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Isolation and localization of DNA segments from specific human chromosomes

(DNA hybridization/human chromosome 11/reiterated DNA/recombinant DNA/bacteriophage λ Charon 4A)

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ABSTRACT **Recombinant DNA techniques have been combined with somatic cell genetic methods to identify, isolate, and amplify fragments of human DNA localized at specific regions of human chromosome 11 selected as a model system. A library of genomic DNA segments has been constructed, in λ Charon 4A bacteriophage, from the DNA of a somatic cell hybrid carrying a portion of human chromosome 11 on a Chinese hamster ovary cell background. Using a nucleic acid hybridization technique that distinguishes human and Chinese hamster interspersed, repetitive DNA, we have been able to distinguish recombinant phages carrying DNA segments of human origin from recombinant phages carrying DNA segments of Chinese hamster origin. We have isolated 50 human DNA segments thus far and have characterized 5 in detail. For each DNA segment characterized, a subsegment that carries no repetitive human DNA sequences has been identified. These segments have been used as hybridization probes in experiments that localize the DNA fragment on the chromosome. In each case an unequivocal chromosomal localization has been obtained with reference to a panel of hybrid cell clones each of which carries a deletion of a portion of the short arm of chromosome 11. At least one DNA segment has been identified which maps to each of the four regions on the short arm defined by the panel of hybrid cell clones used. The approaches described here appear to be general. They can be extended to produce a fine structure map of human chromosome 11 and other human chromosomes. This approach promises implications for human genetics generally, for the human genetic diseases, and possibly for understanding of gene regulation in normal and abnormal differentiation.**

There are two principal reasons for attempting to achieve as complete as possible fine-structure analysis of the human genome. First, delineation of the DNA sequence of individual genes and construction of the corresponding probes now can be used to detect the presence of human genetic disease. These procedures can be applied *in utero* (1, 2) so that birth of tragically defective babies can be prevented. Second, and perhaps of even greater ultimate importance to biomedical science, definition of DNA sequences over large chromosomal regions, including sequences specifying protein structures as well as the noncoding intervals within and between these sequences, appears to promise greater understanding of physiological and biochemical mechanisms of human gene regulation. The genetic data so obtained should illuminate many aspects of medicine and developmental biology including situations not usually considered to lie within the narrow scope of the term "genetic disease."

The application of recombinant DNA techniques has already had a significant impact on human genetics. The isolation of cloned human globin genes (3, 4) has been instrumental in the analysis of the fine structure of these genes (5–7) and in determining the physical basis of the genetic lesions responsible for the α and β thalassemia syndromes (8–10). Cloned globin probes also have been used to exploit polymorphism in restriction enzyme recognition sites as a basis of antenatal diagnosis of sickle cell anemia (1, 2). However, a general method for producing DNA probes for specific chromosomal regions has not been available. At present more than 300 genetic markers have been assigned to specific chromosomal locations (11, 12), but these are not enough to permit linkage studies in many cases in which such information would be important to the intrauterine diagnosis of genetic disease. In addition these markers are so widely distributed over the genome that achievement of fine-structure mapping of a specific genetic region has not appeared feasible.

In this paper, we describe an approach that uses recombinant DNA methods in combination with somatic cell genetics (*i*) to isolate cloned DNA segments from defined regions of human chromosome 11, which has been selected as a model system, and (*ii*) to map these on the chromosome. The approach is general and appears to be applicable to the isolation of cloned DNA segments of other human chromosomes. The construction of a fine-structure map of a human chromosomal region at the DNA level appears to be possible by this technology. The ability to achieve this goal should have a significant impact on human genetics at various levels.

