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Anonymous DNA Probes to Human Chromosome 16 Derived from a Flow-purified Library

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Summary

Anonymous DNA probes specific for human chromosome 16 were isolated from a flow-purified human chromosome 16 library. The library was constructed at the Lawrence Livermore National Laboratory. Twenty-nine clones containing a unique or low-copy DNA insert were isolated. Of these, six were assigned to chromosome 16 and regionally mapped and 12 were shown not to map to chromosome 16. One clone mapped to 16pter→16p13.1, one clone to 16p11.1→16q13, one clone to 16q13→16q22.1, and three clones to 16q22.1→16q24. An additional clone from the same library was mapped to 16q13→16q22.1.

Introduction

Chromosome 16 has not been extensively mapped, and up to the time of Human Gene Mapping 8 (Cox and Gedde-Dahl 1985) only two unique and one repeat cloned anonymous DNA fragments had been mapped to this chromosome. Apart from these there were 27 genes mapped to this chromosome (Cox and Gedde-Dahl 1985), perhaps the most important ones being those for the α-globin complex, adult polycystic kidney disease, and adenine phosphoribosyltransferase (Sutherland et al. 1987). Further cloned DNA fragments are required for construction of a detailed genetic map of this chromosome and to detect RFLPs linked to disease genes (Botstein et al. 1980).

Chromosome-specific libraries have been used to enrich for clones mapping to specific chromosomes (Davies et al. 1981; Krumlauf et al. 1982; Kunkel et al. 1982, 1985; Muller et al. 1983; Lalande et al. 1984), and flow-purified chromosomes have been used to construct phage libraries under a U.S. Department of Energy contract to the Lawrence Livermore National Laboratory and the Los Alamos National Laboratory (Fuscoe et al. 1986; Van Dilla et al. 1986). We have used the Lawrence Livermore chromosome 16–specific library to isolate unique and low-copy anonymous DNA probes and have regionally mapped them to specific intervals of chromosome 16 by using a somatic hybrid cell panel that divides the chromosome into six regions (Callen 1986).

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Material and Methods

Cell Lines

A hybrid clone panel containing various translocation products of chromosome 16 in mouse A9 cells has been constructed (Callen 1986). This panel enabled the following six intervals to be defined: 16pter→16p13.11, 16p13.11→16p11.1, 16p11.1→16q13, 16q13→16q22.1, 16q22.1→16q24, and 16q24→16qter. The portion of chromosome 16 present in the various cell lines is summarized in table 1.

DNA Isolation and ^{32}P Labeling

Genomic DNA was isolated from mouse fibroblasts, hybrid clones, and human blood (Wyman and White 1980). DNAs were digested with *Hin*dIII or *Taq*I (New England Biolabs). The restricted DNAs were electrophoresed in 0.8% or 1.0% agarose gels (Pharmacia) and blotted to GeneScreen Plus membranes (New England Nuclear) (Southern 1975). Human DNA was labeled with ^{32}P-dCTP (BRESA) to a specific activity of 2×10^8 cpm/μg by means of nick-translation (Rigby et al. 1977). Recombinant phage

Table I

Portion of Human Chromosome 16 in the Mouse/Human Hybrid Cell Lines That Was Used for Mapping

Portion of Chromosome 16	Cell Line	Reference
16pter→16qter	CY18	Callen 1986
16pter→16qter	CY16	Koeffler et al. 1981
16pter→16q24	CY3	Callen 1986
16p13.11→16qter	CY13	Callen 1986
16p11.1→16qter	CY11	Koeffler et al. 1981
16q13→16qter	CY7	Callen 1986
16q22.1→16qter	CY5	Callen 1986
16q24→16qter	CY2	Callen 1986

