

*Original investigations***An alphoid DNA sequence conserved in all human and great ape chromosomes: evidence for ancient centromeric sequences at human chromosomal regions 2q21 and 9q13****Antonio Baldini^{1,2,3}, Thomas Ried¹, Viji Shridhar⁴, Keiko Ogura¹, Leonardo D'Aiuto⁵, Mariano Rocchi⁶, David C. Ward¹**¹ Department of Human Genetics, Yale University School of Medicine, 333 Cedar Street, New Haven, CT 06510, USA² CNR Institute of Molecular Genetics, Porto Conte Research and Training Laboratories, Alghero, Italy³ Imperial Cancer Research Fund, 44 Lincoln's Inn Fields, London WC2A 3PX, UK⁴ Department of Molecular Biology and Genetics, Wayne State University School of Medicine, Detroit, Michigan, USA⁵ Istituto di Anatomia Umana Normale, Università di Modena, Modena, Italy⁶ Istituto di Genetica, Università di Bari, Bari, Italy

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Abstract. Using vector-CENP-B box polymerase chain reaction (PCR) we isolated and cloned from a human chromosome 21-specific plasmid library, a 1 kb DNA sequence, named α H21. In *in situ* hybridization experiments, α H21 hybridized, under high stringency conditions, to the centromeric region of all the human, chimpanzee, gorilla and orangutan chromosomes. On human chromosomes α H21 also identified non-centromeric sequences at 2q21 (locus D2F33S1) and 9q13 (locus D9F33S2). The possible derivation of these sequences from ancestral centromeres is discussed. Sequence analysis confirmed the alphoid nature of the whole α H21 insert.

Introduction

The centromeric region of primate chromosomes contains long arrays of tandemly repeated DNA sequences, referred to as alpha satellite DNA or alphoid DNA (Maio 1971; Kurnit and Maio 1973; Manuelidis 1978; Rosenberg et al. 1978; Willard and Waye 1987). The common feature of this DNA is the length of the repeat unit, which is about 170 bp. Alphoid repeats have been studied widely, especially in human, where subsets highly specific for a single chromosome or shared by several chromosomes have been documented. Other subsets are distinguishable from each other using very high stringency conditions of hybridization or by restriction analysis (see Choo et al. 1991 for review). A human alphoid

DNA sequence that hybridizes to every human and great ape centromere under fairly high stringency conditions, has also been reported (clone p82H, Mitchell et al. 1985; Alexandre et al. 1987; Miller et al. 1988). Chromosome-specific organization and sequence conservation has been shown for a single gorilla- and a single chimpanzee-derived alphoid DNA sequence (Durfy and Willard 1990; Baldini et al. 1991). However, the evolutionary studies on alpha satellite DNA among human and great apes have not yet collected enough data to draw general conclusions. The highest sequence similarity so far reported between human and great ape alphoid sequences is 91% (Baldini et al. 1991), much lower than the expected similarity for selectively neutral sequences (see Goodman et al. 1989 for review), suggesting that this DNA is rapidly changing. However, sequence comparisons between tandem repeats from different species are of limited value owing to obvious difficulties in identifying orthologous DNA segments. No function has so far been assigned to the alpha satellite DNA. It contains a 17-bp protein binding domain, referred to as the "CENP-B box" owing to its ability to bind the CENP-B protein (reviews in Pluta et al. 1990; Rattner 1991). The CENP-B box is also present in the mouse minor satellite (Wong and Rattner 1988), which is located at the centromere of mouse chromosomes but is otherwise unrelated to the alpha satellite DNA. We used the CENP-B box sequence as a primer, in combination with a vector-specific primer, to amplify alpha satellite sequences from the DNA of a chromosome-specific library, using the polymerase chain reaction (PCR). With this method, we isolated and cloned a 1-kb alphoid DNA sequence, named α H21, that hybridized, *in situ*, to the centromeric region of all the human and great ape chromosomes. Further, α H21 identified non-centromeric sequences on human chromosomes that could be the remnants of ancestral centromeres.

GenBank accession number, M64321

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Materials and methods

Polymerase chain reaction

The chromosome 21-specific plasmid library, pBS21A, was kindly provided by J. Gray (University of California, San Francisco). It consists of HindIII fragments derived from flow sorted chromosomes 21, ligated in the HindIII site of the Bluescribe plasmid vector (Collins et al. 1991). These inserts can be amplified by PCR using standard primers hybridizing to the T3 and T7 promoters located at the two extremities of the vector polylinker sequence.