MATERIALS AND METHODS

Preparation of DNAs. DNA was prepared from the HeLa and Chinese hamster ovary (CHO-K1) cell lines, and from the previously described human–CHO-K1 hybrids J1, J1-7, J1-10, J1-11, and J1-23 (13). Digestions were performed as described (13, 14) with *Eco*RI (Boehringer Mannheim) and other restriction endonucleases (New England BioLabs). λ Charon 4A bacteriophage was propagated in liquid culture and its DNA was prepared as described (14). λ Charon 4A arms were separated from the internal fragments by sucrose gradient centrifugation of *Eco*RI-digested phage DNA (15). DNA fragments [15–20 kilobases (kb)] from CHO-K1 and J1-11 were isolated after partial *Eco*RI digestion by the method of Maniatis *et al.* (15). These DNAs were ligated to the λ Charon 4A arms as described by Varsanyi-Breiner *et al.* (14). An *in vitro* encapsidation procedure (14, 16) was used to obtain viable phage containing the ligated DNA. These were propagated to obtain J1-11 and CHO-K1 libraries as described by Varsanyi-Breiner *et al.* (14). The total human library used was provided by T. Maniatis (15). Grids of recombinant phages were obtained by transfer, with sterile toothpicks, of individual plaques from plates with 100–300 plaque-forming units onto a fresh lawn on a 150-mm bacterial dish.

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Abbreviation: kb, kilobase(s).

Screening of J1-11 Library. Duplicate nitrocellulose filters were prepared from each grid as described by Benton and Davis (17) and hybridized to total nick-translated (18) HeLa cell DNA or CHO-K1 DNA respectively (10^6 cpm/filter; specific activity, 2×10^8 cpm/μg) as described by Varsanyi-Breiner *et al.* (14). Clones containing human DNA sequences were chosen by virtue of their hybridization to HeLa DNA but not to CHO-K1 DNA. The corresponding phages were cloned by plaque formation, and DNA was prepared from them as for λ Charon 4A (14) except that the disabled host DP50 (*supF*) was used (19). All manipulations involving viable recombinant phage were performed at the P2-EK2 level of containment in accordance with the National Institutes of Health *Guidelines for Recombinant DNA Research*.

Preparation of Clone-Specific Hybridization Probes. Each putative human–Charon 4A recombinant DNA was digested with *Eco*RI and a number of other restriction enzymes (*Bam*HI, *Sac* I, *Hin*dIII, *Xba* I, *Hae* II) and the resulting fragments were first resolved by agarose gel electrophoresis and then transferred to nitrocellulose (20). Filters were hybridized (13) to nick-translated radioactive HeLa cell DNA to locate fragments containing repetitive human sequences. For each clone, a fragment not hybridizing to the HeLa DNA probe was chosen for use as probe against similar filters containing *Eco*RI-digested DNA of cell lines CHO-K1, J1, J1-7, J1-10, J1-11, and J1-23. This fragment was prepared by the freeze-squeeze procedure (21) and nick-translated to high specific activity as described by Rigby *et al.* (18).

RESULTS

To isolate DNA sequences from human chromosome 11, we used the following strategy. The methods of somatic cell genetics have been used to prepare a series of human–Chinese hamster cell hybrids containing chromosome 11 or deletion mutants of this chromosome as their only human chromosomal component (22–24). A library of recombinant clones of DNA from the hybrid cell containing the short arm and the centromeric region of chromosome 11 was constructed by using a procedure similar to that originally described by Maniatis *et al.* (15). DNA fragments 15–20 kb in length were isolated from the hybrid cell DNA by partial digestion with *Eco*RI and size fractionation of the partially digested DNA on a sucrose gradient. These fragments were then cloned in the λ phage vector Charon 4A. Each recombinant phage had one 15- to 20-kb fragment of DNA of human or Chinese hamster origin.

Our next step was to develop species-specific probes that would distinguish those phages carrying cloned human DNA segments from those carrying a Chinese hamster DNA segment. The following considerations were invoked. Previous work has indicated that repetitive DNA sequences are interspersed throughout the genome many thousand times (25, 26). Evidence has also suggested that substantial evolutionary divergence of these repetitive sequences exists in mammals (27). We reasoned, therefore, that a repetitive DNA probe might be species-specific. Hybridization of such a probe to DNA from clones of a library constructed from genomic DNA of a hybrid cell containing both human and Chinese hamster chromosomes might distinguish between cloned DNA segments of human and of Chinese hamster origin.