DNA was labeled with ^{32}P-dCTP (BRESA) to a specific activity of 5×10^8 cpm/μg by using the random-primer extension method (Feinberg and Vogelstein 1983). The GeneScreen Plus membranes were hybridized using a modification of the dextran sulfate protocol (Wahl et al. 1979). The denatured probe was added to a concentration of 10 ng/ml of hybridization solution, SSPE (1 × SSPE = 180 mM NaCl, 10 mM NaH_2 PO_4, 1 mM EDTA, pH 7.4) was substituted for SSC, and the SDS concentration was increased to 2%. Final washes were at 65 C for 2 × 15 min in 0.1 × SSPE, 1% SDS. Filters were exposed to Kodak X-OMAT X-ray film at −70 C for 1–7 days; an intensifying screen was used.

Phage Library

The chromosome 16–specific gene library (ID code LL16NS02) used in this work was constructed at the Lawrence Livermore National Laboratory. The construction of this library has been described elsewhere (Fuscoe et al. 1986). The human DNA had been inserted into the *Hin*dIII site of bacteriophage lambda Charon 21A. The clone 16B11 (*D16S6*) was obtained from S. Reeders, who had localized it to 16q. The clone had been derived from another aliquot of the same flow-purified chromosome 16 library used in the present study.

An aliquot was plated on *Escherichia coli* LE392. Plaques showing no hybridization to total human DNA were picked and placed in 300 μl of SM buffer (50 mM Tris HCl, pH 7.5, 10 mM $MgSO_4$, 100 mM NaCl, 0.1% gelatin). The phage stock was amplified to ≥1 × 10^8 plaque-forming units (pfu)/ml. A 20-μl aliquot of the phage stock was spotted on a 20-ml (0.5% LM agarose) overlay of *E. coli* LE392, such that an area of 150 mm^2 was confluent. The area of confluent growth in the agarose was extracted, resuspended in 200 μl SM buffer, vortexed for 1 min, and left at room temperature for 30 min. DNase and RNase were added to concentrations of 10 μg/ml and 1 μg/ml, respectively, and incubated at 37 C for 30 min, and ammonium acetate and magnesium chloride were then added to 0.3 M and 10 mM. The aqueous phase was extracted with phenol:chloroform:isoamylalcohol (50:49:1). DNA was precipitated overnight at −20 C by addition of 2 vol of 95% ethanol, centrifuged, and washed in 70% ethanol. The dried DNA was resuspended in 19 μl of *Hin*dIII buffer and digested for 3 h with 20 units of *Hin*dIII (New England Biolabs). The digested DNA was electrophoresed on a 0.8% agarose minigel. A 15-cm confluent plate was used to prepare larger quantities of the recombinant phage DNA.

Results

An aliquot of the chromosome 16 library was screened with ^{32}P-labeled total human DNA to isolate clones containing unique DNA inserts that would not bind sufficient ^{32}P-labeled DNA during hybridization to give a signal after autoradiography. Two rounds of screening with ^{32}P-labeled total human DNA were carried out. A total of 98 plaques were selected from the first screen and respotted on a 15-cm plate such that an area of 50 mm^2 was confluent for each clone. A replicate filter was probed with ^{32}P-labeled total human DNA, and 69 of the 98 clones gave no signal after autoradiography. DNA was prepared from the 69 clones, digested with *Hin*dIII, and electrophoresed on a 0.8% minigel. Twenty-nine clones contained a visible insert with sizes ranging from 0.5 to 5 kb.

The 18 clones with the largest inserts were then mapped to or excluded from human chromosome 16. This was done by means of hybridization of the clone to a panel containing *Taq*I digests of human, mouse A9, and CY18 DNA. A clone mapped to chromosome 16 if DNAs of similar molecular weight were detected in human and CY18 DNA and were not present in A9 DNA. The clones ACH208 and ACH224 illustrate this, as shown in figure 1. A clone was excluded from chromosome 16 if homologous DNA of similar molecular weight was detected in human DNA but not in CY18 or mouse A9 DNA. The clones ACH136 and ACH163 illustrate this, as shown in figure 1. Of the 18 clones analyzed, six were assigned to human chromosome 16. The six clones mapped to chromosome 16 were ACH92 (*D16S3*),