PCR reactions were performed using 100 ng of pBS21A and primer pairs *NotI*- α and T3 or *NotI*- α and T7. The *NotI*- α sequence, 5'-GCCGCGCCCTTCGTTGGAAACGGGA3', contains 5' to the 17-mer CENP-B box the *NotI* recognition sequence (italicized) for other cloning purposes. The T3 and T7 primer sequences were 5'ATTAAACCCTCACTAAAG3' and 5'AATACGACTCACTATAG3', respectively. Each primer was used at a final concentration of 0.5 μ M. PCR mixtures contained 1.5 mM MgCl₂, 10 mM TRIS-HCl, 50 mM KCl, 0.001% gelatin, 130 μ M each of the four dNTPs and 2 U of *Taq* polymerase (Perkin Elmer/Cetus). After initial denaturation at 95°C for 3 min, 32 cycles of PCR were carried out with denaturation at 94°C for 1 min, annealing at 55°C for 1 min and extension at 72°C for 4 min (last cycle 7 min). The thermocycling was performed using a commercially available automatic machine (Ericomp). The products were ethanol precipitated in the presence of 2 M ammonium acetate and resuspended in TE (10 mM TRIS, 1 mM EDTA).

PCR products were isolated from low melting temperature agarose. A 1-kb fragment amplified by the T7-*NotI*- α primers was cloned using a commercially available kit (TA cloning system, Invitrogen) and the clone was named α H21. The kit is based on the non-template dependent activity of *Taq* polymerase, which adds single deoxyadenosines to the 3' end of duplex molecules produced by PCR, creating A-overhangs. The products are ligated to a linearized plasmid vector (named pCR1000) that has T-overhangs.

DNA sequencing

The nucleotide sequence of double-stranded DNA was determined with the dideoxy method using T7 DNA polymerase (Pharmacia) following the procedure suggested by the manufacturer. [³⁵S]-dATP was the labeled deoxynucleotide. M13 forward and reverse primers were used to sequence the inserts in the plasmid vectors. Two *HincII* fragments were subcloned from α H21 in order to obtain the entire sequence. Sequence data were handled using the University of Wisconsin Genetics Computer Group (UWCGC) software package (Devereux et al. 1984) and compared with the sequences recorded in the GenBank (Release 70.0, 12/91) and EMBL (Release 29.0, 12/91) databases using previously described algorithms (Wilbur and Lipman 1983).

In situ hybridization

Standard metaphase spreads were obtained from peripheral blood lymphocytes of a human male donor and from lymphoblastoid cell lines from gorilla (*Gorilla gorilla*, individual Machi, female), chimpanzee (*Pan troglodytes*, individual Tank, male) and orangutan (*Pongo pygmaeus*, individual Jari, female). The primate cell lines were derived by D. A. Lawlor (Stanford, Calif.) from blood samples provided by O. R. Ryder (San Diego, Calif.). Probes were labeled with biotin-11-dUTP or digoxigenin-11-dUTP using nick translation. Probe labeling and fluorescence in situ hybridization were performed essentially as described by Lichter et al. (1990). Three post-hybridization washes at 42°C in 2 \times SSC 50%, formamide were followed by three washes in 0.1 \times SSC at 60°C. Chromosome identification was based on in situ hybridization banding using digoxigenin-11-dUTP (Boehringer Mannheim) labeled Alu-PCR products as a co-hybridization probe (about 2 μ g/ml). This produces an R-banding pattern suitable for gene mapping studies (Baldini and Ward 1991). Biotin-labeled DNA was detected using fluorescein isothiocyanate (FITC)-conjugated avidin DCS (5 μ g/ml) (Vector Laboratories); digoxigenin-labeled DNA was detected using a rhodamine-conjugated anti-digoxigenin antibody (Boehringer Mannheim). Ten to 20 metaphase spreads were analysed per species. In some cases

chromosome identification was performed by simultaneous 4',6-diamidino-2-phenylindole (DAPI) staining, which produces a Q-banding pattern. The nomenclature and presumptive homologies of great ape chromosomes used in this paper are according to the International System for Human Cytogenetic Nomenclature (ISCN 1985).

Digital images were obtained using a cooled charge-coupled device (CCD) camera (Photometrics) as previously described (Baldini and Ward 1991).

Southern blot analysis

Southern blot analysis of genomic DNA of human and somatic cell hybrids was performed as described (Baldini et al. 1992). Hybridization was carried out in 50% formamide, 4 \times SSC, 5 \times Denhardt's solution, 0.5% SDS, 10 mM EDTA, pH 8 and 100 μ g/ml sonicated herring sperm DNA at 42°C for 16–18 h. Post-hybridization wash conditions were 65°C in 1 \times SSC, 0.1% SDS, twice for 15 min. Filters containing DNA from somatic cell hybrids were washed under higher stringency conditions, i.e., at 65°C in 0.1 \times SSC, 0.1% SDS, twice for 15 min.

