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Precise localization of human β-globin gene complex on chromosome 11*

(DNA hybridization/hemoglobin β chain/hemoglobin δ chain/regional gene mapping)

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ABSTRACT **Cloned DNA probes were used in combination with a panel of five hybrid cell clones containing a series of different terminal deletions in human chromosome 11 to map precisely the human hemoglobin β and δ chain structural genes contained on this chromosome. The region of deletion in each clone of the panel has been defined by biochemical, immunologic, and cytogenetic markers. DNA from clones containing successively larger terminal deletions was tested with appropriate DNA probes to determine the point on the chromosome at which DNA for these two closely linked hemoglobin genes is deleted. These genes, and by inference the closely linked $^{G}\gamma$ and $^{A}\gamma$ globin genes as well, have been assigned to the intraband region 11p1205 → 11p1208 on the short arm of chromosome 11, an interval containing approximately 4500 kilobases of DNA. The approach appears to have potential for even greater resolution and reasonably wide applicability for gene mapping.**

The ability to map human genes at precise chromosomal locations is likely to have important implications at both the practical and the theoretical level. The accumulation of precise mapping information may prove relevant to investigations of linkage and position effects, to study of developmental processes in which particular genes are sequentially activated, and to diagnosis and understanding of diseases with abnormal patterns of gene activation.

Significant progress has been achieved in the assignment of human genes to specific chromosomes (1). Somatic cell hybrids have played an important role in such identifications (2). Although occasional regional assignment of such genes to sections of an arm have been reported, systematic mapping of the human genes with a precision comparable to that available in *Drosophilia* or the mouse has not yet been possible.

The development of techniques with a high degree of resolution of restriction endonuclease digestion, nucleic acid hybridization, and sequence determination as well as the ability to purify and amplify specific human DNA sequences by using recombinant DNA methods opens the prospect of mapping the DNA of a human chromosome with a much greater precision than has previously been possible (3–5). In the present report, these molecular approaches have been combined with somatic cell genetics (6, 7) to achieve precise chromosomal mapping of human genes. So far, most mammalian genes that have been localized in specific chromosomes are those present in cells capable of synthesizing the corresponding mRNA or protein. Recently, human genes for α and β chains of the hemoglobin molecule have been identified by molecular hybridization in solution and have been assigned to human chromosome 16 (8) and 11 (9), respectively, by using complementary DNA probes for human α-globin and β-globin gene sequences in human–mouse cell hybrids containing various combinations of human chromosomes. The recent development of molecular hybridization of nucleic acids transferred from agarose gels to nitrocellulose filters offers another powerful method for detecting specific gene sequences (10). This method is highly sensitive and requires much less DNA than does molecular hybridization of nucleic acid in solution.

In order to localize the β-globin gene complex precisely within the chromosome, we have applied this technique to a panel of deletion clones derived from a hybrid containing chromosome 11 as its only human component. Previously we described (11) this highly stable hybrid clone, designated A_L-J1 or J1, which contains the entire genome of the Chinese hamster ovary cell (CHO-K1) plus the single human chromosome 11. In further studies, five subclones of the A_L-J1 hybrid were prepared by treatment with chromosome-breaking agents and are designated as J1-7, -9, -10, -11, and -23 (12, 13). The treatment was followed by selection in antisera specific to particular human cell surface antigens plus complement. The use of these specific antisera is possible because of the large immunologic differences in the cell surface antigenic activity between human and Chinese hamster cells (14, 15). These antisera made possible selection of clones that had lost various combinations of the antigen markers SA11-1, -2, and -3. Biochemical analysis for lactate dehydrogenase A (EC 1.1.1.27) (LDH_A) and acid phosphatase 2 (EC 3.1.3.2) (ACP_2) and cytogenetic analysis with banding were also performed. Five clones were selected, each of which displayed a different terminal deletion of chromosome 11 (Fig. 1*A*) and a corresponding loss of specific markers. Thus, this panel of clones divides chromosome 11 into six regions, each of which is characterized by cytogenetic and gene markers (Fig. 1*B*). Genes known to lie on chromosome 11 can be readily assigned to one of these regions by appropriate assays.

