GENOMICS 35, 353–360 (1996)
ARTICLE NO. 0367

A 500-kb Physical Map and Contig from the Harvey *ras-1* Gene to the 11p Telomere

MARK W. RUSSELL,*,† DAVID J. MUNROE,‡ EVA BRIC,‡ DAVID E. HOUSMAN,‡ JEANNE DIETZ-BAND,§ HAROLD C. RIETHMAN,¶ FRANCIS S. COLLINS,† AND LAWRENCE C. BRODY†,[1]

*Departments of Pediatrics and Communicable Diseases, Human Genetics, and Internal Medicine, University of Michigan, Ann Arbor, Michigan 48109; †Laboratory of Gene Transfer, National Center for Human Genome Research, National Institutes of Health, Bethesda, Maryland 20892-4470; ‡Center for Cancer Research, Massachusetts Institute of Technology, Cambridge, Massachusetts 02139; §Department of Research and Development, Oncor Incorporated, Gaithersburg, Maryland 20877; and ¶The Wistar Institute, Philadelphia, Pennsylvania 19104

Received February 14, 1996; accepted April 25, 1996

A contiguous physical map was constructed from the Harvey ras-1 (*HRAS1*) gene to the 11p telomere. The contig spans approximately 500 kb and is minimally composed of a telomere-containing YAC and P1 and cosmid clones. Included in the contig are 11 sequence-tagged sites derived from P1 and cosmid ends. Three genes were placed on the contig in the following order: telomere–ribonuclease/angiogenin inhibitor (*RNH*)–Harvey *ras-1* (*HRAS1*)–HRAS1-related complex (*HRC*). Two novel tetranucleotide repeats (heterozygosity of 66 and 68%) and a complex CA repeat (heterozygosity of 78%) were isolated and characterized.

INTRODUCTION

Physical mapping of the human genome is an essential component in identifying and mapping genes associated with human diseases. The majority of the physical mapping efforts thus far have utilized YACs to cover large portions of the human genome (Cohen *et al.*, 1993; Hudson *et al.*, 1995). One of the several problems of YAC clones is that telomeric and subtelomeric DNA sequences are significantly underrepresented, causing gaps of unknown size between the most distal mapped marker and the end of the chromosome. This is particularly troublesome given the increasingly recognized importance of the structure and functions of the telomere (Zakian, 1995). Telomere shortening has been associated with cellular senescence (Harley *et al.*, 1992), and the stabilization of telomere length by the activation of telomerase has been demonstrated in ovarian carcinoma (Counter *et al.*, 1994). Physical mapping of telomeres has led to an increased knowledge of telomere function and may aid the identification and understanding of proteins with telomere-binding activity (Zhong *et al.*, 1992). The terminal portions of chromosomes are reported to have the highest gene concentrations in the human genome (Saccone *et al.*, 1992), and targeted mapping of these regions may be particularly important to positional cloning and transcript identification efforts. The terminal portion of 11p is particularly interesting due to the mapping of a tumor suppressor gene to this region (Bepler and Garciablanco, 1994) and the large number of CpG islands in proximity to the Harvey *ras-1* (*HRAS1*) gene (Weitzel *et al.*, 1992) on 11p15.5.

Physical and genetic mapping of 11p telomeric to the *HRAS1* gene has been hampered by the paucity of published informative genetic markers and physical mapping reagents. In this report we describe a completed physical map and contig of 11p15.5, from the telomere to the *HRAS1* gene. The contig is anchored at one end by the 11p telomeric YAC, yRM2209 (approximately 130 kb), which had been isolated from a YAC library enriched for human telomeric DNA (Riethman *et al.*, 1989). Preliminary studies had determined that this YAC contained the subterminal repeat element TelBam3.4 (Brown *et al.*, 1990), and fluorescence *in situ* hybridization (FISH) using yRM2209 DNA as a probe demonstrated a strong signal on the 11p telomere and a weak signal on 10p14 (Browne *et al.*, 1995). With this YAC as a telomeric boundary, we used cosmid fingerprinting and sequence-tagged site (STS) content mapping to develop a cosmid- and P1-based contig from *HRAS1* to the 11p telomere, a distance of approximately 500 kb. In addition we describe three new simple sequence repeat polymorphisms: two highly informative tetranucleotide repeat markers and one CA/CT repeat marker. These markers will aid the genetic mapping of the 11p telomere and, in addition to D11S2071 (Browne *et al.*, 1995), provide a genetic boundary for 11p. The physical mapping of this interval will allow complete analysis of the 11p telomeric region in positional cloning efforts.

[1] To whom correspondence should be addressed at Laboratory of Gene Transfer, National Center for Human Genome Research, 49 Convent Drive, Room 3A14, Bethesda, MD 20892-4470. Telephone: (301) 496-7824. Fax: (301) 402-4929. E-Mail: lbrody@helix.nih.gov.