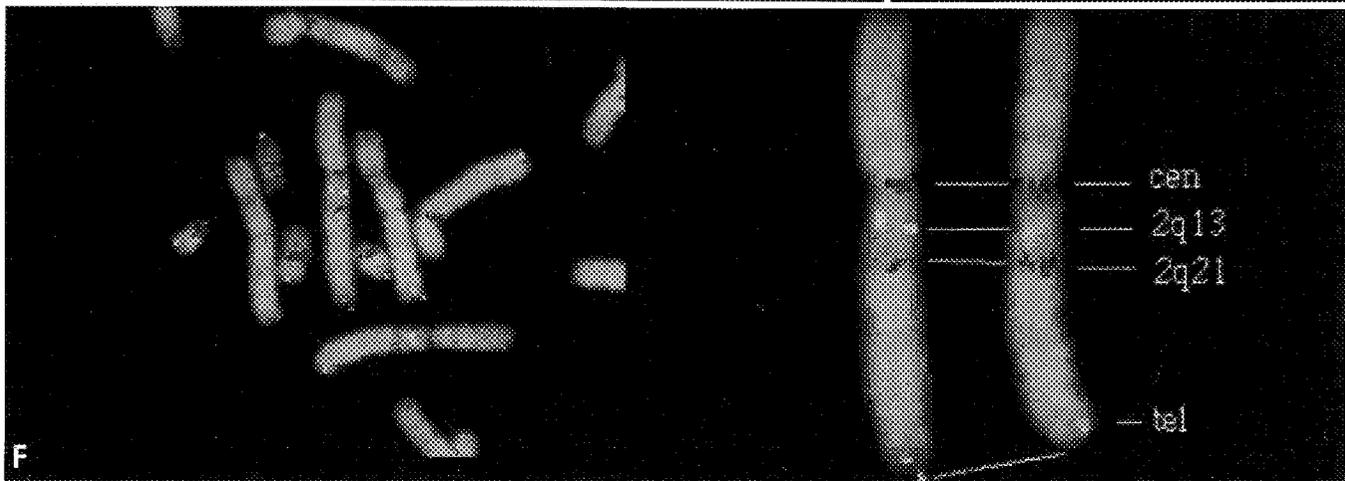
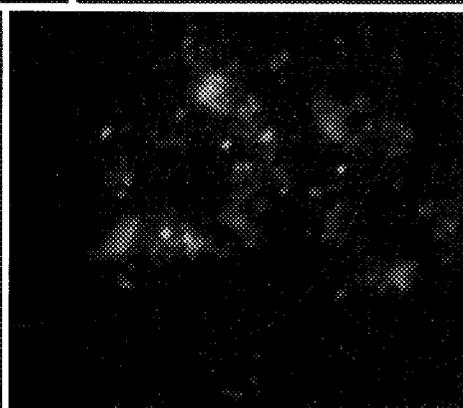
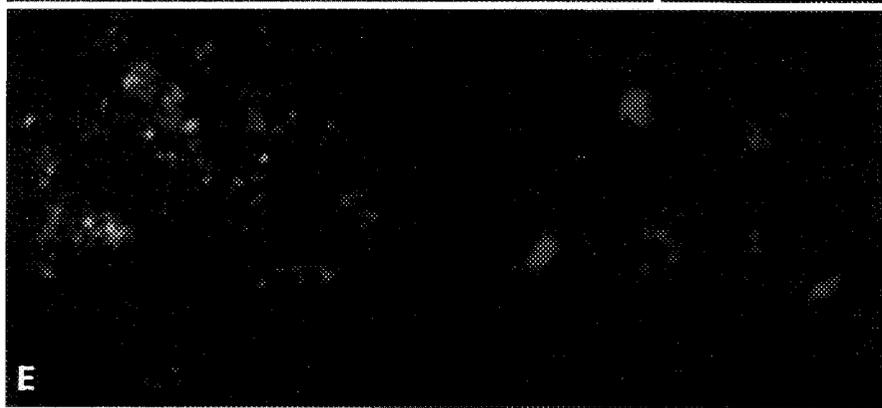
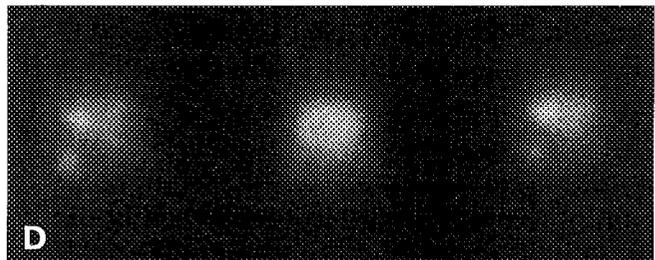
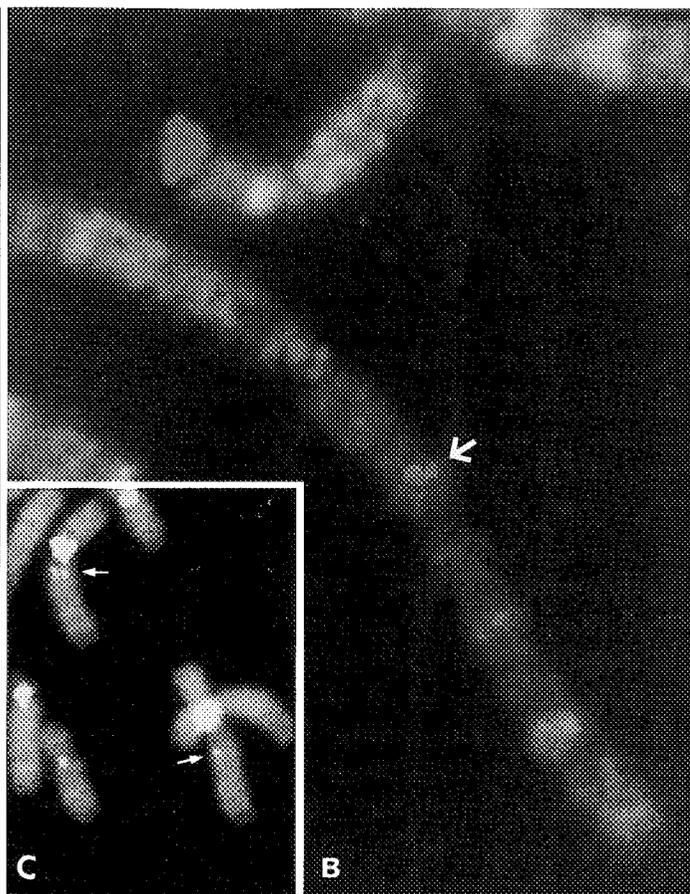
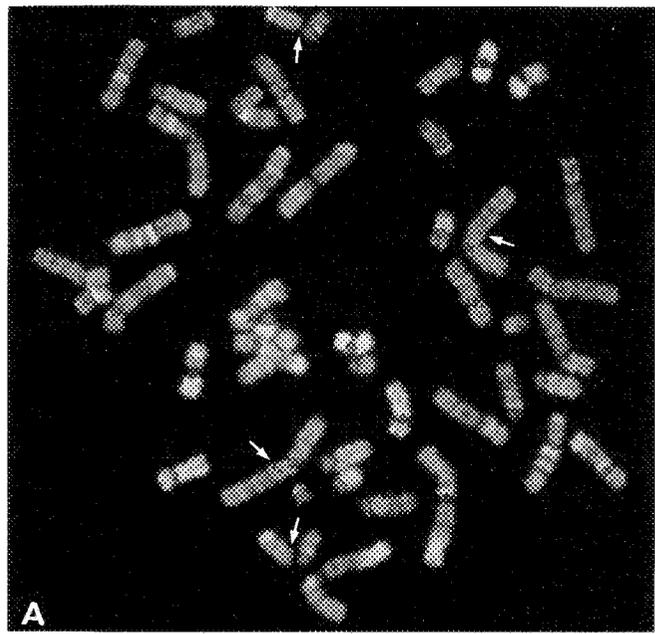
Results