Direct assay by DNA·DNA hybridization was required because the globin genes are not expressed in these hybrid cells. In the current study we used cloned DNA fragments of the β- and δ-globin genes isolated by recombinant DNA techniques from the genomic DNA as probes in the Southern hybridization procedure (10). This assay procedure provided an unequivocal test for the presence or absence of the DNA sequences of the β- and δ-globin genes with a sample of DNA of 20 μg or less.

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Abbreviations: kb, kilobase pair(s); $NaDodSO_4$, sodium dodecyl sulfate; NaCl/Cit, standard saline citrate (0.15 M NaCl/0.015 M Na citrate, pH 7); LDH_A, lactate dehydrogenase-A; ACP_2, acid phosphatase 2.

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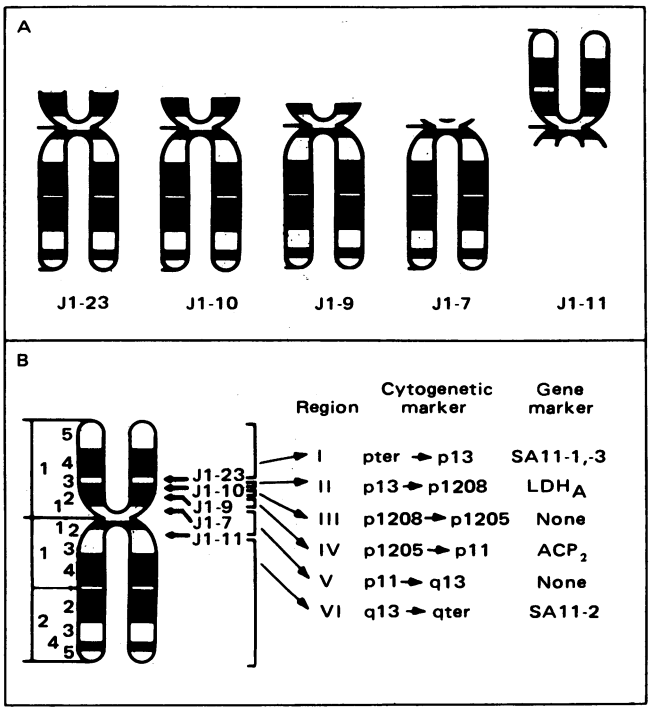

FIG. 1. (*A*) Diagram showing the various terminal deletions of human chromosome 11 in the five clones. (*B*) Scheme of human chromosome 11; arrows indicate the breakpoints at which terminal deletions occurred in the five clones. These five breakpoints divide chromosome 11 into six regions, each of which is characterized by cytogenetic and gene markers as indicated.

The parental cells and the panel of hybrid clones containing the various deletions of chromosome 11 were then tested in this manner for the presence or absence of the globin genes. The experiments described here permit mapping of the β and δ hemoglobin genes (Hb β and δ) on a specific segment of the short arm of human chromosome 11 which contains 4500 kilobase pairs (kb) of DNA.

MATERIALS AND METHODS

Cells. The HeLa cell and human fibroblasts were used as sources of human DNA. The Chinese hamster ovary cell (CHO-K1), hybrid clone J1 (11), and a panel of regional hybrid clones J1-7, J1-9, J1-10, J1-11, and J1-23 (13) were used as clones containing varying amounts of human chromosome 11. These cells were routinely cultivated in F-12 medium supplemented with 10% fetal calf serum. The phenotypes and the karyotypes of each of these panel members have been characterized by immunological, isozymic, and cytogenetic methods (12, 13).

Preparation of DNA. Cells were grown in α medium (16) plus 15% fetal calf serum (GIBCO) in Corning T-150 flasks to an approximate cell density of 2×10^8 cells per flask. The cells were washed several times with phosphate-buffered saline and harvested by trypsinization. The cells were collected by centrifugation and washed again with phosphate-buffered saline. The cell pellet was gently resuspended in 10 ml of buffer A (100 mM NaCl/10 mM Tris pH 8.0/1 mM EDTA). An equal volume of buffer A containing 0.5% sodium dodecyl sulfate (NaDodSO$_4$) was added slowly, and the solution was mixed gently. Proteinase K (EM Laboratories, Elmsford, NY) was then added to a final concentration of 200 μg/ml. This solution was incubated for 19 hr at 37°C and then extracted once with an equal volume of buffer-saturated phenol and three times with chloroform/isoamyl alcohol, 24:1 (vol/vol). The aqueous phase was removed with a wide-mouth pasteur pipette after the final extraction, and 2.5 vol of isopropyl alcohol was added and gently mixed. The DNA precipitate was removed with a curved tip without spooling. The excess isopropyl alcohol was drained and the DNA was transferred to a tube containing 2 ml of 10 mM Tris, pH 8.0/100 mM NaCl, incubated for 12 hr at 25°C without shaking, and stored at 4°C.