To test this hypothesis the experiment shown in Fig. 1 was performed. Our probe in this experiment was total human DNA labeled to high specific activity with ^{32}P by the nick-translation procedure. Molecular hybridization conditions were arranged such that only repetitive DNA sequences would register a detectable hybridization signal. The species specificity of the procedure was tested in experiments in which the ^{32}P human DNA probe was hybridized to a series of cloned DNA segments

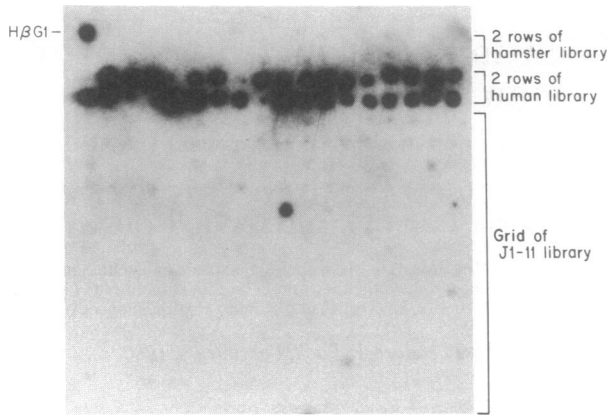

FIG. 1. Isolation of recombinants containing human DNA sequences from a library of human–Chinese hamster hybrid cell DNA. Recombinant λ Charon 4A phages from the indicated libraries were spotted into an array. A nitrocellulose replica was made and hybridized to 10^6 cpm of ^{32}P-labeled HeLa cell DNA. One phage plaque from the J1-11 library showed intense hybridization. A parallel filter (data not shown) was hybridized to ^{32}P-labeled Chinese hamster DNA to confirm the identification of these human DNA-containing clones.

from human and Chinese hamster cells. The hybridization technique used was similar to the method described by Benton and Davis (17). Individual cloned DNA segments from libraries of Chinese hamster DNA, human DNA, and a human–Chinese hamster hybrid cell were grown by transferring phage from single well-isolated plaques to a grid on a fresh lawn of host bacteria. After growth of the phage, the DNA from the various clones selected was transferred to nitrocellulose filters. Each filter was hybridized to the ^{32}P-labeled human DNA probe.

As shown in Fig. 1, the hybridization technique was effective in discriminating cloned DNA segments of human origin. The first two rows of the grid consisted of λ Charon 4A clones carrying segments of Chinese hamster origin. None of these clones hybridized to the probe. The next two rows contained clones picked at random from a library constructed from human DNA in λ Charon 4A. Almost all of these clones hybridized to the human DNA probe. Included in this array was the phage HβG1 carrying the coding regions for the human β- and δ-globin genes described by Lawn *et al.* (3), the DNA of which hybridized intensely with the probe. These control experiments (and others to be reported in detail elsewhere) confirm the species specificity of the hybridization method.

In the remaining rows of the grid, cloned DNA segments derived from the human–Chinese hamster cell line J1-11 were spotted. This cell line carries the entire short arm of human chromosome 11, the centromere of chromosome 11, and a small segment of the long arm of chromosome 11 adjacent to the centromere, against a background of Chinese hamster ovary chromosomes (23) (Fig. 2). Of the clones in this array 1 of 234 clones tested hybridized to the human DNA probe. A parallel hybridization of the DNA of these clones to a Chinese hamster DNA probe showed that this clone did not hybridize to the Chinese hamster DNA probe whereas >80% of the recombinant clones picked from the library constructed from J1-11 DNA did hybridize to the Chinese hamster probe (data not shown). A systematic survey of more than 20,000 clones from this library yielded ≈50 recombinants of putative human origin.

Five clones were selected at random from the initial 50 for further characterization. The goal of these studies was to determine the distribution of reiterated DNA sequences within the cloned DNA segment and, if possible, to isolate from such clones DNA fragments that contained only single-copy DNA. The analysis of the DNA from one of the five clones, H11-3, is shown in Fig. 3. The DNA of this clone was digested with *Eco*RI