Isolation and sequence analysis of α H21

Vector-CENP-B box PCR amplification of the chromosome 21-specific plasmid library led to products ranging from 1 kb to about 200 bp in size (data not shown). These amplified sequences, isolated as separate bands from a gel, hybridized in situ to chromosome 21, but none of them showed chromosome 21 specificity. However, all of them were located at the centromere as expected for alphoid DNA sequences. The 1-kb amplified fragment was cloned as described (see Materials and methods) and further characterized. It was chosen because it was the longest amplified fragment.

The sequence of the α H21 insert (recorded in the GenBank/EMBL databases under the accession number M64321) revealed the alpha satellite nature of the whole clone. The sequence shows the characteristic alphoid structure composed of ~171-bp repeats and aligns well with alphoid DNA consensus sequences (Waye and Willard 1987; Choo et al. 1991). Sequence database searches revealed that the most

Fig. 1A–F. Fluorescence in situ hybridization of α H21 (shown in red; yellow in C, D and E) to human chromosomes (in yellow-green; blue in C and F). The digital images (see Materials and methods) were pseudocolored to distinguish the different fluorochromes. Photographs were taken from the computer screen. **A** Chromosome spread from a human male donor; arrows indicate the non-centromeric hybridization sites. Chromosomes were R-banded by in situ hybridization banding (see Materials and methods). **B** Human chromosome 2. The arrow indicates the extracentromeric signal at 2q21. **C** Extracentromeric signals at 9q13. Chromosomes were counterstained with 4',6-diamidino-2-phenylindole (DAPI). **D** Hybridization of alpha satellite centromeric repeats at the centromeric region of chromosome 3. *Left*, α H21; *center*, pAE0.68 (identifying the locus D3Z1); *on the right*, the two signals are merged. Note the different morphology of the two signals. **E** A lymphocyte nucleus hybridized with α H21 (on the left, in yellow) and with pC1.8 (in the center, in red; this is an alpha satellite DNA clone hybridizing to the centromere of chromosomes 1, 5 and 19). On the right, the two images are merged. The α H21 signal is more grainy and dispersed than the pC1.8 signal. **F** *Left* detail of a metaphase spread hybridized with α H21 (in red) and with c8.1 (in yellow, identifying the fusion point of the two ancestral chromosomes that formed human chromosome 2; c8.1 also hybridizes to several telomeric sites). *On the right*, karyotyped and magnified chromosomes 2 are shown (chromosomes were counterstained with DAPI).



similar sequence present in the GenBank and EMBL databases is that of the clone pTRA7 (Vissel and Choo 1991; 82% sequence similarity).

