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DNA restriction endonuclease analysis for localization of human β- and δ-globin genes on chromosome 11

(chromosome mapping/somatic cell hybrids/X/autosome translocation)

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ABSTRACT **DNA from a clone of a mouse–human hybrid that retained a human chromosome consisting of the major part of chromosome 11 and region q25-26–qter of the X chromosome was digested with various restriction endonucleases, subjected to electrophoresis in agarose gels, and transferred to nitrocellulose filters. The restriction digest pattern of the clone, when hybridized with a ^{32}P-labeled plasmid fragment containing human β-globin gene sequences, was a composite of the normal human and mouse (A9) patterns. When back-selected in 6-thioguanine to eliminate the 11 translocation chromosome, the hybrid cells showed only the A9 restriction pattern. These results substantiate the localization of β- and δ-globin genes to human chromosome 11 and exclude the region 11q23–qter as the site.**

During the past few years, somatic cell hybrids have been used to assign many human genes to chromosomes on the basis of detection of structural or functional differences in the proteins of the parent cells (1). A few genes whose protein products cannot be differentiated but for which mRNA is available have been detected by nucleic acid hybridization (2, 3). Using solution hybridization, Deisseroth *et al.* (4) assigned the β- and δ-globin genes to chromosome 11. Others, on the basis of *in situ* hybridization, had previously assigned these genes to chromosome 2, 4, or 5 (5–8). Because of this uncertainty, we sought to confirm the assignment by an alternate method. We report here the use of restriction endonuclease digestions of DNA from hybrid and parent cells as a method for mapping genes when a specific DNA probe is available. This confirmation was facilitated by a hybrid cell line with an X/11 translocation which permitted back-selection and elimination of the pertinent chromosome.

METHODS

Cells. The clone of hybrid cells (clone 13) used for these experiments was derived from the fusion of cells from a mouse line (A9) deficient in hypoxanthine phosphoribosyltransferase (HPRT; IMP:pyrophosphate phosphoribosyltransferase, EC 2.4.2.8) and human skin fibroblasts carrying a reciprocal translocation between chromosomes X and 11, obtained from a female with gonadal dysgenesis [46,X,t (X;11)(q25-26;q23)] (9). The apparently balanced translocation involves break points q25-26 on the X chromosome and q23 on chromosome 11, resulting in two translocation chromosomes. In effect, chromosomes 11 and X had exchanged the terminal segments of their long arms. The single intact human chromosome in the hybrid cells was the derivative 11 translocation chromosome (der-11) consisting of the major part of chromosome 11 attached to the terminal segment of the X chromosome (q25-26–qter) at the long arm telomere (Fig. 1). This segment of the X contains the human genes for glucose-6-phosphate dehydrogenase (G6PD; EC 1.1.1.49) and HPRT but not phosphoglycerate kinase (PGK; EC 2.7.2.3). The HPRT locus on this chromosome was responsible for the retention of the der-11 chromosome in the hybrid cells selected and maintained in ouabain enriched minimal essential medium with hypoxanthine, amethopterin, thymidine, glycine, and fetal calf serum (10). Hybrid clone 13 was subjected to back-selection in medium containing 6-thioguanine (60° μM) to eliminate the der-11 chromosome. The resistant subclones, which had lost HPRT, were propagated in the selective medium.

Enzyme Analysis. The hybrids were analyzed for the presence of G6PD and lactate dehydrogenase (LDH; EC 1.1.1.27) as described (10).

DNA Methods. Nuclear DNA was prepared (11) from blood from a normal human male and from A9 cells, hybrid cells with the der-11 chromosome, and thioguanine-resistant hybrid cells. DNA was digested with several restriction endonucleases according to the recommended conditions of the commercial supplier. Thereafter, 10-μg aliquots were subjected to electrophoresis (10 mA, 12 hr) in 0.8% agarose gels. Gels were denatured and transferred to nitrocellulose membranes (Schleicher & Schuell, type B-85) according to the method of Southern (12) as modified by Jeffreys and Flavell (13). The membranes were then incubated overnight at 37°C in polyethylene bags with a prehybridization mix containing 50% deionized formamide (buffered to pH 6.8 with 20 mM sodium phosphate), 0.45 M NaCl, 0.045 M sodium citrate, 10 mM Hepes, 0.1% sodium pyrophosphate, 0.2%, Ficoll, 0.2% polyvinylpyrrolidone (M_r, 300,000), 0.2% bovine serum albumin, sonicated salmon sperm DNA (100 μg/ml), yeast RNA (200 μg/ml), 1 mM Na_2EDTA, and 0.1% sodium dodecyl sulfate. Hybridizations were carried out for 3 days at 37°C in the same solution to which polyadenylic acid (10 μg/ml) and ^{32}P β probe (2×10^6 cpm/ml) had been added. Filters were washed according to the procedure of Kan and Dozy (14) and autoradiographed for 1–3 days.