Digestion of DNA with Restriction Enzyme. Aliquots (20 μg) of DNA were digested with restriction enzymes in 0.1 ml final volume as follows.

(*i*) *Digestion with Xba I.* To each DNA aliquot was added 15 units of *Xba* I (New England Biolabs) in the presence of 0.15 M NaCl/6 mM Tris, pH 7.9/6 mM $MgCl_2$/6 mM 2-mercaptoethanol.

(*ii*) *Digestion with* EcoRI. To each DNA aliquot was added 60 units of *Eco*RI (Boehringer Mannheim) in the presence of 100 mM Tris, pH 7.5/50 mM NaCl/10 mM $MgCl_2$/2 mM 2-mercaptoethanol. Digestion with either enzyme was carried out for 2 hr at 37°C. The restriction enzyme was inactivated by incubation of the sample for 4 min at 65°C.

Gel Electrophoresis and Hybridization of DNA. The sample was made 8% in Ficoll (M_r, 400,000) (Sigma) and loaded on a 1% agarose gel in a Blaircraft gel apparatus. Electrophoresis was at 40 V for 15 hr. The gel was stained with ethidium bromide (0.5 μg/ml) for 30 min, and the DNA was visualized with ultraviolet light. The gel was then incubated for 45 min in 1 M NaOH with gentle shaking and then for two successive 20-min periods in 1 M Tris, pH 7.6/1.5 M NaCl. The gel was then transferred to nitrocellulose as described by Southern (10) except that 20-fold concentrated standard saline citrate (NaCl/Cit) was used instead of 6-fold concentrated. After transfer, any agarose attached to the filter was removed by washing with 3-fold concentrated NaCl/Cit. Filters were then baked for 2 hr under reduced pressure at 80°C. The filter was prehybridized in a sealed plastic bag (Seal 'n Save, Sears) at 67°C for 3 hr in a solution containing 10 ml of 6-fold concentrated NaCl/Cit, 10-fold concentrated Denhardt's solution (0.2% bovine serum albumin/0.2% polyvinylpyrollidone/0.2% Ficoll), 100 μg of denatured salmon sperm DNA per ml, and 0.1% NaDodSO$_4$. The filter was then rinsed with 3-fold concentrated NaCl/Cit and air dried.

For hybridization, the filter was placed in a plastic bag with one end open. Hybridization solution (17 μl/cm^2) [the mixture of NaCl/Cit, Denhardt's solution, denatured salmon sperm DNA, and NaDodSO$_4$ mentioned above and 3×10^6 cpm of ^{32}P-labeled hybridization probe per ml (specific activity, 2×10^8 cpm/μg)] was added. Air bubbles and excess hybridization solution were removed by rolling a test tube over the bag. The area of the bag containing the filter was sealed. Hybridization was carried out at 67°C for 24 hr. The filter was then washed at 67°C in 500-ml portions as follows: 1 hr with 3X NaCl/Cit/10X Denhardt's/0.1% NaDodSO$_4$; three times for 1 hr with 2X SSC/0.1% NaDodSO$_4$; and 1 hr with NaCl/Cit/0.1% NaDodSO$_4$. The filters were blotted dry and autoradiographed with XR-5 x-ray film (Kodak), with a Dupont Cronex intensifying screen, at −70°C for 2–7 days.