In situ hybridization of p α H21

Clone p α H21 was hybridized in situ to banded human, chimpanzee, gorilla and orangutan chromosomes under high stringency conditions (see Materials and methods). On human chromosomes, p α H21 hybridized to every centromere as well as to bands 2q21 and 9q13 (Fig. 1A–C). The intensity of the hybridization signals at the non-centromeric loci was lower than in the centromeric regions. In situ hybridization experiments were also performed using two subclones of p α H21. The signals had the same distributions but were of lower intensity (data not shown). In situ hybridization experiments under the same conditions were also performed using the probe pTRA7 (Vissel and Choo 1991) whose sequence is similar to that of p α H21 (see above, sequence analysis). The hybridization signal had a different chromosomal distribution and did not detect extracentromeric loci (data not shown).

On human chromosome 3, the hybridization signal often showed a characteristic morphology, i.e., discrete, punctate signals at the periphery of the centromere (Fig. 1D, on the left). In contrast, the hybridization signal of a chromosome 3-specific alphoid DNA clone appears more compact and centrally located (Fig. 1C, in the center, same chromosome hybridized with clone pAE0.68 identifying the locus D3Z1; Baldini et al. 1989a). The two signals are superimposed on the right-hand side of Fig. 1D. These data suggest that on this chromosome, p α H21-like sequences are located peripherally compared with the tandem arrays of chromosome-specific alpha satellite repeats. In interphase nuclei the p α H21 hybridization signals showed different degree of condensation, often appearing as grainy and dispersed. Figure 1E shows a nucleus hybridized in a dual labeling experiment with p α H21 (Fig. 1E, on the left, shown in yellow) and the multi-loci alphoid DNA clone pC1.8 (hybridizing to the centromere of chromosomes 1, 5 and 19; Baldini et al. 1989b) (Fig. 1E, in the center, shown in red). On the right hand side of Fig. 1E the two images are superimposed. There is a clear difference in appearance between the signals from the two probes, p α H21 being more grainy and dispersed. The signal from pC1.8 appears more compact and, therefore, more typical of long arrays of tandem repeats.

On chimpanzee, gorilla and orangutan chromosomes, p α H21 hybridized to every centromere but no extracentromeric location was apparent (data not shown). Because the gorilla and orangutan individuals that we studied were both female, we do not know whether p α H21 hybridizes to the Y chromosome in these species; lymphocyte lines from males were not available for study.

Southern blot analysis

p α H21 was hybridized to a Southern blot containing human genomic DNA cut with *Eco*RI, *Stu*I, *Hin*FI, *Hind*III, *Bam*HI, *Pst*I and *Bcl*I (Fig. 2). Results show a complex pattern with no indication of a higher order repeat. However, bands of 2.7,

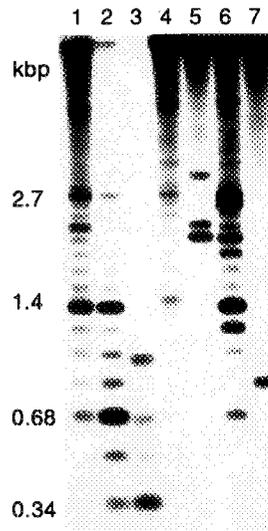


Fig. 2. Autoradiograph of a Southern blot containing human genomic DNA cut with: lane 1, *Eco*RI; lane 2, *Stu*I; lane 3, *Hin*FI; lane 4, *Hind*III; lane 5, *Bam*HI; lane 6, *Pst*I and lane 7, *Bcl*I. The filter was hybridized with p α H21 under the stringency conditions described (see Materials and Methods)

1.4 and 0.68 kb are predominant in *Eco*RI, *Stu*I and *Pst*I digested DNA (Fig. 2, lanes 1, 2 and 6) suggesting a basic tetrameric organization.

In order to determine whether sequences detected by p α H21 are highly conserved in different chromosomes, we performed a Southern blot analysis under higher stringency conditions (see Materials and methods) using a standard mapping panel of human-hamster somatic cell hybrids (Rocchi et al. 1986). Higher stringency conditions were not tested on in situ hybridization experiments because they would strongly reduce the efficiency of signal detection. The results of the hybridization to the somatic hybrids panel are shown in Fig. 3 where lanes 1–18 contain DNA from hybrids, lane Hu contains total human genomic DNA and lane CHO, hamster DNA. DNA was cut with *Eco*RI. The chromosome content of each hybrids is listed in Table 1. Two major bands are present in the human genomic DNA, one of about 2.7 kb and the other of about 1.4 kb. The two bands are not always present in the same hybrid indicating that they are present in different chromosomal subsets. The 1.4-kb band is 100% concordant with the presence of chromosome 18. The 2.7-kb band could not be assigned to a specific chromosome suggesting that this fragment is present in multiple chromosomes. However, the hybridization signal in certain hybrids is very weak or absent (lanes 6, 7 and 12). This suggests that in some chromosomes p α H21-like sequences may be present as a single copy. In other chromosomes p α H21-like sequences, although detected by in situ hybridization, are not detected by Southern blot analysis (performed under higher stringency conditions), probably due to slight sequence divergence from the probe.

