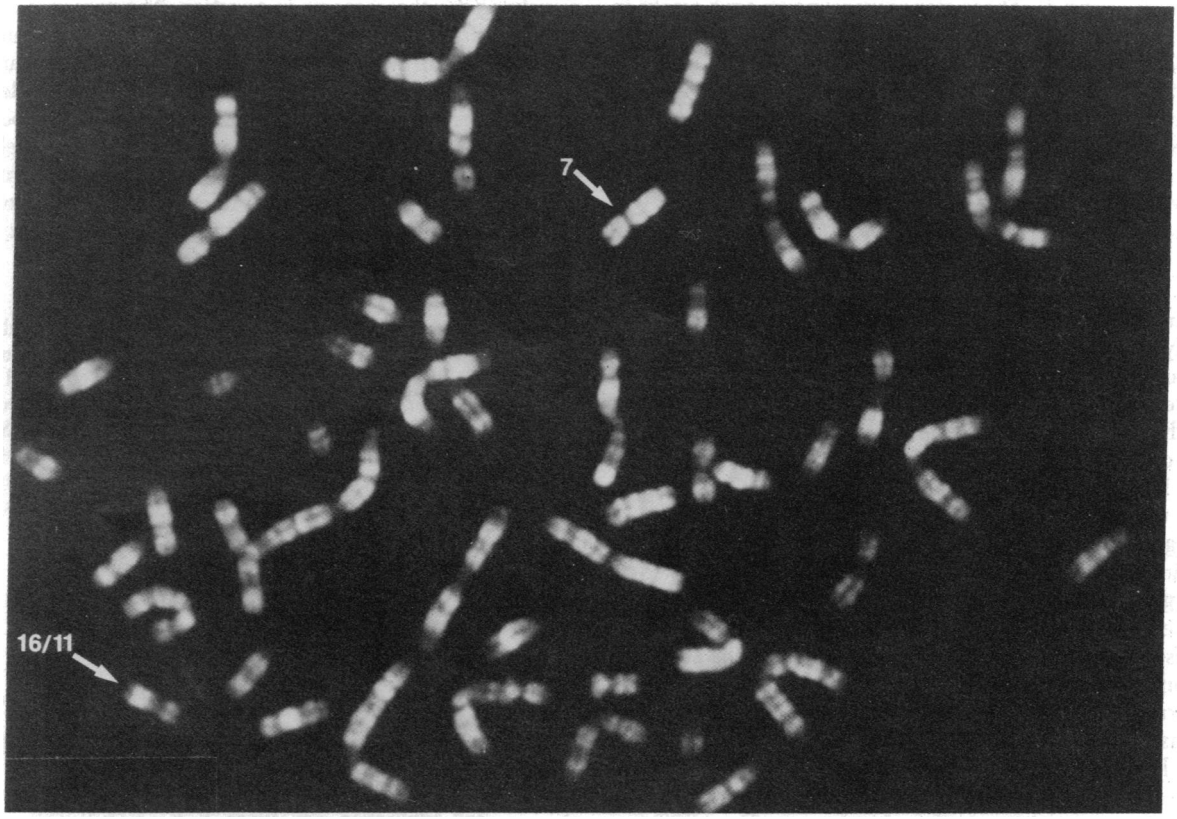

FIG. 1. Q-Banded metaphase spread from a human hybrid clone retaining the translocated chromosome (16/11) which consists of the long arms of human chromosomes 16 and 11 (arrow). The only other human chromosome in this spread is chromosome 7 (arrow).

larly treated *Hin*dIII-digested DNA from two clones (52-56 and 52-46-6) of mouse–human hybrid cells that contained the long arm but not the short arm of HC16 showed only the background mouse bands. Autoradiographs of *Hin*dIII-digested DNA from the three mouse–human clones (52-28, 52-30, 52-46-5) that retained the entire HC16 showed the human α-globin chromosomal pattern of 19-, 4.5-, and 3.7-kb bands as well as the background mouse bands.

Loss of the α-Globin in Hybrids that Lost Human Chromosome 16 After Counterselection. Mouse–human hybrid cells grown in medium that contained the purine analog diaminopurine died if the cells had APRT; hybrid cells that lost HC16 with its gene for APRT were able to grow in the presence of diaminopurine. Mouse–human hybrid clones 52-58 and 52-30 contained HC16 in 90% and 97%, respectively, of their cells. The DNA of these cell clones contained the human α-globin

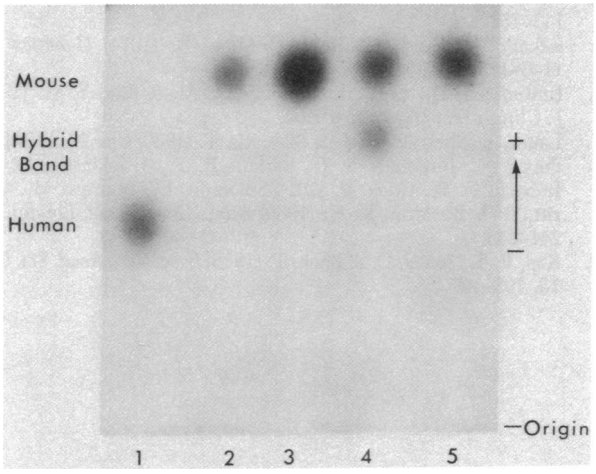

FIG. 2. Results of PGPase electrophoretic analysis. Lanes: 1 and 5, human and mouse patterns, respectively; 2 and 3, hybrids negative for human enzyme expression (hybrid 52-26 and hybrid 52-46-6, respectively); 4, positive for human enzyme expression (hybrid 52-30).