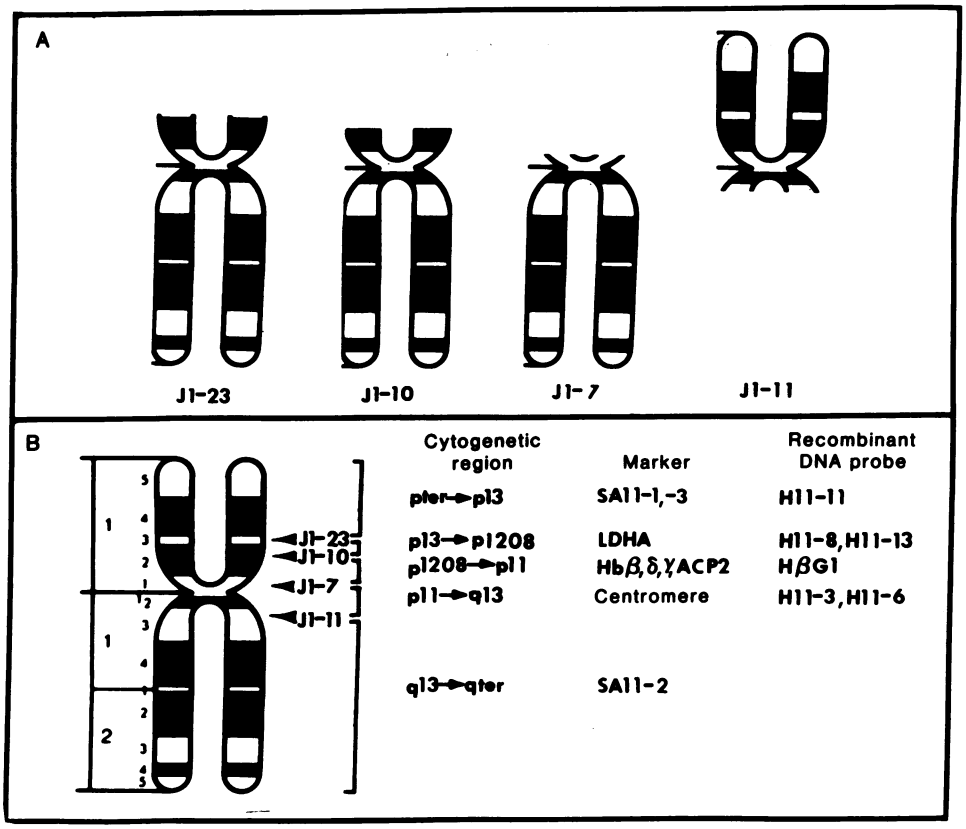

FIG. 2. (*A*) Diagram showing the various terminal deletions of human chromosome 11 in the four cell hybrids. In our previous paper (13) five different terminal deletion mutants involving chromosome 11 were used. In the present study, clone J1-9 was not included. (*B*) Schematic representation of human chromosome 11 with arrows indicating the breakpoints at which terminal deletions occurred in the four clones. The four breakpoints divide chromosome 11 into five cytogenetic regions, each of which is characterized by various markers as indicated. The map positions of the recombinant DNA probes characterized in this study are indicated.

and *Bam*HI and fractionated on a 1% agarose gel. The DNA fragments were then visualized by staining with ethidium bromide. *Eco*RI digestion of clone H11-3 DNA (lane A) produced four fragments: the λ Charon 4A arms (x and z), a dimer of the λ Charon 4A arms hybridized at the terminal cohesive ends (w), and a single inserted fragment (y) of 15 kb. *Bam*HI digestion (lane B) produced nine fragments, of which four consisted entirely of DNA from the λ Charon 4A arms (c, d, e, and h) and five contained at least some of the human cloned sequence (a, b, f, g, and i).

After visualization of the DNA fragments, the DNA was transferred to a nitrocellulose filter and hybridized to high-specific-activity ^{32}P human DNA (2×10^8 cpm/μg). The results are shown in Fig. 3 *Right*. Although fragment y from the *Eco*RI digestion and two of the DNA fragments (b and f) from the *Bam*HI digestion hybridized to the human DNA probe and therefore presumably contained some reiterated human DNA sequences, three fragments (a, g, and i) produced by *Bam*HI digestion of clone H11-3 did not hybridize to the probe under these conditions and therefore were presumed to contain only *single-copy* DNA. One of these fragments, g, was chosen as a probe for further hybridization experiments.

To localize this cloned DNA segment to a specific region of chromosome 11 the experiment shown in Fig. 4 was performed. DNA was isolated from the following cell lines: CHO-K1, the parent cell line of all the hybrids used in this study (22, 23); J1, which carries the entire human chromosome 11 on a Chinese hamster background; and the four cell lines J1-11, J1-23, J1-10, and J1-7, which contain specific deletions in chromosome 11 (Fig. 2). Based on the pattern of restriction fragments shown by clone H11-3, we expected to observe hybridization to *Eco*RI-digested DNA of cell lines that carried this 15-kb DNA fragment. As expected, hybridization was observed in this region on the gel in DNA samples from clones J1 and J1-11. The pattern of hybridization to clones J1-7, J1-10, and J1-23 can be used to ascertain the chromosomal location of the DNA fragment isolated and cloned in H11-3 (13). The presence of intense hybridization in DNA isolated from all three cell lines indicates that the H11-3 DNA segment is located in the centromere-linked region delineated by the breakpoints of clones J1-11 and J1-7 (Fig. 2).