p α H21 is a PCR clone and therefore its sequence may not reproduce exactly the genomic sequence. It is known that *Taq* DNA polymerase lacks proof-reading activity. However, our clone is unlikely to be an artifact because: (a) its sequence completely aligns with alpha satellite consensus se-

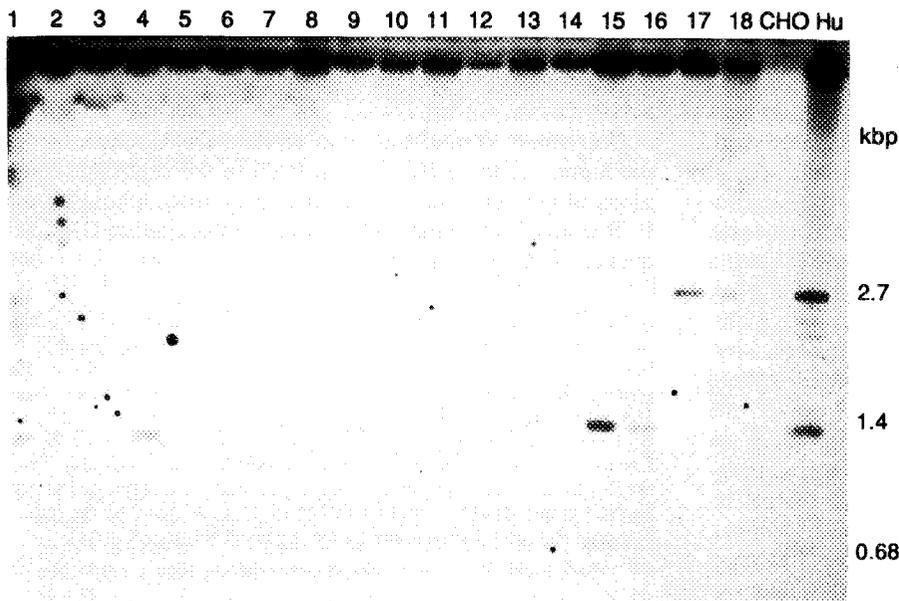


Fig. 3. Autoradiograph of a Southern blot containing *EcoRI*-cut genomic DNA from a standard mapping panel of human-hamster somatic cell hybrids (Rocchi et al. 1986) (lanes 1-18) as well as total human genomic DNA (lane Hu) and hamster DNA (lane CHO). Experiments were performed under high stringency conditions (see Materials and methods). The chromosome content of each hybrid is listed in Table 1

Table 1. Chromosome content of hamster-human somatic cell hybrids of the panel used in Fig. 3. This panel has been extensively used for gene mapping studies (Rocchi et al. 1986). Lane numbers are referred to in the autoradiograph shown in Fig. 3

Chromosome	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
1	-	-	-	-	-	-	-	-	-	-	-	+	-	-	+	+	-	-
2	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-
3	-	-	-	-	-	-	-	+	-	+	-	-	-	-	+	-	+	-
4	-	-	-	-	-	-	-	-	+	-	-	-	-	-	+	-	+	-
5	-	+	+	+	-	+	-	+	-	-	-	-	+	-	-	-	+	-
6	-	-	-	+	+	-	+	-	+	-	-	+	-	-	+	-	+	+
7	-	-	-	-	-	-	+	-	+	-	-	-	+	+	-	-	+	-
8	-	-	+	+	-	-	+	-	-	-	-	-	+	-	+	-	-	-
9	-	-	-	-	-	+	-	-	-	-	-	-	+	+	-	-	-	-
10	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	+	+
11	-	-	+	-	-	-	-	+	-	-	-	-	-	-	+	+	+	+
12	+	+	+	-	-	+	-	-	-	-	+	+	-	-	+	+	+	-
13	+	-	-	+	+	-	-	-	-	+	-	-	-	+	-	-	-	-
14	+	+	+	+	-	-	-	+	+	-	-	-	-	-	+	+	-	-
15	+	-	-	-	+	-	-	-	-	-	-	+	+	-	+	-	-	-
16	-	-	-	-	+	-	+	-	-	-	-	-	-	-	-	-	+	+
17	-	+	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-
18	+	+	-	+	-	-	-	-	+	-	-	-	+	-	+	+	-	-
19	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	-
20	+	-	-	+	-	-	-	-	-	-	-	-	-	-	-	+	-	-
21	-	-	+	-	+	-	-	-	-	+	-	+	+	-	+	-	-	-
22	-	-	-	-	-	-	+	+	+	-	+	-	-	-	+	-	+	-
X	q-	+	+	+	+	+	+	+	+	+	-	-	+	+	+	+	+	-
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18

quences; (b) two different subclones gave identical chromosome distribution in fluorescent in situ hybridization experiments; (c) the clone clearly identifies homologous sequences under high stringency conditions on Southern blot experiments.