0888-7543/96 $18.00

MATERIALS AND METHODS

DNA preparation. Yeast cells were prepared for pulsed-field gel analysis according to standard methods (Chandrasekharappa *et al.*, 1992), and YAC DNA was isolated by pulsed-field gel electrophoresis on a 1% low-melting-point agarose gel. A 130-kb band corresponding to yRM2209 was confirmed to contain YAC vector sequence by Southern hybridization and isolated from the gel for use as a probe. P1 DNA was prepared for PCR and sequencing as follows: (1) single colonies were isolated and grown in 2 ml LB broth with kanamycin (25 μg/ml) overnight at 37°C; (2) 10 ml of LB with kanamycin was inoculated with 200 μl of the overnight culture; (3) after 1 h the culture was induced with 80 μl of isopropyl-β-D-thiogalactopyranoside (25 mg/ml); (4) after 8 h at 37°C, the bacteria were pelleted by centrifugation, resuspended in 400 μl of proteinase K (PK) lysis solution (10 m*M* Tris–HCl, pH 7.5, 1 m*M* EDTA, and 50 μg/ml PK), and incubated at 55°C for 30 min; and (5) the lysate was incubated at 80°C for 15 min and placed on ice for 1 min, and the debris was pelleted by microcentrifugation for 3 min. The P1 DNA containing supernatant was then used directly in subsequent reactions (Southard-Smith *et al.*, 1994). For use as probes on cosmid library filters, P1 DNA was prepared using the alkaline–lysis method (Sambrook *et al.*, 1989). Cosmid DNA was prepared using the Qiagen miniprep (tip 20) kit (Qiagen Inc., Chatsworth, CA).

Library screening. High-density grid filters of the LA11NC01 cosmid library (Smith *et al.*, 1993; L. Deaven, Los Alamos National Laboratory) were screened sequentially using the following probes: (1) yRM2209; (2) cosmid LT6 end-probes; (3) 96F2 (whole P1 DNA prep); (4) HRAS1 3′ (STS); (5) D11S483 (STS); (6) 135F4 (whole cosmid prep); and (7) 53A1 (whole P1 prep). STS and end probes derived from the cosmid and P1 clones were amplified by PCR and resolved on 1% low-melting-point agarose gels (Life Technologies Inc., Gaithersburg, MD) in 1× TAE buffer. The amplified DNA was excised and labeled with [α-^{32}P]dCTP (Rediprime, Amersham Inc.) as directed by the manufacturer. For DNA in solution from cosmid or P1 preparations, approximately 1 μg of DNA was labeled. Probes derived from gel-isolated YAC, cosmid, or P1 DNA preps were annealed with 600 μl of sheared human placental DNA (10 mg/ml; Sigma Chemical Inc., St. Louis, MO), 30 μl Cot-1 DNA (1 mg/ml; Gibco BRL, Gaithersburg, MD), and 5 μg of cosmid DNA as follows. The mixture was heated (100°C) for 10 min and then incubated at 65°C for 1 h. The probe was then added to Church–Gilbert hybridization solution (Sambrook *et al.*, 1989) and hybridized to the filters overnight at 65°C.

The P1 library (DMPC-HFF #1; Shepard *et al.*, 1994) was screened both by hybridization of a high-density grid and by a PCR-based strategy. Hybridization screening was performed as described above using the following probes: (1) HRC (STS); (2) 1F7 cosmid end probe; and (3) D11S483 (STS). PCR screening was performed and positive clones isolated by Genome Systems (St. Louis, MO).

Generation of cosmid end probes. Cosmid end probes were generated using *Alu*–vector PCR as previously described (Liu *et al.*, 1993). Briefly, 5′ or 3′ *Alu*-specific primers were used in combination with T3 and T7 primers to generate *Alu*–vector products from both ends of the cosmid. The products present only in the *Alu*–vector reactions and not in the *Alu*–*Alu* reactions were used as probes. The location of the probe in the contig was determined using Southern blot hybridization of cosmid digests. In some cases, a specific *Alu*–vector product could not be generated, and the cosmid end was sequenced directly.

Sequencing of cosmid and P1 ends. The sequencing protocol was adapted from Southard-Smith *et al.* (1994). Cosmid DNA (3 μg) or P1 DNA (7 μl), prepared as above, was sequenced using the fmol cycle sequencing kit (Promega Inc., Madison, WI). For each sequencing reaction, 1.5 pmol of primer was end-labeled with 2.5 μCi of [γ-^{32}P]ATP at 37°C for 2 h, followed by 2 min at 90°C. The sequencing reaction components were combined on ice and aliquoted into the d/ddNTP reaction mix as per the fmol protocol. The reactions were then cycled in a prewarmed Perkin–Elmer 9600 (Perkin–Elmer Inc., Branchburg, NJ) thermal cycler under cycling conditions as described by Wang and Keating (1994). Products were resolved on 6% polyacrylamide sequencing gels (Sequagel-6; National Diagnostics, Atlanta, GA).

Contig construction and verification. The genomic sequences were analyzed using the Genquest server (q@ornl.gov), and regions matching the human repetitive sequences database were identified. PCR primers were constructed from nonrepetitive regions and used to screen the contig. If an STS was not represented in the contig other than in the clone from which it was derived, it was used to screen the P1 library by PCR. The accuracy of the contig was verified using the clone end and gene-based STSs to map the clones on the contig. Any P1 clone whose position in the contig could not be verified by an STS content assay was not included in the contig and was not used for further "walking." Regions of the contig developed using hybridization were verified by STS content mapping and/or restriction fragment fingerprinting. For fingerprint analysis, 3 μg of cosmid was digested with *Sac*I, *Eco*RI, and *Bam*HI in separate reactions. The restriction fragment sizes and Southern hybridization patterns (using *Alu*–vector end probes or gel-purified STS probes) were used to determine regions of overlap.