Preparation of Hybridization Probes. Two cloned DNA fragments were used as hybridization probes. Both were derived from the human β-globin genomic clone HβG-1 isolated by Maniatis and coworkers (17). A probe specific for the β-globin coding region (Hβ1) and a probe specific for the δ-globin coding region (Hδ1) were obtained by digestion of the HβG-1 clone with restriction enzyme *Pst* and subcloning the appropriate fragments in the plasmid pBR322. These clones were kindly provided by T. Maniatis and his coworkers. DNA was prepared from Hβ1 and Hδ1 exactly as described by Wilson *et al.* (18). The plasmid DNA was labeled with ^{32}P to a specific

activity of approximately 2×10^8 cpm/μg by the procedure of Rigby *et al.* (19).

RESULTS

Precise Mapping of the LDH_A and ACP_2 Genes. In early experiments it became apparent that these two genes are critical members of the set of markers to be used for localization of the β-globin gene. Thus, it was necessary to locate their positions as accurately as possible.

Clones J1-9 and J1-10 have the same phenotype with respect to the markers studied here (Table 1) but differ in the extent of their respective deletions as determined by cytogenetic analysis. The breakpoint in J1-9 lies in the middle of the band p12, whereas the breakpoint in J1-10 lies near the top of this same band. Following the recommendation of the Paris Conference Supplement (20) for designation of breakpoints within a chromosomal band, the band is subdivided into 10 equal parts (numbered 01, 02, . . ., 10), the 01 region being that closest to the centromere. The breakpoint for J1-9 is designated as p1205 and that for J1-10 is p1208. Such intraband designations are approximate and may involve an uncertainty of 10% or possibly 20% of the band width. LDH_A is present in J1-23 but is absent from J1-10, J1-9, and J1-7. Therefore, its location may be assigned to the region between p1208 and p13. Francke *et al.* (21) have assigned LDH_A to the intraband region p1203 → p1208. The interval common to these two regions is the 10% band interval, p1208, which we therefore take as the locus of LDH_A, with an uncertainty of 10% or 20% of the band width.

ACP_2 is present in J1-9 but absent from J1-7, as described (13). Because the breakpoint of J1-9 appears to occur at p1205, the mapping position of ACP_2 can be placed within the interval p1205 → p11.

Demonstration of the Absence of the Hemoglobin β Gene in CHO-K1 and Its Presence in the J1 Hybrid. In our initial experiments we wished to determine if the Southern hybridization procedure could be used to detect specifically the presence or absence of a single copy of the human β-globin gene in a human–hamster hybrid cell. DNA was isolated from the human–hamster hybrid cell line J1 and from the parental hamster cell line CHO-K1, digested with *Xba* I, fractionated by agarose gel electrophoresis, transferred to a nitrocellulose filter, and hybridized to a cloned DNA probe for the human β-globin gene. A typical experiment is shown in Fig. 2. An intense hybridization band was evident in the 12- to 13-kb region for J-1 (lane 2) but not for CHO-K1 (lane 1). Hybridization in this region was also observed with a sample of human DNA treated in a similar manner. These results indicate the presence of the human β-globin gene in J1 and its absence from CHO-K1, thus confirming the original assignment of the gene to human chromosome 11 by Deisseroth *et al.* (9).

Demonstration that β-Globin Gene Lies in the Interval Between Respective Deletions of Clones J1-9 and J1-10. All three *Xba* I-digested DNA samples from clones J1-10, J1-11, and J1-23 hybridized to human β-globin probe showed hybridization in the 12- to 13-kb region, indicating the presence of the human β-globin gene in these clones (Fig. 2, lanes 5–7). These results imply that the β-globin gene is not present on the portion of the short arm deleted in J1-10. With DNA from clones J1-7 and J1-9, no significant hybridization was observed in the 12- to 13-kb region (Fig. 2, lanes 3 and 4).

These results are summarized in Table 1, which shows that the β-globin gene is lost when terminal deletion of the short arm starting from p1205 occurs but is present when a terminal deletion in the short arm occurs starting from p1208 or when a terminal deletion in the long arm occurs which includes almost