The β probe was prepared by double digestion (*Hin*dIII/*Mbo*II) of the recombinant plasmid JW102 (generously supplied by B. Forget) into which human β-globin sequences had been inserted (15). The resultant 1200-base-pair fragment, 50% β-globin gene, was recovered from agarose gels (16) and labeled to a specific activity of approximately 10^8 cpm/μg with [α-^{32}P]dATP and [α-^{32}P]dCTP by the "nick translation" function of *Escherichia coli* DNA polymerase I (17).

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Abbreviations: HPRT, hypoxanthine phosphoribosyltransferase; G6PD, glucose-6-phosphate dehydrogenase; PGK, phosphoglycerate kinase; LDH, lactate dehydrogenase.

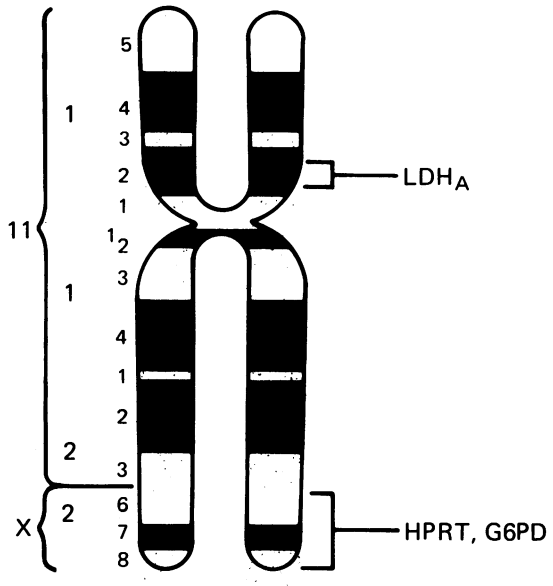

FIG. 1. Diagram of the derivative 11 translocation der (11), t(X;11), showing breakpoint and location of enzyme markers.

RESULTS

A representative karyotype of the resistant hybrid clone used in these experiments appears in Fig. 2. The clone has a modal number of 58 chromosomes, of which 57 are mouse. One of these is a translocation chromosome with a fragment of human material, identified by Giemsa 11 staining. The single intact human chromosome present is the der-11 chromosome. This

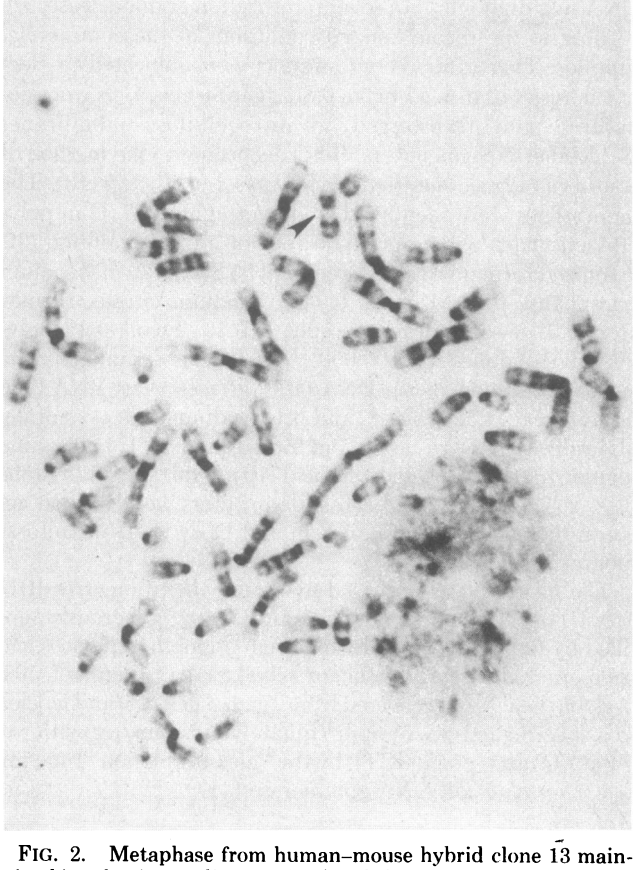

FIG. 2. Metaphase from human–mouse hybrid clone 13 maintained in selective medium, stained with Giemsa. Note the presence of the der-11 chromosome (arrowhead).