Identification of polymorphic markers. Cosmids covering the telomeric 150 kb of the contig were restricted, separated by electrophoresis, and transferred to a nylon membrane as described above. Southern blots were then consecutively probed with (1) a CA/GT probe (Pharmacia Biotech, Piscataway, NJ); (2) a pool of 10 trinucleotide-repeat oligonucleotides (~24–30 bp); and (3) a pool of 27 tetranucleotide-repeat oligonucleotides (28 bp) (Flejter *et al.*, 1995). The CA/GT probe was labeled using the Rediprime kit according to the kit instructions and hybridized to the membranes overnight at 65°C. Oligonucleotide probes (100 ng) were end-labeled individually with 10 μCi or [γ-^{32}P]ATP (Amersham Inc., Arlington Heights, IL) using T4 polynucleotide kinase. Hybridization was performed overnight at 42°C in Church–Gilbert solution.

Cosmids containing repeats were subcloned into the pBluescript (pBS) cloning vector (Stratagene Inc., La Jolla, CA). Two micrograms of each cosmid was restricted with *Alu*I, *Hae*III, and *Sau*3A in independent reactions. Endonucleases were inactivated for 15 min at 65°C. Digested cosmid DNAs (120 ng) were ligated to phosphatased (120 ng) pBS vector (12°C, overnight) in a total volume of 12 μl. A 1-μl aliquot of the ligation reaction was used to transform DH5αTM Max Efficiency cells (Gibco BRL). Insert-containing subclones were transferred to a 96-well plate containing 100 μl LB broth with 100 μg/ml ampicillin. A 96-pin duplicating device was used to transfer bacteria to nylon membranes on LB–ampicillin plates. After overnight growth and standard preparations (alkaline denatured, neutralized, UV cross-linked), duplicate colony filters were hybridized with the CA/GT or oligo probes as described previously. DNA from the positive subclones was prepared using the Qiagen miniprep protocol and sequenced using cycle sequencing as described. Primers to amplify the repeats were chosen using the PrimerSelect program (DNAStar Inc.).

Amplification of polymorphic markers. Marker D11S2071 was amplified using the following primers: D11S2071F, AGGGCAATGAGGACATGAAC and D11S2071R, ATGTGGCTGGTCCACCTGGTT (sequence in boldface not included in previously published primers). Primer sequences for 146C2.T1, 146C2.T2, and LT6.CA are shown in Table 1. One primer (20 pmol per 20-μl reaction) for each polymorphism was end-labeled with ^{32}P as for sequencing. Standard buffer conditions and dNTP concentrations were used in the reaction mix with 1 unit of Perkin–Elmer *Taq* polymerase per 20-μl reaction. For the 146C2.T2 marker, 0.2 units of Perfect Match (Stratagene Inc.) was added per reaction. After denaturing (5 min, 94°C), the reactions were subject to 35 cycles (Perkin–Elmer 9600) using the following conditions: 1 min at 94°C, 30 s at the annealing temperature, and 2 min at 72°C. After a final elongation step of 10 min at 72°C, the products were denatured in formamide and resolved on a 6% acrylamide sequencing gel. Observed heterozygosity was determined based on the number of unrelated CEPH parents (n = 80) that were heterozygous for that marker. Allele sizes and frequencies have been deposited in GDB.

TABLE 1

Novel Polymorphic Markers from the Telomeric End of Chromosome 11p

GDB No.	Marker	Sequence	Annealing temp. (°C)	Allele sizes	Heterozygosity (%)
D11S4893	LT6.CA	5′ CCATGGGTGGGGCTGGACAC 5′ACCCAGCACCAGGGGACTCAGA	60	163–183	78
D11S4894	146C2.T1	5′ AGATGCACGGATGGCTGGAT 5′ TCTCATGTCTGACTTGGCTTTGT	60	376–404	66
D11S4895	146C2.T2	5′ CACTGAATCTTTGGAGCCTGGAG 5′ GTCCGTCCGTCCATGAATCTAT	60	400–420	68

Note. Heterozygosity and allele size ranges are based on genotyping of 80 unrelated CEPH parents.

Fluorescence in situ hybridization. Two-color FISH was performed as has been described previously (Ijdo *et al.,* 1992). Briefly, cosmid LT6 DNA was prepared for FISH by biotin labeling followed by competitive hybridization with human C_ot-1 DNA (Gibco BRL). The biotin-labeled cosmid was detected with Texas red–avidin (Oncor, Gaithersburg, MD). Cosmids containing *HRAS1* (84B6) and the tyrosine hydroxylase gene (148G3) were labeled with digoxigenin and detected with fluorescein-conjugated anti-digoxigenin (BMB, Indianapolis, IN). All slides were counterstained with propidium iodide-antifade (Oncor) and photographed under UV light epi-illumination using a multipass filter.

RESULTS

To construct a contig from *HRAS1* to the telomere, we began with the telomere-containing YAC yRM2209 and several STSs, including D11S483, *RNH,* and *HRC,* that had been mapped in prior studies near or telomeric to *HRAS1* (Redeker *et al.,* 1994; Higgins *et al.,* 1994). yRM2209 DNA was labeled and hybridized to a gridded chromosome 11 cosmid library, yielding 16 positive cosmids. The cosmids were digested with *Sac*I, *Eco*RI, and *Bam*HI in separate reactions. Fingerprint analysis demonstrated that there were two nonoverlapping sets of cosmids that were spanned by a third set (data not shown). Based on the fingerprints, this cosmid contig spanned approximately 105 kb. The centromeric extent of this cosmid contig was determined to contain marker D11S483 by hybridization and PCR assay (Table 2), although yRM2209 was negative for this STS. By fingerprint analysis there is no gap found in the cosmid contig from 1F7 to D11S483.