Discussion

Alpha satellite DNA has been demonstrated at the centromeric region of every human chromosome. Owing to the divergence of the chromosomal subsets of this repetitive

DNA, a given alphoid clone will preferentially hybridize to a single or to a limited number of chromosome pairs. To date, only one alphoid clone (p82H) has been shown to hybridize to every human and great ape chromosome under fairly high stringency conditions (Mitchell et al. 1985; Aleixandre et al. 1987; Miller et al. 1988). In this paper we describe a second alphoid DNA clone, p α H21, that hybridizes to the centromeric region of every human and great ape chromosome (however, with the caveat that the Y chromosome in gorilla and orangutan could not be evaluated). The high stringency conditions of the in situ hybridization experiments (see Materials and methods) should prevent any non-specific cross-hybridization of p α H21 with the numerous, diverged alphoid DNA subsets that are chromosome specific. However, the p α H21-like sequences located on different chromosomes are likely to be slightly divergent from one another owing to independent evolution. This appears to be true for the p82H-like sequences. In fact sequences identical, or quasi-identical, to the cloned one were only demonstrated at a very low copy number in a single chromosome (Waye et al. 1988). Our experiment of Southern hybridization, under high stringency conditions, to a somatic cell hybrid panel suggests that p α H21 identifies a limited number of sequences located on multiple chromosomes.

p α H21 also hybridizes to the non-centromeric bands 2q21 and 9q13 in human. Human chromosome 2 is derived from the end-to-end fusion of two ancestral chromosomes. The fusion point has been recently identified at 2q13 (Ijdo et al. 1991). The centromere of one of the two chromosomes has been lost or inactivated. Standard cytogenetic techniques, such as C-banding, failed to detect any heterochromatic block on the long arm of human chromosome 2, suggesting that the bulk of the centromeric repetitive DNA has been lost. In this paper we demonstrate centromeric repeats at 2q21 that could be the remains of the ancestral centromere. This is supported by the fact that p α H21 hybridizes at the centromeres of both chromosomes that are the origin of human chromosome 2. In Fig. 1F a dual labeling hybridization with clones p α H21 and c8.1 (identifying the fusion point, Ijdo et al. 1991) shows the relative location of the remnants of the two chromosomal structures. Although definitive demonstration would require further comparative mapping in this region using more specific probes, our data are nevertheless compatible with data obtained by comparative banding analysis (Yunis and Prakash 1982) and add a new element to the delineation of the chromosome 2 phylogeny. No alphoid clone so far reported identifies repeats at 2q21. This could be explained by the relative rarity of p α H21-like sequences compared with chromosome-specific alphoid subsets. However, Bellis et al. (1991) showed that yeast artificial chromosome (YAC) clones from the centromeric region of chromosome 21 hybridized in situ to the same region of chromosome 2 as p α H21. The sequences hybridizing to chromosome 2 are still to be characterized.

Non-centromeric sequences on 9q13 might be due to residual alphoid repeats that persisted after the pericentric inversion that this chromosome underwent during the evolution from the great apes. Alternatively, alphoid sequences could have remained isolated after the amplification and/or generation of the non-alphoid heterochromatic block at 9q11-q12 (absent in the homologous great ape chromosomes). Alphoid

sequences at this region have also been demonstrated using p82H (Aleixandre et al. 1987) but could not be detected by the chromosome 9-specific alphoid clone pMR9A (Rocchi et al. 1991).

Our results demonstrate that alphoid DNA sequences in the human genome are not restricted to the centromeric regions of chromosomes. We also showed that alphoid-vector PCR is an effective method to isolate alpha satellite DNA sequences from genomic libraries.