A similar set of experiments was performed for each of the other four clones chosen for analysis. In each case, a single-copy DNA was selected for mapping of the corresponding 15- to 20-kb fragment on chromosome 11. The map positions of these DNA sequences are listed in Table 1. Each clone could be localized unequivocally by using this technique and, together with β-globin gene sequences, these recombinant clones provide markers for each of the regions delineated by the current panel of cell hybrids. Fig. 2*B* summarizes the markers on human chromosome 11 used in this study and the map positions of the recombinant DNA probes characterized.

The steps of the procedure are summarized as follows: (*i*) preparation of total DNA from the J1-11 hybrid; (*ii*) partial *Eco*RI digestion and sucrose gradient fractionation to produce 15- to 20-kb DNA fragments; (*iii*) ligation to the λ Charon 4A arms, *in vitro* encapsidation to produce viable phage containing the ligated DNA, and amplification of the phages to obtain a J1-11 library; (*iv*) identification of phages carrying human DNA by molecular hybridization with human DNA under conditions such that only reiterated DNA is hybridized; (*v*) endonuclease digestion of the DNA from each phage clone to produce subfragments; (*vi*) identification of the subfragments by molecular hybridization testing for the presence of human reiterated or single-copy DNA; (*vii*) ^{32}P-labeled single-copy DNA probe production by nick-translation; and (*viii*) localization of the single-copy DNA probe on human chromosome 11 by Southern blot hybridization with DNAs from a battery of mutant hybrid cell clones containing successively larger terminal deletions of human chromosome 11.

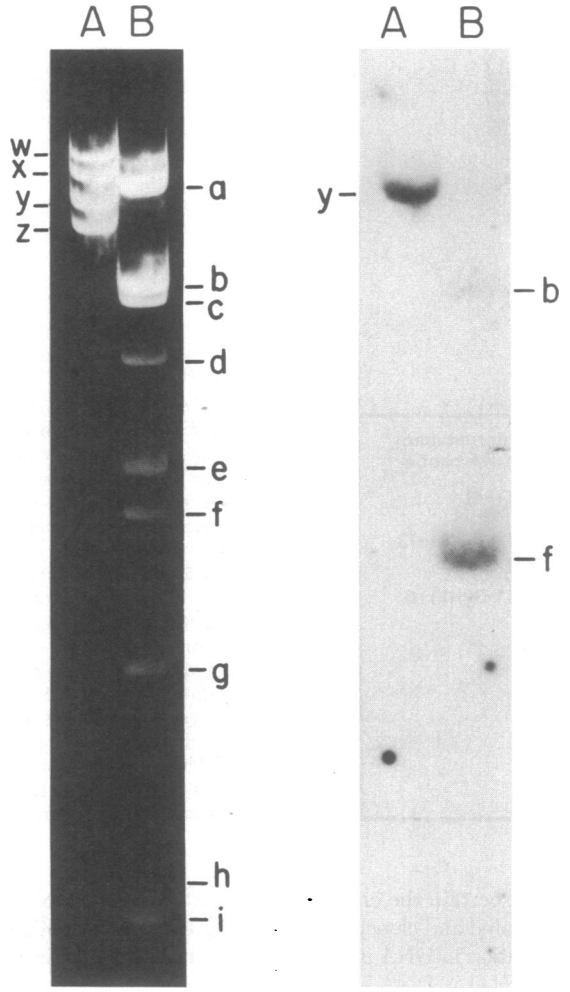

FIG. 3. (*Left*) Ethidium bromide-stained restriction fragments of clone H11-3. Lanes: A, *Eco*RI; B, *Bam*HI. Restriction fragments were resolved on a 1% agarose gel and visualized by staining with ethidium bromide at 0.5 μg/ml. Fragment y is the single 15-kb human *Eco*RI fragment inserted in this clone. (*Right*) Hybridization of restriction fragments of clone H11-3 to ^{32}P-labeled HeLa cell DNA. The fragments shown in *Left* were transferred to nitrocellulose and hybridized to ^{32}P-labeled HeLa cell DNA. Fragments b and f contained some human repetitive DNA. Fragments c, d, e, and h consisted entirely of λ Charon 4A DNA. Fragments a, g, and i showed no hybridization to the ^{32}P-labeled HeLa DNA. Fragment g was chosen for use as a unique sequence probe.