Three gene-based STSs, HRAS1, HRC1, and RNH, were used to isolate cosmids and P1s from the centromeric side of the contig (Fig. 1). A cosmid contig was developed using fingerprint analysis and verified using STS mapping and Southern hybridization with RNH, HRAS1, HRC, 135F4, and 96F2 as probes. Based on a previous mapping study (Higgins *et al.,* 1994), RNH was determined to be telomeric to HRAS1, so the "walk" was continued in that direction. Unfortunately, the telomeric P1 isolated with the RNH probe 96F2 contained a 30-bp segment repeated 10 times at the telomeric end of the clone. Therefore, the entire P1 was used to probe the gridded cosmid library. This hybridization yielded approximately 10 positive clones, 7 of which were already contained in the HRAS1 cosmid contig. Two of the three remaining cosmids, including 135F4, were demonstrated by hybridization to contain the 30-bp repeats. Cosmid 135F4 DNA was then used to probe the gridded cosmid library, yielding two new cosmids including 125C1. An STS made from the telomeric end of this cosmid was used to screen a P1 library by PCR. This screening yielded three P1s. Successive "walks" using an STS derived from the most telomeric extent of the P1s were performed until the telomeric contig was positive for that STS, indicating contig closure.

In total, 21 STSs were used in contig construction. A minimal set of 19 (11 cosmid or P1 ends, 3 novel polymorphisms, 3 genes, D11S2071, and D11S483) was used to confirm the contig (Table 3). The three gene-based STSs were used to place *HRC, HRAS1,* and *RNH* on the contig with an order of telomere–*RNH*–*HRAS1*–*HRC*–centromere. A minimal set of reagents covering this contig was negative for a DRD4 STS (data not shown), consistent with genetic mapping of this gene centromeric to HRAS1 (Petronis *et al.,* 1993).

The cosmids telomeric to and including LT6 were screened for microsatellite repeats. One novel CA repeat and two tetranucleotide repeats were isolated. The CA-positive subclone was derived from LT6 and contained a complex CA/CT repeat in which the CA repeat varied from 10 to 20 and the CT repeat varied from 1 to 5. Both of the tetranucleotide repeats were isolated from cosmid 146C2 and were surrounded by shorter, complex tetranucleotide repeats. In each case, these complex tetranucleotide repeats precluded the design of primers to yield shorter PCR products that would allow the alleles to be resolved with nonisotopic methods. Instead, both tetranucleotide repeats could be scored only by PCR amplification in which one of the primers was end-labeled with ^{32}P. Eighty unrelated individuals from the CEPH DNA collection were genotyped for LT6.CA, 146C2.T1, and 146C2.T2, yielding observed heterozygosity measurements of 78, 66, and 68%, respectively (Table 1). Cosmid 146C2 contained markers D11S2071, 146C2.T1, and 146C2.T2, indicating that all three of these markers are no more than 40 kb from each other. Physical mapping of the markers determined that D11S2071 was the most telomeric of the markers, with a marker order of telomere–

TABLE 2

Overlaps among YAC, P1, and Cosmid Clones

CLONE	Vector	Source	1F7	55G2	141F4	168H3	D11S2071	146C2	146C2.T1	146C2.T2	D11S483	56D8.T7	13T7	LT6.CA	5835SP6	56D8.SP6	5162T7	5835T7	4217T7	5162SP6	4219T7	125T3	125T7	4217SP6	135F4	96F2	RNH	HRAS	91G2	HRC
yRM2209	Y	Wis	H				S		S	-	-			-																
5D12	P	S	H																											
130F6	P	S	H																											
1F7	C	LA	H/F	F	F		-																							
55G2	C	LA	F	F	F	F	-		-	-																				
141F4	C	LA	F	F	F	F	-		-	-																				
168H3	C	LA	F	F	F	F	S	F	-	-																				
146C2	C	LA				F	S	F	S	S	S	-	-	-																
80F7	C	LA						F	S	S	S																			
74C1	C	LA							-	-	S																			
156C5	C	LA					S	F	S	S	-	-	-	-																
116D6	P	S							S	S	S/H	S	S	S																
56D8	P	S									S/H	S	S	S	S	S														
13F10	C	LA											S	S	-	-														
LT6	C	MIT										S	S	S	-	-														
1097H10	P	GS													S	S	S	S	-											
0857D5	P	GS															S	S	S	S										
0295E8	P	GS																	S	S	S	S								
0431D12	P	GS																		S	S	S		S						
0352G5	P	GS																				S		S						
125C1	C	LA																				S	S	S	H					
140C8	C	LA																					S		H	H	S			
135F4	C	LA																					S		H	H	S			S
109E7	C	LA																					-			H	S			
96F2	P	S																					-		H	H	S	S		S
53A1	P	S																							H	H	S	S	H	S
84B6	C	LA																					-				-	S	H	-
74B7	C	LA																					-				-	S	H	-
66H8	P	S																									-	S	H	S
119H12	P	S																									-	S	H	S
91G2	C	LA																										S	H	S
66E9	C	MIT																									-	S	H	S

Note. YAC (Y), P1 (P), and cosmid (C) clones from the 11p telomeric region are listed. Clones are determined to be positive for a given marker by STS content (S), hybridization (H), or fingerprint analysis (F). MIT and Wis denote clones isolated by D. Munroe and H. Riethman, respectively. A clone that was tested for a marker and was negative is represented as a minus sign (−). Blank spaces indicate that no test of overlap was made. Cosmid clones were derived from the LA11NC01 cosmid library (LA). The P1 clones were either derived from the NCHGR copy of the N. Sternberg (S) P1 library or obtained from Genome Systems Inc. (GS) using PCR-based screening.