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References

- Aleixandre C, Miller DA, Mitchell AR, Warburton DA, Gersen SL, Disteche C, Miller OJ (1987) p82H identifies sequences at every human centromere. *Hum Genet* 77:46-50
- Baldini A, Ward D (1991) In situ hybridization banding of human chromosomes with Alu-PCR products: a simultaneous karyotype for gene mapping studies. *Genomics* 9:770-774
- Baldini A, Smith DS, Rocchi M, Miller OJ, Miller DA (1989a) Cloning and analysis of 100 kbp of pericentromeric repetitive (alphoid) DNA from human chromosome 3. *Am J Hum Genet* 45:501
- Baldini A, Smith DS, Rocchi M, Miller OJ, Miller DA (1989b) A human alphoid DNA clone from the EcoRI dimeric family: genomic and internal organization and chromosomal assignment. *Genomics* 5:822-828
- Baldini A, Miller DA, Miller OJ, Ryder OA, Mitchell AR (1991) A chimpanzee derived chromosome-specific alpha satellite DNA sequence conserved between chimpanzee and human. *Chromosoma* 100:156-161
- Baldini A, Archidiacono N, Carbone R, Bolino A, Shridhar V, Miller OJ, Miller DA, Ward DC, Rocchi M (1992) Isolation and comparative mapping of a human-chromosome 20-specific alpha-satellite DNA clone. *Cytogenet Cell Genet* 59:12-16
- Bellis M, Charlieu JP, Carter D, Orti R, Marcias B, Viegaspequignot E, Roizes G (1991) The use of the YAC technology to find new polymorphic markers to study the alzheimers-disease gene and the centromeric region of human chromosome-21. *Am J Hum Genet* 49:366
- Choo KH, Vissel B, Nagy A, Kalitsis P (1991) A survey of the genomic distribution of alpha satellite DNA on all human chromosomes, and derivation of a new consensus sequence. *Nucleic Acids Res* 19:1179-1182
- Collins C, Kuo WL, Segraves R, Fuscoe J, Pinkel D, Gray JW (1991) Construction and characterization of plasmid libraries enriched in sequences from single human chromosomes. *Genomics* 11:997-1006
- Devereux J, Haerberly P, Smithies O (1984) A comprehensive set of sequence analysis programs for the VAX. *Nucleic Acids Res* 12:387-395
- Durfy SJ, Willard HF (1990) Concerted evolution of primate alpha satellite DNA. Evidence for an ancestral sequence shared by gorilla and human X chromosome alpha satellite. *J Mol Biol* 216:555-566
- Goodman M, Koop BF, Czelusniak J, Fitch DHA, Tagle DA, Slightom JL (1989) Molecular phylogeny of the family of apes and humans. *Genome* 31:316-335
- Ijdo JW, Baldini A, Ward DC, Reeders ST, Wells RA (1991) Origin of human chromosome 2 - an ancestral telomere-telomere fusion. *Proc Natl Acad Sci USA* 88:9051-9055
- ISCN (1985) An international system for human cytogenetic nomenclature. Harnden DG, Klinger HP (eds) Published in collaboration with Cytogenet Cell Genet. Karger, Basel New York

- Kurnit DM, Maio JJ (1973) Subnuclear distribution of DNA species in confluent and growing mammalian cells. *Chromosoma* 42:23-36
- Lichter P, Tang Chang C-J, Call K, Hermanson G, Evans GA, Housman D, Ward DC (1990) High-resolution mapping of human chromosome 11 by in situ hybridization with cosmid clones. *Science* 247:64-69
- Maio JJ (1971) DNA strand reassociation and polyribonucleotide binding in the African green monkey, *Cercopithecus aethiops*. *J Mol Biol* 56:579-595
- Manuelidis L (1978) Chromosomal location of complex and simple repeated human DNAs. *Chromosoma* 66:23-32
- Miller DA, Sharma D, Mitchell AR (1988) A human derived probe, p82H, hybridizes to the centromeres of gorilla, chimpanzee, and orangutan. *Chromosoma* 96:270-274
- Mitchell AR, Gosden Jr, Miller DA (1985) A cloned sequence, p82H, of the alphoid repeated DNA family found at the centromeres of all human chromosomes. *Chromosoma* 92:369-377
- Pluta AF, Cooke Ca, Earnshaw WC (1990) Structure of the human centromere at metaphase. *Trends Biochem Sci* 15:181-185
- Rattner JB (1991) The structure of the mammalian centromere. *Bioessays* 13:51-56
- Rocchi M, Roncuzzi L, Santamaria R, Archidiacono N, Dente L, Romeo G (1986) Mapping through somatic cell hybrids and cDNA probes of protein C to chromosome 2, factor X to chromosome 13, and alpha 1-acid glycoprotein to chromosome 9. *Hum Genet* 74:30-33
- Rocchi M, Archidiacono N, Ward DC, Baldini A (1991) A human chromosome 9-specific alphoid DNA repeat spatially resolvable from satellite 3 DNA by fluorescent in situ hybridization. *Genomics* 9:517-523
- Rosenberg H, Singer M, Rosenberg M (1978) Highly reiterated sequences of Simian. *Science* 200:394-402
- Vissel B, Choo KH (1991) Four distinct alpha satellite subfamilies shared by human chromosomes 13, 14 and 21. *Nucleic Acids Res* 19:271-277
- Waye JS, Willard HF (1987) Nucleotide sequence heterogeneity of alpha satellite repetitive DNA: a survey of alphoid sequences from different human chromosomes. *Nucleic Acids Res* 15:7549-7569
- Waye JS, Mitchell AR, Willard HF (1988) Organization and genomic distribution of "82H" alpha satellite DNA. Evidence for a low-copy or single-copy alphoid domain located on human chromosome 14. *Hum Genet* 78:27-32
- Wilbur WJ, Lipman DJ (1983) Rapid similarity searches of nucleic acid and protein data banks. *Proc Natl Acad Sci USA* 80:726-730
- Willard HF, Waye JS (1987) Hierarchical order in chromosome-specific human alpha satellite DNA. *Trends Genet* 3:192-198
- Wong AKC, Rattner JB (1988) Sequence organization and cytological localization of the minor satellite of mouse. *Nucleic Acids Res* 16:11645-11661
- Yunis JJ, Prakash O (1982) The origin of man: a chromosomal pictorial legacy. *Science* 215:1525-1530