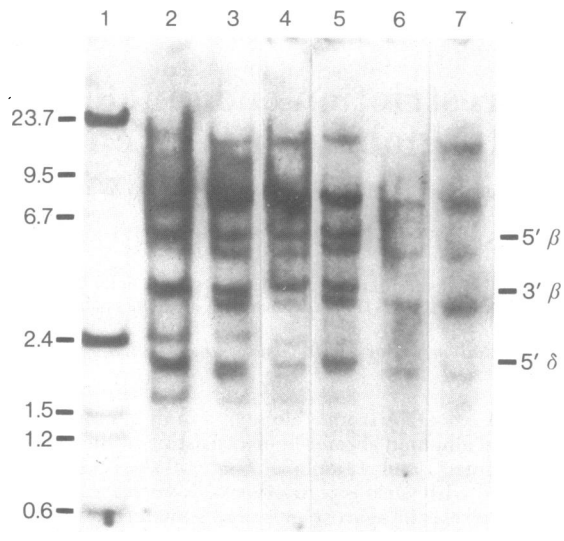

FIG. 3. *Eco*RI restriction endonuclease patterns of DNA from human leukocytes (lane 2), hybrid clone with der-11 chromosome (lanes 3, 4, and 5), 6-thioguanine-resistant subclone of hybrid clone 13 lacking der-11 (lane 6), and A9 mouse cells (lane 7). Markers (lane 1) are a *Hin*dIII digest of λ phage DNA and an *Eco*RI/*Ava* I/*Hin*dIII triple digest of the plasmid PβG1 (13). Size scale is shown in kilobases. Note that the selected hybrid clones have the 5′β (5.2 kilobases), 3′β (3.2 kilobases), and 5′δ (2.25 kilobases) human fragments seen in lane 2 whereas these are absent in the back-selected cells. The mouse fragments correspond in size to 16.4, 8.1, 4.6, 2.9 and 2.0 kilobases.

hybrid clone expressed human G6PD and HPRT, specified by genes known to reside on the distal long arm of the human X (18, 19) and did not express human PGK. Human LDH$_A$, assigned to the short arm of chromosome 11 (11 p123–p128) (20), was also present and served as a marker for this chromosome. After back-selection the hybrid cells no longer expressed human G6PD or LDH$_A$, indicating the loss of the der-11 chromosome. Karyotypes of the 6-thioguanine-selected cells confirmed the loss of der-11 and the continued presence of the mouse chromosome containing a human fragment.

Of the restriction endonucleases surveyed, *Eco*RI provided the DNA fragment banding pattern best able to discriminate between the human and mouse β-like genes (Fig. 3). The hybrid cell line showed a composite of both human and mouse bands. Equivalent results were obtained with *Pst* I, but similarities in the fragment sizes of human and A9 DNAs made interpretation difficult. In the 6-thioguanine-resistant hybrids the three major human *Eco*RI fragments were absent. One mouse band also was missing from the back-selected cells, perhaps as a result of incomplete transfer of larger DNA fragments from the gel or loss of a relevant mouse chromosome.

DISCUSSION

Recent detailed maps of the restriction enzyme sites in the β–δ gene cluster (21, 22) have allowed us to identify the three major human bands in the hybrid digest as those corresponding in size to the 5′ and 3′ portions of the β gene and the 5′ portion of the δ gene along with their flanking regions. The absence of these bands in DNA from back-selected cells, lacking the der-11 chromosome and markers for both the X chromosome and chromosome 11, strongly suggests that these globin genes are associated with the der-11 chromosome.

Thus, our results confirm those of Deisseroth *et al.* (4) who used solution hybridization with a partially purified β-globin cDNA and synteny analysis of hybrid clones for the β gene assignment. Like the earlier assignment of the α-globin loci to chromosome 16 (23), but unlike earlier analyses of the β genes

(4), our localization of the β and δ genes to chromosome 11 is strengthened by the presence of a selectable marker on the der-11 chromosome. In addition, because the der-11 lacks the terminal segment of the long arm of chromosome 11, we can conclude that the β and δ genes are not present in the region q23–qter. Because of close sequence homology, the β probe hybridizes with the δ gene under the conditions used but inconsistently so with the less-similar γ genes. However, the known close linkage of the β, δ, and γ genes (22) makes it likely that the γ genes are also present on the der-11 chromosome.

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