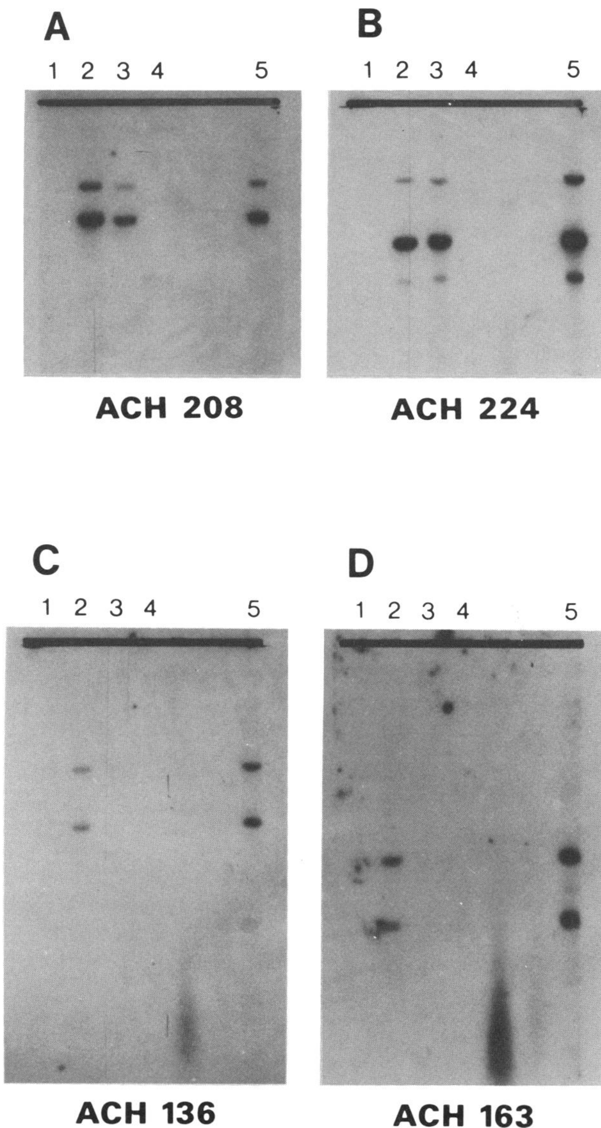

Figure 1 Assignment of clones to chromosome 16 by means of Southern blot hybridization analysis of DNA from cell lines. The panels contained *Taq* I-digested DNA of human (lanes 2 and 5), CY18 (lane 3), and A9 (lane 4). The panel was hybridized with ACH208 (*A*), ACH224 (*B*), ACH136 (*C*), and ACH163 (*D*). *Eco*RI-digested SPP-1 standards were present in lane 1 and were used to determine kilobase sizes. The autoradiogram was not reproduced for the SSP-1 DNA standards. The SPP-1 bacteriophage *Eco*RI fragments were 7.84, 6.96, 5.86, 4.69, 3.37, 2.68, 1.89, 1.80, 1.45, 1.33, 1.09, 0.88, 0.66, 0.48, and 0.38 kb.

ACH127 (*D16S13*), ACH202 (*D16S14*), ACH207 (*D16S4*), ACH208 (*D16S15*), and ACH224 (*D16S5*). The screening process involved in mapping these clones is summarized in the Appendix.

The human chromosome 16 hybrid cell panel was used to map the clones to specific regions of chromosome 16. The DNAs of the panel were digested with *Hin*dIII or *Taq*I. The use of two digests of the DNAs was necessary for ACH202, since a DNA band of similar molecular weight was seen in both human and mouse DNAs for the *Hin*dIII digest but not for the *Taq*I digest. Three other clones—ACH127, ACH207, and ACH208—produced more easily interpretable autoradiograms with the *Taq*I-digested panel.