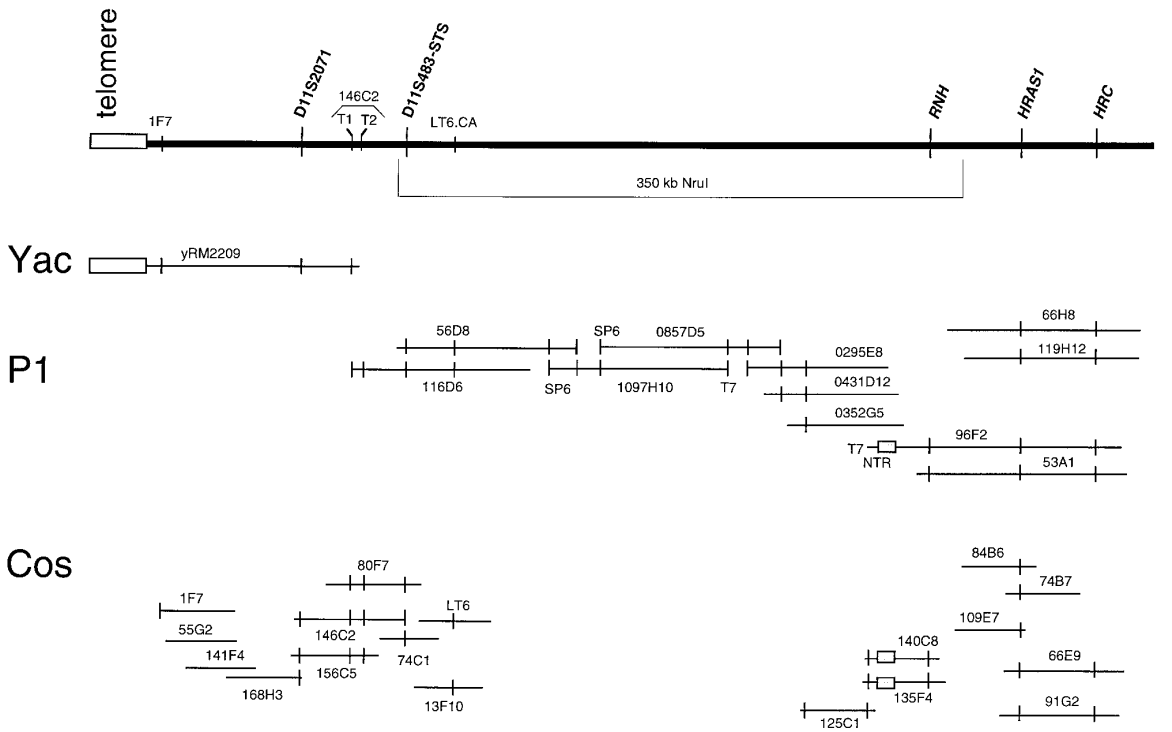

FIG. 1. The contiguous map of the 11p telomeric region. (Top) The representation of the 11p telomeric region from the telomere to the *HRC* gene. YAC, P1, and cosmid (Cos) clones that are positive for each marker are represented by a vertical line directly below the marker. The end of the P1 clones are indicated as SP6 or T7 based on the vector primer sequence. NTR refers to the 30-bp tandem repeats that are present at the T7 end of the 96F2 clone. The P1s 0295E8, 0431D12, and 0352G5 have not been tested for the presence of this repeat or the 125T7 STS.

D11S2071–146C2.T1–146C2.T2–LT6.CA–HRAS1–centromere.

Interphase FISH was used to confirm the location of one of the cosmids, which contained a CA repeat. Based on two-color FISH analysis with cosmid probes for cosmid LT6, tyrosine hydroxylase, and *HRAS1*, it was determined that LT6 was telomeric to *HRAS1* (data not shown). This cosmid was later joined to the telomere cosmids isolated by the yRM2209 probe, using STS mapping and end probe hybridizations to cosmid "walk."

DISCUSSION

This contig creates a physical cap to the p arm of chromosome 11. YAC-based mapping efforts on chromosome 11p, at the University of Buffalo, have created an extensive physical map of chromosome 11p (Internet site: http://www.shows.med.buffalo.edu/). However, perhaps due to the instability of the telomere and the *HRAS1* region in YAC vectors (unpublished observations), this mapping effort has not yet completed contiguous coverage of the *HRAS1* region. The most distal marker physically mapped by the chromosome 11 Genome Center at the University of Texas Southwestern Medical Center is D11S922 (Internet site: http://www.mcdermott.swmed.edu/). The nearest anchored marker on the recently published physical map of the human genome is D11S1318 (Hudson *et al.*, 1995; Internet site: http://www-genome.wi.mit.edu/). This marker is 3–6 cM proximal to the *HRAS1* gene.