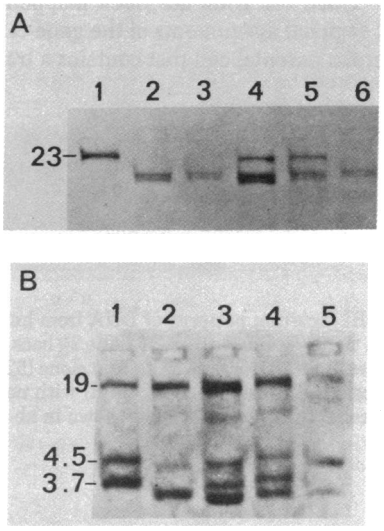

FIG. 3. (*A*) *Eco*RI digestion patterns of DNA from human leukocytes (lane 1), hybrid clones (52-26, 52-46-6) with der(16) chromosome (lanes 2 and 3), hybrid clones (52-28, 52-30) with HC16 (lanes 4 and 5), and A9 mouse cells (lane 6). (*B*) *Hin*dIII digestion patterns of DNA from human leukocytes (lane 1), hybrid clone (52-26) with der(16) chromosome (lane 2), hybrid clones (52-28, 52-30) with HC16 (lanes 3 and 4), and A9 mouse cells (lane 5). Size scale is shown in kb. Exposure time, 3 days.

genes (Figs. 3 and 4). These clones were grown for 8 weeks in medium containing diaminopurine, and the sublines 52-28R and 52-30R were obtained. The sublines lost the HC16 as shown by karyotype and PGPase isozyme analyses (Table 1), and the DNA from these sublines also lost the 23-kb human α-globin gene sequence after *Eco*RI digestion (Fig. 4). The presence of human α-globin gene sequences in the seven hybrid clones analyzed was concordant only with the short arm of HC16.

DISCUSSION

Prior studies have shown that the α-globin genes are on HC16 by analyzing cDNA·DNA reassociation kinetics between partially purified α-globin cDNA and DNA from mouse–human hybrid clones (2). Our studies confirm this assignment and localize the human α-globin genes to the short arm (p11→pter) region of HC16. Analysis of nucleotide sequences of cloned human DNA has previously shown linkage of two-globin genes and an α-globin-like pseudogene (ψα1) to the two normal α-globin genes (17). Therefore, it is likely that the whole ζ-ψα1, α-globin gene complex is on the short arm of HC16.

The gene locus for PGPase has been assigned to human chromosome 16 by several laboratories (3). Studies by Povey *et al.* (18) raise the possibility that PGPase may be on the short arm of chromosome 16. Our results clearly localize PGPase to the short arm of HC16 and thus the observations of Povey *et al.* (18) are consistent with this assignment.

The localization of the human α-globin genes was possible by using a human cell line that had a translocation of the long arm of chromosome 16 to another chromosome. The APRT gene is on the long arm of HC16 and is a selectable marker when hybridized with APRT⁻ mouse cells and grown in medium that selects for cells retaining APRT. Using this strategy we were able to isolate hybrids that retained HC16 or HC16q.

The use of techniques of nucleic acid hybridization to analyze somatic cell hybrids is not affected by the phenotype of the hybrid cells. Any purified human gene can be used as a probe to help assign the gene to a specific human chromosome by analysis of mouse–human hybrids using nucleic acid hybridization methods. Once the gene has been mapped to a specific chromosome, regional assignments of the gene can be made by selecting a human parental cell that contains a translocation involving the chromosome in question. The presence of a selectable marker in the rearranged chromosome, as in our studies, facilitates the mapping of the gene. A library of human cell cultures is available (Human Genetic Mutant Cell Repository) which contains various cell lines with various translocations. It is also possible to induce new translocations and deletions *in vitro* (19). The ability to assign genes to precise chromosomal locations provides knowledge relevant to the study of sequentially activated genes, of linkage and position effect of genes, and of diagnosis and understanding of diseases.

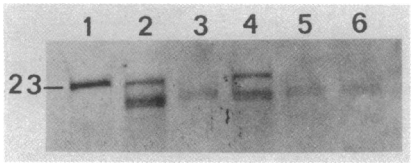

FIG. 4. *Eco*RI digestion patterns of DNA from human leukocytes (lane 1), hybrid clone (52-28) with HC16 (lane 2), back-selected hybrid clone (52-28R) with no HC16 (lane 3), hybrid clone (52-30) with HC16 (lane 4), back-selected hybrid clone (52-30 R) with no HC16 (lane 5), and A9 mouse cells (lane 6). Size scale is shown in kb. Exposure time, 2 days.

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