The hybridization results are shown in figures 2 and 3 and are summarized in table 2. ACH92, which contained one insert of 3.0 kb and one insert of 1.5 kb, had the 1.5-kb insert mapped to 16pter→16p13.11 and the 3.0-kb insert excluded from chromosome 16. ACH127 mapped to 16p11.1→16q13, and homologous DNA sequences were detected on other human chromosomes. ACH207 mapped to 16q13→16q22.1. 16B11 contained a 3.7-kb insert that also mapped to 16q13→16q22.1, but the DNA preparation was contaminated by a clone containing a 2.7-kb insert; this 2.7-kb insert was excluded from chromosome 16. ACH202, ACH208, and ACH224 mapped to 16q22.1→16q24. The large-molecular-weight DNA fragments seen in figures 2 and 3 were lambda DNA–specific sequences. Three of the clones—ACH202, ACH224, and 16B11—detected a homologous DNA fragment in mouse A9 DNA.

Discussion

The flow-purified chromosome 16 library provided a source of cloned unique anonymous DNAs. The screening protocol was effective, since 17 of the 18 clones analyzed were clones of unique, single-copy DNA. ACH127 was repetitive and had many homologous DNA fragments both in 16p11.1→16q13 and in other chromosomes. Of the 69 clones isolated, 29 contained a visible insert; and the remaining 40 may have contained an insert that was too small to be detected by ethidium bromide staining and UV illumination of a miniagarose gel. Nonetheless, this served to isolate inserts of a minimum size of 0.5 kb. The larger the insert the more likely it is that an RFLP will be detected for any given DNA fragment. The 18

Table 2

Summary of Mapping Using Cell Lines

Probe and Insert Size (kb)	Hybridization to DNA in Cell Lines										Location on Chromosome 16
	A9	Human	CY18	CY16	CY3	CY13	CY11	CY7	CY5	CY2	
ACH92:											
1.5	–	+	+	+	+	–	–	–	–	–	16pter→16p13.11
3.0	–	+	–	–	–	–	–	–	–	–	
ACH127:											
3.3	–	+	+	+	+	+	+	–	–	–	16p11.1→16q13[a]
ACH207:											
4.7	–	+	+	+	+	+	+	+	–	–	16q13→16q22.1
16B11:											
3.7	–	+	+	+	+	+	+	+	–	–	16q13→16q22.1[b]
2.7	–	+	–	–	–	–	–	–	–	–	
ACH202:											
3.2	–	+	+	+	+	+	+	+	+	–	16q22.1→16q24[b]
ACH224:											
5.0	–	+	+	+	+	+	+	+	+	–	16q22.1→16q24[b]
ACH208:											
3.7	–	+	+	+	+	+	+	+	+	–	16q22.1→16q24

[a] Additional homologous DNA fragments were detected on other human chromosomes.
[b] Clone also hybridized to a mouse homologous-DNA fragment.