The terminal portion of 11p may be of particular importance due to the density of CpG islands near the *HRAS1* gene (Weitzel *et al.*, 1992). Recently, Thäte *et al.* (1995) isolated several CpG island clones for chromosome 11p. Two of these clones are contained in cosmids (152C2 and 12F7) that map to the interval covered by our contig but have not been further sublocalized (data not shown). This suggests that there may be several genes in this region in addition to *HRAS1*, *RNH*, and *HRC*. Further studies using this set of physical reagents can now be used to search for novel transcripts.

The structure of the 11p telomeric region is similar to the 1q (Negorev *et al.*, 1994), 2q (Macina *et al.*, 1994), 16p (Wilkie *et al.*, 1991), and Xq/Yq telomeric regions (Kvaloy *et al.*, 1994). The terminal 50–80 kb appears to contain a high concentration of low-copy repeats. A region approximately 100 kb from the 11p telomere contains three CA repeats, D11S2071, LT6.CA, and a nonpolymorphic repeat; a very complex series of tetranucleotide repeats that includes 146C2.T1 and 146C2.T2; and a polymorphism near the D11S483 STS that is detected by the probe cCI-330 (Tokino *et al.*, 1991). A similar stretch of highly polymorphic DNA has not yet been described for other telomeres but would not be unexpected due to the repetitive nature of subtelomeric DNA and the high frequency of recom-

TABLE 3

STSs Used to Contstruct the 11p Telomeric Contig

STS	Sequence	Annealing temp. (°C)	Product size
13T7	5′ GAACGGTTGTGGGGCCTGGAGTG	60	142
	5′ GGGACCACAAGGTCATCTGTTAGTG		
5835SP6	5′ TTGTAGAATATCACTCAGTTTGTGTT	48	64
	5′ GGCCATAACCTCGTTCCA		
5835T7	5′ GTTCTTGACCCCCATCCGTCTTG	62	97
	5′ CGCAGGAGCTTCCAGAGTGAGAGG		
5162T7	5′ TGCTTCCCACAAGGCTACAGGTT	58	95
	5′ GCTCCACCATCTGCCCTCTACTTATT		
5162SP6	5′ CCTGGGATGGGGGCTCTCACTCT	62	76
	5′ CACCGCGCCACGTCTTCTTCAG		
4217T7	5′ TACAACGCCACCGGTTCCTCA	60	84
	5′ AGGCGGAGTCCCCACAGTGAC		
4217SP6	5′ GCTGCCCACCCCACGATTCC	60	77
	5′ TAGCATACCGCCTGACCCTGACA		
4219T7	5′ TTCAATCCCAAAAACCTACC	60	136
	5′ AGCCCATCTCCCTGTGACC		
56D8.SP6	5′ TGGGATTACAGGCGTGAGC	52	97
	5′ GAGAGGGAAAGGCAGCATAC		
56D8.T7	5′ TCCGTCCCCATTAAACAGCAACT	54	99
	5′ ATTAGAACAGCAAATCCGTAGAGACT		
125T3	5′ CTAAGTGAGAGGAGCCAGTTGAG	52	87
	5′ ACTACCACATCCATATTGCCTCTT		
125T7	5′ GGGTCTGGCCAAGCCTGAGG	60	75
	5′ AGTGCTCCCGTGACCCATCTG		
HRAS1	5′ TGGGTGGCTTCAACAGGA	60	405
	5′ CCAGCCCCGGGAAGACCAGATG		
HRC	5′ GGCCCCCATGACGCAGAA	58	458
	5′ TCGGGCAGTTTATTGAGGT		

bination that has been observed near the telomere. In this regard, it is notable that although D11S2071 is within 400 kb of *HRAS1*, the maximum likelihood recombination fractions between these two markers were 0.05 in males and 0.00 in females (Browne *et al.*, 1995). Another interesting feature of the 11p telomere is a region approximately 150 kb from the telomere that appears by hybridization to the cosmid and P1 libraries to be repeated elsewhere on chromosome 11 (unpublished observations). Like the subtelomeric repeated unit from 2q (Macina *et al.*, 1994), this region may contain a CpG island but the existence of a repeated unit and the precise locations of the CpG islands in this contig will need to be confirmed by additional studies.

The completed contig reflects the transition from hybridization and fingerprint-based mapping, which marked the initial stages of the project, to STS-based P1 walking. The STS-based walking approach proved to be more rapid and reliable, particularly for the interval between D11S483 and *RNH*, which contains a repeated element that made hybridization screening problematic. One of the major obstacles to this approach has been the difficulty preparing P1 DNA for sequencing. In this study, we used a proteinase K preparation of the P1 for sequencing. This proved to be a technically simple and rapid method that allowed us to construct an STS from the end of every P1 used in this study. Once the contig is established, DNA can be prepared from a minimal set of P1s and used to isolate cosmids or used directly in transcript identification studies such as direct selection (Morgan *et al.*, 1992) or exon trapping (Buckler *et al.*, 1991).

The contig spans 500–600 kb based on the sizes of the cosmid contigs and the number of nonoverlapping P1s. D11S483 is on the same cosmid as the centromeric extent of yRM2209, which contains approximately 100–120 kb of telomeric 11p DNA. *RNH* is within two cosmids of *HRAS1*, and *RNH* and D11S483 are separated by three nonoverlapping P1s. This size is consistent with previous pulsed-field mapping studies that determined that D11S483 and *RNH* are present on the same 350-kb *Nru*I fragment and that *RNH* is within 90 kb of *HRAS1* (Schneider *et al.*, 1992). Our contig is also consistent with long-range restriction mapping studies that placed *RNH* and D11S483 telomeric to *HRAS1*. We were unable to place *DRD4* on this contig, suggesting that *DRD4* is centromeric to *HRAS1* as proposed by recombination analysis (Petronis *et al.*, 1993).