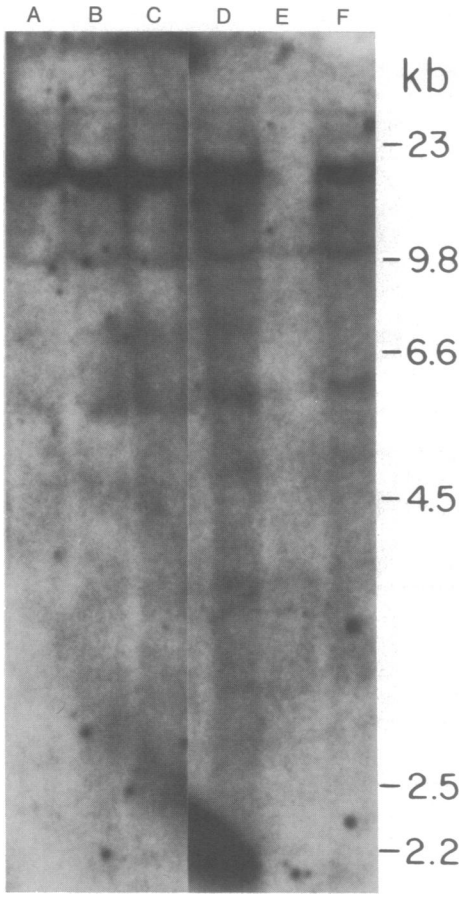

FIG. 4. Localization of clone H11-3 on chromosome 11. *Eco*RI-digested DNA from cell lines J1-11 (lane A), J1-23 (lane B), J1-10 (lane C), J1-7 (lane D), CHO-K1 (lane E), and J1 (lane F) was loaded onto a 1% agarose gel. Electrophoresis was at 40 V for 15 hr, and then the DNA was transferred to nitrocellulose. The filter was hybridized to nick-translated fragment g from a *Bam*HI digest of clone H11-3 DNA (see Fig. 3). A *Hin*dIII digest of λ cI857 Sam 7 was run in a parallel lane to provide approximate size markers.

DISCUSSION

The previous paper of this series (13) demonstrated how cloned, radioactive DNA probes could be used with a panel of specific deletion mutants of a hybrid cell containing a single human chromosome to secure high-resolution chromosomal mapping of genes present in the DNA of the probe. The present paper shows that a library can be constructed from the single human chromosome of such a hybrid and that the various DNA segments of this library can be mapped on the corresponding human chromosome. No RNA isolation is necessary. The method appears to be general and is based on the high species-specificity of the reiterated mammalian chromosomal DNA.

Table 1. Localization of cloned DNA sequences on human chromosome 11

DNA clone	Cell line						Locus
	CHO-K1	J1	J1-11	J1-23	J1-10	J1-7	
H11-3	−	+	+	+	+	+	p11 → q13
H11-6	−	+	+	+	+	+	p11 → q13
HβG1	−	+	+	+	+	−	p1208 → p11
H11-8	−	+	+	+	−	−	p13 → p1208
H11-13	−	+	+	+	−	−	p13 → p1208
H11-11	−	+	+	−	−	−	pter → p13

Mammalian gene mapping has made significant progress in recent years, particularly by means of cytogenetic identification of relatively gross regions on each chromosome (28) and by development of powerful methods for determining DNA sequences (29, 30). However, one of these techniques operates at the level of millions of base pairs whereas the other is limited to handling of thousands. Therefore, a large gap in resolving power exists which must be bridged before these techniques can be combined for systematic high-resolution mapping of the human genome. The work of this and the previous paper of this series (13) demonstrates how this gap can be filled. It now appears possible to isolate, from specific human chromosomes, DNA segments of a size permitting complete sequence analysis and to map these segments precisely on the chromosome.