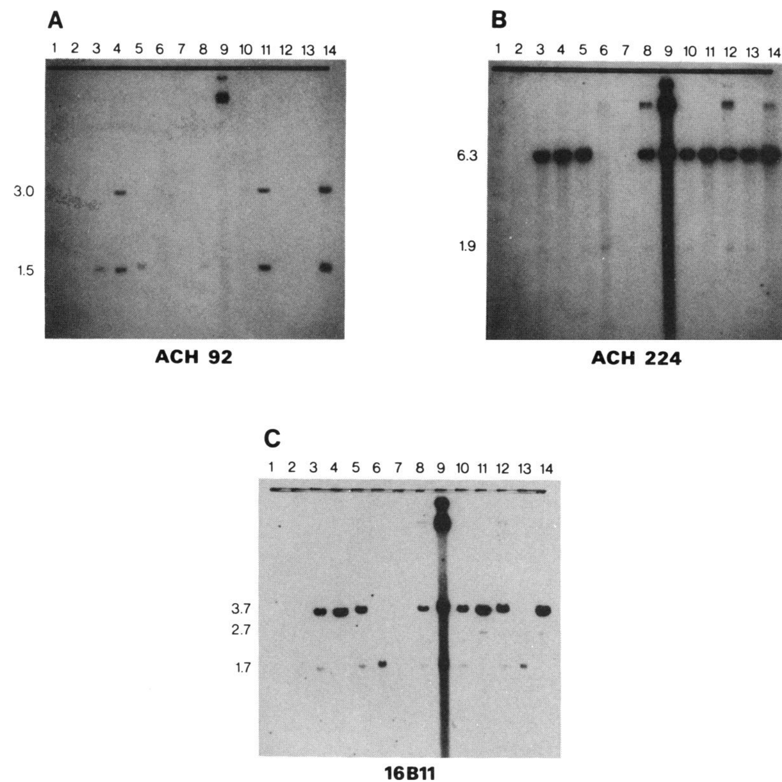

Figure 2 Localization of clones to intervals on chromosome 16 by Southern blot hybridization of DNA from cell lines. The panel contained *Hin*dIII-digested DNA of A9 (lane 2), CY18 (lane 3), human (lanes 4, 11, and 14), CY3 (lane 5), CY2 (lane 6), CY16 (lane 8), CY7 (lane 9), CY13 (lane 10), CY11 (lane 12), and CY5 (lane 13). The panel was hybridized with ACH92 (*A*), ACH224 (*B*), and 16B11 (*C*). *Eco*RI-digested SPP-1 standards were present in lanes 1 and 7 (see legend to fig. 1). The large-molecular-weight DNA fragments in the autoradiograms were due to contamination of the cell-line DNAs with lambda DNA.