Contig closure from the cosmid 1F7 to *HRAS1* has been verified using STS content mapping. We cannot exclude the possibility that the YAC yRM2209 has a small internal deletion telomeric to 1F7 (Fig. 1) but centromeric to the telomere (since this YAC requires a functional human telomere). However, as previously mentioned, the size of the contig is consistent with previous estimates on the distance from *HRAS1*

to the telomere, and we therefore expect that if there were a deletion in yRM2209, it would be relatively small.

Efforts to complete a physical map of chromosome 11p can now be focused on the region centromeric to *HRAS1*. Included in our contig are three new polymorphisms, which, combined with D11S2071, will aid the evaluation of the 11p telomere as a candidate region for human disease genes in this region, including the tumor suppressor locus described by Bepler and GarciaBlanco (1994). Furthermore, the physical mapping reagents gathered during the course of this study (1) can be used to isolate novel transcripts that may have implications for human disease and (2) may aid in the understanding of the structure and function of the telomeric and the subtelomeric regions.

ACKNOWLEDGMENTS

The authors thank Settera Chandrasekharappa and the members of the NCHGR physical mapping core for their assistance with the cosmid and P1 libraries, Michelle Southard-Smith for her advice concerning P1 preparation and sequencing, and Yusuke Nakamura for supplying the cosmid probe, cCI-330. This study could not have been performed without the use of the chromosome 11 cosmid library (LA11NC01), provided by Larry Deaven, Los Alamos National Laboratories. This work was supported by a National Research Service Award (M.W.R.).

REFERENCES

Bepler, G., and Garciablanco, M. A. (1994). Three tumor-suppressor regions on chromosome 11p identified by high-resolution deletion mapping in human non-small-cell lung cancer. *Proc. Natl. Acad. Sci. USA* 91: 5513–5517.

Brown, W. R. A., MacKinnon, P. J., Willasante, A., Spurr, N., Buckle, V. J., and Dobson, M. J. (1990). Structure and polymorphism of human telomere-associated DNA. *Cell* 63: 119–132.

Browne, D. L., Smith, E. A., Dietz-Band, J., Riethman, H. C., Phromchotikul, T., and Litt, M. (1995). Dinucleotide repeat polymorphism at the human chromosome 11p telomere (D11S2071). *Genomics* 25: 600–601.

Buckler, A. J., Chang, D. D., Graw, S. L., Brook, J. D., Haber, D. A., Sharp, P. A., and Housman, D. E. (1991). Exon amplification: A strategy to isolate mammalian genes based on RNA splicing. *Proc. Natl. Acad. Sci. USA* 88: 4005–4009.

Chandrasekharappa, S. C., Marchuk, D. A., and Collins, F. S. (1992). Analysis of yeast artificial chromosome clones. *In* "Methods in Molecular Biology, Pulse Field Gel Electrophoresis" (M. Burmeister and L. Ulanovsky, Eds.), Vol. 12, pp. 235–255. Humana, Totowa, NJ.

Cohen, D., Chumakov, I., Weissenbach, J., *et al.* (1993). A first generation physical map of the human genome. *Nature* 16: 698–701.

Counter, C. M., Hirte, H. W., Bachetti, S., and Harley, C. (1994). Telomerase activity in human ovarian carcinoma. *Proc. Natl. Acad. Sci. USA* 91: 2900–2904.

Flejter, W. L., Bennett-Baker, P., Barcroft, C. L., Kiousis, S., and Chamberlain, J. S. (1995). Region-specific cosmids and STRPs identified by chromosome microdisection and FISH. *Genomics* 25: 413–420.

Harley, C. B., Vaziri, H., Counter, C. M., and Allsopp, R. C. (1992). The telomere hypothesis of cellular aging. *Exp. Gerontol.* 27: 375–382.

Higgins, M. J., Smilinich, N. J., Sait, S., Koenig, A., Pongratz, J., Gessler, M., Richard, C. W., III, James, M. R., Sanford, J. P., Kim, B.-W., Cattlelane, J., Nowak, N. J., Winterpacht, A., Zabel, B. U., Munroe, D. J., Bric, E., Housman, D. E., Jones, C., Nakamura, Y., Gerhard, D. S., and Shows, T. B. (1994). An ordered *Not*I fragment map of human chromosome band 11p15. *Genomics* 23: 211–222.

Hudson, T. J., Stein, L. D., Gerety, S. S., Ma, J., Castle, A. B., *et al.* (1995). An STS-based map of the human genome. *Science* 270: 1945–1954.

Ijdo, J. W., Lindsay, E. A., Wells, R. A., and Baldini, A. (1992). Multiple variants in subtelomeric regions of normal karyotypes. *Genomics* 14: 1019–1025.

Kvaloy, K. F., Galvagni, F., and Brown, W. R. A. (1994). The sequence organization of the long arm pseudoautosomal region of human sex chromosomes. *Hum. Mol. Genet.* 3: 771–778.

Liu, P., Siciliano, J., Seong, D., Craig, J., Zhao, Y., deJong, P. J., and Siciliano, M. J. (1993). Dual Alu polymerase chain reaction primers and conditions for isolation of human chromosome painting probes from hybrid cells. *Cancer Genet. Cytogenet.* 65: 93–99.