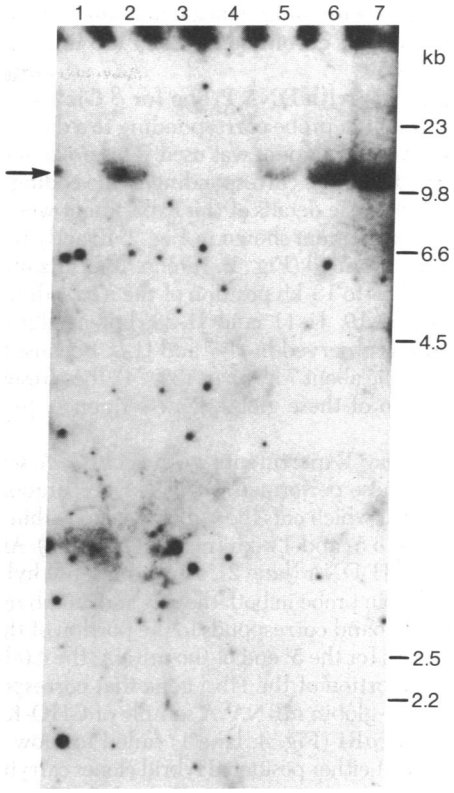

FIG. 2. Hybridization of a human β-globin probe to *Xba* I-digested DNA isolated from hybrid cells. DNA extracted from the cell line indicated was digested with *Xba* I, fractionated by agarose gel electrophoresis, and hybridized to a [^{32}P]DNA probe specific for the human β-globin gene (plasmid Hβ1). DNA derived from bacteriophage λ digested with *Hin*dIII (22) was run in an adjacent channel; the fragments ran at the positions indicated at the left and serve as approximate size markers. A sample of normal human DNA was run in another parallel channel and the position of the major areas of hybridization, corresponding to the major fragments produced by *Xba* I digestion described by Lawn *et al.* (17), is indicated by the arrow. Lanes: 1, CHO-K1; 2, J1; 3, J1-7; 4, J1-9; 5, J1-10; 6, J1-11; 7, J1-23.

Table 1. Demonstration of relationships between amount of chromosome 11 lost through terminal deletions in various mutant hybrid clones and presence of human β-globin gene

Clone	Terminal deletion	Presence of specific human markers					Assay for human Hbβ gene
		SA11-1	SA11-3	LDH_A	ACP_2	SA11-2	
J1	None	+	+	+	+	+	+
J1-23	p13 → pter	−	−	+	+	+	+
J1-10	p1208 → pter	−	−	−	+	+	+
J1-9	p1205 → pter	−	−	−	+	+	−
J1-7	p11 → pter	−	−	−	−	+	−
J-11	q13 → qter	+	+	+	+	−	+

all of that arm. These results indicate that the β-globin gene lies on the short arm of chromosome 11 in the intraband region p1208 → p1205.

Hybridization with DNA Probe for β Globin. In another set of experiments a probe corresponding to a different region of the 12-kb *Xba* I fragment was used. This probe included the section of the fragment corresponding to the coding sequences of the δ globin. Basic details of this experiment were similar to those of the experiment shown in Fig. 2. Exactly the same set of results was obtained (Fig. 3). Intense hybridization was observed at the 12- to 13-kb position of the *Xba* I-digested DNA of clones J1, J1-10, J1-11, and J1-23, but no significant hybridization was observed in J1-7 and J1-9. Because the β- and δ-globin genes lie about 7 kb apart (23, 24), these results confirm the localization of these globin gene sequences to the region indicated.

Confirmatory Experiments with *Eco*RI. A series of experiments was also performed with DNA preparations digested with the *Eco*RI, which cuts the β-globin gene within the coding region to yield 6.5- and 4.0-kb fragments (25, 26). As expected, the sample of J1 DNA (lane 2), showed intense hybridization with the β-globin probe in both the 6.5- and 4.0-kb regions (Fig. 4). The 6.5-kb band corresponds to the portion of the β-globin gene that codes for the 5′ end of the mRNA; the 4.0-kb segment includes the portion of the Hbβ gene that corresponds to the 3′ end of the β-globin mRNA. A sample of CHO-K1 DNA digested with *Eco*RI (Fig. 4, lane 1) failed to show significant hybridization at either position. Hybrid clones carrying partially deleted chromosome 11 exhibited the hybridization pattern