The methods outlined here extend recombinant DNA techniques so that cloned DNA segments can be isolated and characterized based on their genetic map position alone rather than on their ability to code for a specific mRNA. Human chromosome 11 was chosen for our initial study because of the opportunity to apply deletion mapping techniques to the localization of cloned DNA segments. The methods devised by Kao *et al.* (23) can be extended to isolate a large number of deletions of the short arm of chromosome 11. Previously, the

value of a panel of such clones was limited by the availability of genetic markers on chromosome 11 and the ability to distinguish deletion points cytologically. The present study provides an alternative approach to the problem of genetic fine structure which will permit detailed comparison of hybrid cell lines carrying cytologically indistinguishable mutations. Construction of a fine structure map at the DNA level of a specific region of chromosome 11 appears to be feasible in the immediate future by combining the recombinant DNA techniques described here with further deletion mapping. If single-copy DNA sequences exist located more than 30–40 kb from reiterated sequences, their detection by this method would require procedural modifications.

These principles appear to be applicable to any single human chromosome hybrid, of which a reasonable number already have been prepared (22, 31, 32). Indeed, it may well be possible to apply these methods to hybrids containing several human chromosomes.

The present approach appears to be amenable to further development in the direction of materially increasing the resolving power of the mapping process by accumulation of more deletion mutants as well as more DNA probes. Moreover, the probes themselves can furnish a new series of markers for genetic analysis. By use of x-irradiation and other chromosome-breaking agents to increase the number of deletions in the chromosome under study, one can determine the frequency with which members of a pair of markers are lost or retained and relate these frequencies to their distance apart, as has been shown by Goss and Harris (33–35) and Law and Kao (32, 36). The availability of additional markers should aid the quantitation of mutagenic action which has been proposed for the screening of environmental mutagens and carcinogens with the use of the single human chromosome hybrids (37).

Of particular importance is the demonstration of species-specific reiterated DNA. By extension of the methods of this paper, including the use of specific deletion mutants, it may be possible to determine the distribution of such sequences on a particular chromosome and to compare such distributions on different chromosomes. It will be particularly important to examine whether changes in the composition of such sequences can affect the expression of particular genes. Another interesting direction in which exploration may be possible by the technology described here is the determination of which chromosomal regions are exposed and which sequestered in cells in different differentiation states.

These probes also appear to extend powers of diagnosis of genetic disease in man. For the case of diseases due to homozygous recessive defects caused by gene deletion, the procedure is straightforward. For dominant genetic defects involving detectable DNA sequence changes, it should be possible to prepare hybrids containing single copies of the specific chromosomes and test these with appropriate probes. Alternatively, one may be able to distinguish quantitatively, by molecular hybridization with appropriate probes, between situations involving single and double gene doses. Finally, it may be possible to detect different patterns resulting from treatment with particular endonucleases and to use these polymorphisms as genetic markers. A similar procedure should be effective in identifying heterozygous carriers of recessive defects. Obviously, these techniques would be applicable to cell samples obtained by amniocentesis and therefore could serve for prenatal diagnosis and screening. The existence of defects in chromosome 11 that in the heterozygous condition can produce predisposition to Wilms tumor and aniridia (38) furnish interesting possibilities for immediate exploration.