Macina, R. A., Negorev, D. G., Spais, C., Ruthig, L. A., Hu, X.-L., and Riethman, H. C. (1994). Sequence organization of the human chromosome 2q telomere. *Hum. Mol. Genet.* 3: 1847–1853.

Morgan, J. G., Dolganov, G. M., Robbins, S. E., Hinton, L. M., and Lovett, M. (1992). The selective isolation of novel cDNAs encoded by the regions surrounding the human Interleukin 4 and 5 genes. *Nucleic Acids Res.* 20: 5173–5179.

Negorev, D. G., Macina, R. A., Spais, C., Ruthig, L. A., Hu, X.-L., and Riethman, H. C. (1994). Physical analysis of the terminal 270 kb of DNA from human chromosome 1q. *Genomics* 22: 569–578.

Petronis, A., Van Tol, H. H. M., Lichter, J. B., Livak, K. J., and Kennedy, J. L. (1993). The D4 dopamine receptor gene maps 11p proximal to HRAS. *Genomics* 18: 161–163.

Redeker, E., Hoovers, J. M. N., Alders, M., van Moorsel, C. J. A., Ivens, A. C., Gregory, S., Kalikin, L., Bliek, J., de Galan, L., van den Bogaard, R., Visser, J., van der Voort, R., Feinberg, A. P., Little, P. F. R., Westerveld, A., and Mannens, M. (1994). An integrated physical map of 210 markers assigned to the short arm of human chromosome 11. *Genomics* 21: 538–550.

Riethman, H. C., Moyzis, R. K., Meyne, J., Burke, D. T., and Olson, M. V. (1989). Cloning human telomeric DNA fragments into *Saccharomyces cerevisiae* using a yeast-artificial-chromosome vector. *Proc. Natl. Acad. Sci. USA* 86: 6240–6244.

Saccone, S., Desario, A., Dellavalle, G., and Bernardi, G. (1992). The highest gene concentrations in the human genome are in the telomeric bands of metaphase chromosomes. *Proc. Natl. Acad. Sci. USA* 89: 4913–4917.

Sambrook, J., Fritsch, E. F., and Maniatis, T. (1989). "Molecular Cloning: A Laboratory Manual," 2nd ed., Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.

Schneider, R., Higgins, M. J., Kieninger, D., Schneider-Scherzer, E., Hirsch-Kauffmann, M., Schweiger, M., Eddy, R. L., Shows, T. B., and Zabel, B. U. (1992). The ribonuclease/angiogenin inhibitor is encoded by a gene mapped to chromosome 11p15.5, within 90 kb of the HRAS protooncogene. *Cytogenet. Cell Genet.* 59: 264–267.

Shepard, N. S., Pfrogner, B. D., Coulby, J. N., Ackerman, S. L., Vaidyanathan, G., Sauer, R. H., Balkenhol, T. C., and Sternberg, N. (1994). Preparation and screening of an arrayed human genomic library generated with the P1 cloning system. *Proc. Natl. Acad. Sci. USA* 91: 2629–2633.

Smith, M. W., Clark, S. P., Hutchinson, J. S., Wei, Y. H., Churukian, A. C., Daniels, L. B., Diggle, K. L., Gen, M. W., Romo, A. J., Lin, Y., Selleri, L., McElligott, D. L., and Evans, G. A. (1993). A sequence-tagged site map of human chromosome 11. *Genomics* 17: 699–725.

Southard-Smith, M., Pierce, J. C., and MacDonald, R. J. (1994). Physical mapping of the rat tissue kallikrein family in two gene clusters by analysis of P1 bacteriophage clones. *Genomics* 22: 404–417.

Thäte, C., Pongratz, J., Konig, A., Klamt, B., Tsaoussidou, S., Higgins, M., Shows, T., Jones, C., and Gessler, M. (1995). CpG island

clones for chromosome 11p—A resource for mapping and gene identification. *Mamm. Genome* 6: 421–425.

Tokino, T., Takahashi, E.-i., Masaki, M., Tanigami, A., Glaser, T., Park, J. W., Jones, C., Hori, T.-a., and Nakamura, Y. (1991). Isolation and mapping of 62 new RFLP markers on human chromosome 11. *Am. J. Hum. Genet.* 48: 258–268.

Wang, Q., and Keating, M. (1994). Isolation of P1 insert ends by direct sequencing. *BioTechniques* 17: 282–284.

Weitzel, J. N., Kasperczyk, A., Mohan, C., and Krontiris, T. G. (1992). The HRAS1 gene cluster: Two upstream regions recognizing transcripts and a third encoding a gene with a leucine zipper domain. *Genomics* 14: 309–319.

Wilkie, A. O. M., Higgs, D. R., Rack, K. A., Buckle, V. J., Spurr, N. K., Fischel-Ghodsian, N., Ceccherini, I., Brown, W. R. A., and Harris, P. C. (1991). Stable length polymorphism of up to 260 kb at the tip of the short arm of chromosome 16. *Cell* 64: 595–606.

Zakian, V. A. (1995). Telomeres: Beginning to understand the end. *Science* 270: 1601–1606.

Zhong, Z., Shiue, L., Kaplan, S., and de Lange, T. (1992). A mammalian factor that binds telomeric TTAGGG repeats in vitro. *Mol. Cell. Biol.* 12: 4834–4843.