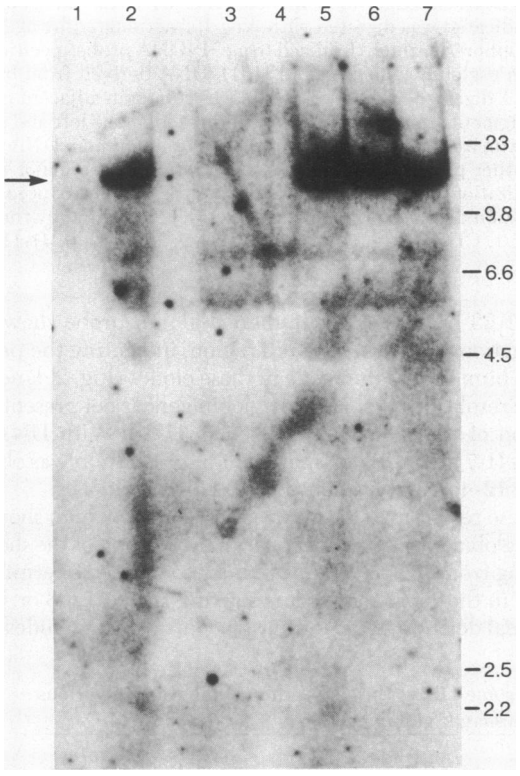

FIG. 3. Hybridization of a human δ-globin probe to *Xba* I-digested DNA isolated from hybrid cells. DNA extracted from the cell lines indicated was treated as in Fig. 2 except that molecular hybridization was carried out with a DNA probe specific for the human δ-globin gene (plasmid Hδ1). Arrow indicates the position of hybridization of an *Xba* I-digested human DNA sample run in a parallel lane to this δ globin probe. Molecular weight markers were run in parallel lanes as in Fig. 2. Lanes: 1, CHO-K1; 2, J1; 3, J1-7; 4, J1-9; 5, J1-10; 6, J1-11; 7, J1-23.

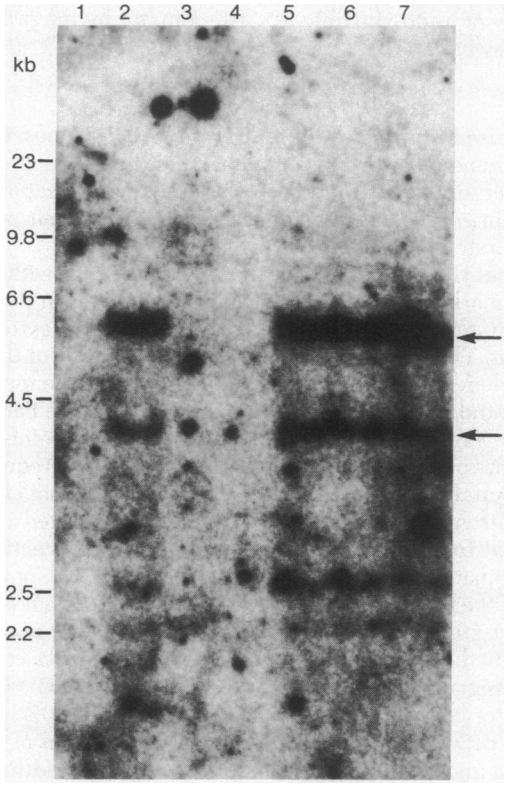

FIG. 4. Hybridization of human β-globin probe to *Eco*RI-digested DNA from hybrid cells. DNA extracted from the cell lines indicated was digested with the *Eco*RI, fractionated by agarose gel electrophoresis, and hybridized to a [^{32}P]DNA probe specific for the human β-globin gene. Arrows show the positions of major regions of hybridization of a normal human DNA sample digested with *Eco*RI and run in a separate lane. Molecular weight markers were run in a parallel lane as in Fig. 2. Lanes: 1, CHO-K1; 2, J1; 3, J1-7; 4, J1-9; 5, J1-10; 6, J1-11; 7, J1-23.

consistent with the pattern previously described as resulting from DNA digested with *Xba* I. Clones J1-10, J1-11, and J1-23 exhibited intense hybridization in the 6.5- and 4.0-kb regions; J1-7 and J1-9 did not exhibit significant hybridization in these areas of the gel. These results, therefore, confirm the previous conclusions.