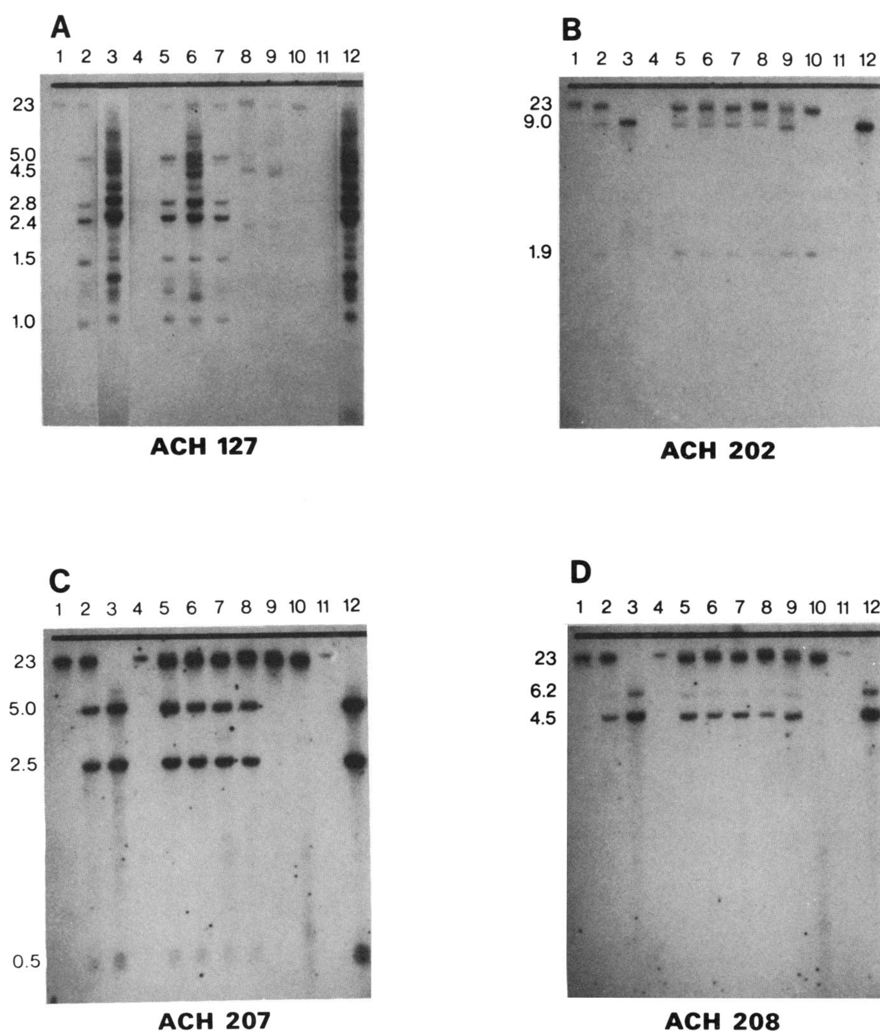

Figure 3 Localization of clones to intervals on chromosome 16 by Southern blot hybridization of DNA from cell lines. The panel contained *Taq*I-digested DNA of A9 (lane 1), CY18 (lane 2), human (lanes 3, 12), CY3 (lane 5), CY13 (lane 6), CY11 (lane 7), CY7 (lane 8), CY5 (lane 9), and CY2 (lane 10). The panel was hybridized with ACH127 (*A*), ACH202 (*B*), ACH207 (*C*), and ACH208 (*D*). *Eco*RI-digested SPP-1 standards were present in lanes 4 and 11 (see legend to fig. 1). The large-molecular-weight DNA fragments in the autoradiograms were due to contamination of the loading buffer of the cell-line digests with lambda DNA.

clones analyzed were selected on the basis of their molecular weight, and higher-molecular-weight inserts were selected first. One-third (six) of these clones were mapped to chromosome 16. The flow purification thus enriched 11-fold for chromosome 16 DNA if it is assumed that chromosome 16 contains 3% of the human DNA content (Southern 1982) and that unique DNA sequences are equally distributed in all chromosomes.

Although only six clones were analyzed, three of these—ACH202, ACH208, and ACH224—have mapped to the interval 16q22.1→16q24, and ACH207 and 16B11 have been mapped to 16q13→16q22.1. The search for RFLPs will enable these clones to be mapped into the gene order cen-*MT-FRA16B-HP-FRA16D-APRT-qter* (Fratini et al. 1986) by means of linkage analysis. In situ hybridization to chromosomes expressing the fragile sites FRA16B or FRA16D (Fratini et al. 1986; Simmers et al. 1986, 1987, and in press) will enable the location relative to the fragile sites to be established without the need for an RFLP and linkage analysis.

One clone, ACH92, contained two inserts, and one of these mapped to 16pter→16p13.11. This is the region to which a highly polymorphic probe to the hypervariable region 3′ to the α-globin gene

(Higgs et al. 1986) mapped (Simmers et al., in press). The 3′-hypervariable region has been tightly linked to the gene for adult polycystic kidney disease (Reeders et al. 1985). The second insert in ACH92 was excluded from chromosome 16. Thus the two cloned DNA fragments are not contiguous in the human genome, unlike a clone containing three *Hin*dIII inserts from a chromosome 18 library (Perlman and Fuscoe 1986).

Three of the seven clones that mapped to 16q hybridized to a DNA fragment in mouse. Conserved linkage between human 16q and mouse 8 has been shown (Lalley and McKusick 1985). These three clones could be used to investigate this conserved linkage and determine over how great an interval the two chromosomes show homology.

A search for RFLPs detected by the seven regionally mapped clones is in progress.

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Appendix

Screening Process Involved in Obtaining Clones Mapping to Chromosome 16

Clones selected on first screen: 98
Clones retained on second screen: 69/98
Clones with visible insert: 29/69
Clones mapping to chromosome 16: 6/18

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