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1. Kan, Y. & Dozy, A. (1978) *Proc. Natl. Acad. Sci. USA* **75,** 5631–5635.
2. Orkin, S., Alter, B., Altay, C., Mahoney, M., Lazarus, H., Hobbins, J. & Nathan, D. (1978) *N. Engl. J. Med.* **299,** 166–196.
3. Lawn, R., Fritsch, E., Parker, P., Blake, G. & Maniatis, T. (1978) *Cell* **15,** 1157–1174.
4. Wilson, J. T., Wilson, L. B., Riel, J., Villa Komaroff, L., Efstradiatis, A., Forget, B. & Weissman, S. (1978) *Nucleic Acids Res.* **5,** 563–581.
5. Flavell, R., Kooter, J., DeBoer, E., Little, P. & Williamson, R. (1978) *Cell* **15,** 25–41.
6. Mears, J., Ramirez, F., Leibowitz, D. & Bank, A. (1978) *Cell* **15,** 15–23.
7. Orkin, S. (1978) *Proc. Natl. Acad. Sci. USA* **75,** 5950–5954.
8. Orkin, S. H., Old, J., Lazarus, H., Attay, C., Gurgey, A., Weatherall, D. & Nathan, D. G. (1979) *Cell* **17,** 33–42.
9. Mears, J. G., Ramirez, F., Leibowitz, D., Makamura, F., Bloom, A., Konotey-Ahulu, F. & Bank, A. (1978) *Proc. Natl. Acad. Sci. USA* **75,** 1222–1226.
10. Orkin, S., Old, J., Weatherall, D. & Nathan, D. (1979) *Proc. Natl. Acad. Sci. USA* **76,** 2400–2404.
11. Lindley, M. (1979) *Nature (London)* **281,** 12.
12. McKusick, V. & Ruddle, F. (1977) *Science* **196,** 390–405.
13. Gusella, J., Varsanyi-Breiner, A., Kao, F., Jones, C., Puck, T., Keys, C., Orkin, S. & Housman, D. (1979) *Proc. Natl. Acad. Sci. USA* **76,** 5239–5243.
14. Varsanyi-Breiner, A., Gusella, J., Keys, C., Housman, D., Sullivan, D., Brisson, N. & Verma, D. P. (1979) *Gene* **7,** 317–334.
15. Maniatis, T., Hardison, R. C., Lowy, E., Lauer, J., O'Connell, C., Quon, D., Sim, G. K. & Efstradiatis, A. (1978) *Cell* **15,** 687–701.
16. Blattner, F. R., Blechl, A., Denniston-Thompson, K., Faber, H., Richards, J., Slighton, J., Tucker, P. & Smithies, O. (1978) *Science* **202,** 1279–1284.
17. Benton, W. & Davis, R. (1977) *Science* **196,** 180–182.
18. Rigby, P., Dieckman, M., Rhodes, C. & Berg, P. (1977) *J. Mol. Biol.* **113,** 237–251.
19. Blattner, F., Williams, B., Blechl, A., Denniston-Thompson, K., Faber, L., Grunwald, D., Kiefer, D., Moore, D., Schuman, J., Sheldon, E. & Smithies, O. (1977) *Science* **196,** 161–169.
20. Southern, E. (1975) *J. Mol. Biol.* **98,** 503–517.
21. Thuring, R., Sanders, J. & Borst, P. (1975) *Anal. Biochem.* **66,** 213–220.
22. Kao, F. T., Jones, C. & Puck, T. (1976) *Proc. Natl. Acad. Sci. USA* **73,** 193–197.
23. Kao, F. T., Jones, C. & Puck, T. (1977) *Somatic Cell Genet.* **3,** 421–429.
24. Jones, C. & Kao, F. T. (1978) *Hum. Genet.* **45,** 1–10.
25. Britten, R. & Kohne, D. (1968) *Science* **161,** 529–540.
26. Davidson, E. & Britten, R. (1979) *Science* **204,** 1052–1059.
27. McConaughy, B. L. & McCarthy, B. J. (1970) *Biochem. Genet.* **4,** 425–446.
28. Bergsma, D., ed. (1972) *Birth Defects Orig. Artic. Ser.* **8,** 7.
29. Sanger, F. & Coulson, A. R. (1975) *J. Mol. Biol.* **94,** 441–448.
30. Maxam, A. M. & Gilbert, W. (1977) *Proc. Natl. Acad. Sci. USA* **74,** 560–564.
31. Moore, E. E., Jones, C., Kao, F. T. & Oates, D. (1977) *Am. J. Hum. Genet.* **29,** 389–396.
32. Law, M. L. & Kao, F. T. (1978) *Somatic Cell Genet.* **4,** 465–476.
33. Goss, S. J. & Harris, H. (1975) *Nature (London)* **255,** 680–684.
34. Goss, S. J. & Harris, H. (1977) *J. Cell Sci.* **25,** 17–37.
35. Goss, S. J. & Harris, H. (1977) *J. Cell Sci.* **25,** 39–58.
36. Law, M. L. & Kao, F. T. (1979) *Cytogenet. Cell Genet.* **24,** 102–114.
37. Waldren, C., Jones, C. & Puck, T. T. (1979) *Proc. Natl. Acad. Sci. USA* **76,** 1358–1362.
38. Riccardi, V. M., Sujansky, E., Smith, A. C. & Francke, U. (1978) *Pediatrics* **61,** 604–610.