DISCUSSION

Human chromosome 11 contains approximately 4.6% of the total human haploid genome (27). The total haploid DNA content of a human somatic cell is equivalent to 3.2×10^9 base pairs (28). Because the intraband region between p1205 and p1208 constitutes 3% of human chromosome 11 by direct measurements, it follows that the region that contains the β- and δ-globin genes represents approximately 0.14% of the total haploid human genome or approximately 4500 kb of DNA, equivalent to the size of the *Escherichia coli* genome.

The experiments presented here make it possible to assign the interval, consisting of approximately 34 kb, containing the cluster of hemoglobin genes $^{G}\gamma$-$^{A}\gamma$-δ-β (29) (Fig. 5*B*) to the region of approximately 4500 kb on the short arm of human chromosome 11, in the intraband position p1205 → p1208 (Fig. 5*A*). The sensitivity of the method and the relatively small number of cells required commend this approach for mapping other genes that are not expressed directly in cells and hybrids.

The present study utilized hybrids containing a single human chromosome and its deletion subclones as a model system to

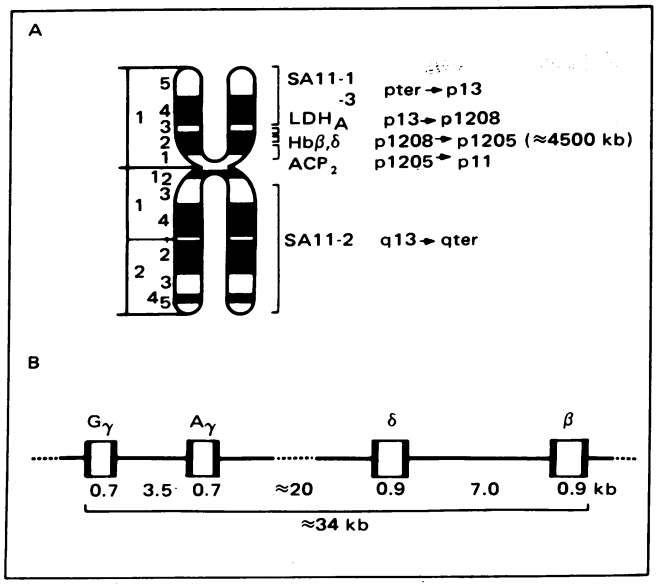

FIG. 5. (*A*) Regional gene map of human chromosome 11 showing the map positions of β- and δ-globin structural genes. (*B*) Tentative gene map taken from Weatherall and Clegg (29) showing the order and distance of the four globin genes, $^{G}\gamma$-$^{A}\gamma$-δ-β. Each gene is composed of exons (dark areas) and introns (light areas).

explore the possibilities of this approach. Two cellular requirements appear to be necessary to perform the type of fine-structure genetic analysis described here. One is the availability of hybrids retaining one or a small number of specific human chromosomes. This requirement has been met by use of the CHO-K1 auxotrophic mutants for fusion with human cells under defined selective conditions (11, 30, 31). The second requirement involves methods for securing a series of subclones with varying terminal deletions in a given chromosome. We have demonstrated that low doses of x-rays in combination with a negative selection against a distal marker such as a cell surface antigen is extremely effective in producing such deletions (12, 13). Accurate timing of 5-bromodeoxyuridine incorporation in the DNA plus subsequent visible light illumination conceivably could also be used to produce such deletions at specific predetermined sites.

The resolving power of the method presented here presumably can be increased by isolating large numbers of deletion clones of chromosome 11 that have lost LDH_A but retained ACP_2. Among such clones will be those that have lost varying amounts of the interval between these two markers. A method has been developed in which a library of human DNA fragments prepared from these deletion subclones is used to achieve a fine-structure map of the DNA in this critical region (unpublished data). The construction of a complete fine-structure map of this genetic region thus becomes feasible. The resolving power that can be achieved by this approach should be at least equal to that of classical crossover mapping procedures in organisms such as *Drosophila* and the mouse. Such a map might be especially valuable in the analysis of the relationship between chromosome structure and gene expression.

At present, three loci have been localized in the area on chromosome 11 accessible to fine-structure mapping: ACP_2, LDH_A, and the β-globin gene complex. Additional polypeptide chains may be assigned to this region as methods for resolving homologous human and Chinese hamster proteins are developed.

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