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RESEARCH

A Palindromic Structure in the Pericentromeric Region of Various Human Chromosomes

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The primate-specific multisequence family chAB4 is represented with ~40 copies within the haploid human genome. Former analyis revealed that unusually long repetition units (>35 kb) are distributed to at least eight different chromosomal loci. Remarkably varying copy-numbers within the genomes of closely related primate species as well as the existence of human specific subfamilies, which most probably arose by frequent sequence exchanges, demonstrate that chAB4 is an unstable genomic element, at least in an evolutionary sense. To analyze the chAB4 basic unit in more detail we established a cosmid contig and found it to be organized as inverted duplications of ~90 kb flanking a noninverted core sequence of ~60 kb. FISH as well as the analysis of chromosome-specific hybrid cell lines revealed a chromosomal localization of chAB4 on chromosomes 1, 3, 4, 9, Y, and the pericentromeric region of all acrocentrics. Furthermore, we can detect chAB4 sequences together with α satellites, β satellites, and satellite III sequences within a single chromosome 22-specific YAC clone, indicating that chAB4 is located in close proximity to the centromere, at least on the acrocentrics. Hence, chAB4 represents an unstable genomic structure that is located just in the chromosomal region that is very often involved in translocation processes.

A considerable part of the mammalian genome is composed of a large number of repetitive DNA families. Among these, low copy number sequences with long repetition units are clearly distinguished from the others regarding their formation, their contribution to the plasticity of the mammalian genome, and their possible functions. The members of such families often are clustered and arranged as direct repeats like the rRNA genes (Sylvester et al. 1986) or the human globin genes (Shen et al. 1981), or as inverted repeats like the human α-amylase gene family (Groot et al. 1990) or the mouse major urinary protein genes (Bishop et al. 1985). Various models originally proposed for the formation of somatically amplified sequences (Hyrien et al. 1988; Windle and Wahl 1992; Nonet et al. 1993) can also explain the formation of sequence duplications or amplifications in the germ line as reported by Koide et al. (1990). Once they are created the members of such families can exchange DNA sequences via unequal homologous recombination or gene conversion-like processes. This phenomenon, for example, could be demonstrated for the rRNA (Krystal et al. 1981) and cyp21 genes (Urabe et al. 1990) and results in the concerted evolution of the family members (for details, see Dover et al. 1982). The process of unequal homologous recombination also leads to an alteration of the copy numbers of a sequence cluster and results in intra- or interspecific variations of the copy numbers of sequence families. For example, four haplotypes of the human α-amylase gene family comprise 3, 6, 9, or 12 genes, respectively (Groot et al. 1990), and the copy numbers of a long range repeat located on chromosome 1 of the mouse vary between 50 and 1800 in closely related strains (Eckert et al. 1991; Purman et al. 1992).

We analyzed a multisequence family, called chAB4, which was originally detected through experiments with extrachromosomal circular DNA from human cells (Assum et al. 1989). Most

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of the features of sequence families described above can also be demonstrated in chAB4. The copy numbers of the family vary considerably between closely related primate species, and the results of frequent exchanges of sequences between family members in one species are obvious. However, whereas most of the families described above are located at single sites in the genome, chAB4 sequences are distributed to at least 10 different loci in the pericentric heterochromatin of various human chromosomes. Pericentromeric regions have been analyzed in detail (Cooper et al. 1992; Trowell et al. 1993). Each of the different families of satellite sequences like alphoid sequences (Choo et al. 1991), satellite III (Fowler et al. 1989; Choo et al. 1990; Vissel et al. 1992), β-satellite sequences (Greig and Willard 1992; Assum et al. 1993) or a 48-bp repeat family, termed p22hom48.4 (Metzdorf et al. 1988), can also be found on various human chromosomes. Therefore, the distribution of chAB4 may be a result of the structure of the family itself. There is also the possibility of chAB4 being distributed by exchanges of flanking satellite sequences. To address this question, which is also important for the understanding of the complex evolutionary history of chAB4, we analyzed the structure and the chromosomal surroundings of chAB4 in greater detail.

RESULTS

In earlier publications (Assum et al. 1991) we described the primate-specific multisequence-family chAB4, which is represented in the haploid human genome with ~40 copies distributed to chromosomes 1, 3, 9, and the short arms of all acrocentrics. At that time our results indicated an apparent length of the chAB4 repetition unit of ~35 kb.

To investigate the structure of the chAB4 basic unit in detail, we isolated chAB4-specific cosmid clones from a chromosome 21- and chromosome 22-specific library [provided by H. Lehrach, Imperial Cancer Research Fund (ICRF), London, UK] by screening with the chAB4-specific probes R2, H1.4, and HS6.4. The relative position of the probes within the chAB4 repetition unit is demonstrated in Figure 2b, below. Assuming repetition units of ~35 kb in length, tandemly arranged in a head-to-tail fashion, one would expect very similar hybridization patterns on the library filters using probes R2 and HS6.4, which were supposed to form the boundaries of the repetition unit. In contrast, we identified only a few cosmid clones detectable with both probes and always being positive with H1.4 too, but the majority of clones that hybridized with HS6.4 differed from those detected with R2. Hence, the length of the chAB4 repetition unit apparently exceeds the 35 kb postulated earlier. To establish a cosmid contig spanning one complete repetition unit, we isolated overlapping cosmid clones from the chromosome-specific libraries by employing vector–insert–junction fragments as probes. These junction fragments were amplified by various PCR-based methods using cosmid DNA as template and were tested for their ability to extend beyond the ends of the region cloned so far. For the identification of new clones in the library we used only those junction fragments as probes, which showed hybridization signals characteristic of moderately amplified sequences (like chAB4) on total human DNA and which did not hybridize to the chAB4-specific cosmids already isolated from the library in a previous step (see Fig. 1). In this way besides the probes already existing from earlier experiments (Assum et al. 1991) we produced probes N, TE1, c8E2, 14E2, BE1, and 18E2.

In cases where the connection between two probes (e.g., Fig. 1, A and D) could only be established on the basis of a single cosmid, we isolated additional vector–insert–junction fragments (Fig. 1E) and hybridized them to a subset of cosmids positive with the flanking probes A and D. In all but one case it was possible to verify the correctness of the contig. In the case of BE1 and 14E2, which could only be connected by several chromosome 22-specific cosmid clones, we confirmed their linkage on chromosome 21 using the Centre d'Etude du Polymorphism Humain (CEPH) sequence-tagged site (STS) G51E07, which turned out to be located between these two markers.

During our efforts, we encountered some problems and peculiarities. (1) When screening both libraries with probe N, we were not able to recover additional cosmid clones beside the ones that had already been identified with probe R2. As N is also under-represented in the libraries, the region beyond N may probably represent a molecular configuration that is resistant to cloning in cosmids, as it is known, for example, for palindromic structures (Looney and Hamlin 1987; Ehrlich 1989; Greener 1990). (2) Another peculiarity we had to deal with was the fact that probes that are too far apart from each other to be

cloned within one single cosmid cohybridized to the same clones. All links between probes that we could establish—including these strange ones—are demonstrated in Figure 2a.

The correct names of the cosmid clones identified by the junction fragments are listed in Table 1. The resulting complex structure represents a composite of various chAB4 copies. It is striking that the region between N and TE1 seems to be very constant with no problems in assembling the cosmid clones into a linear contig. In contrast, the region composed of sequences c8E2, 18E2, BE1, and 14E2 reveals some branches indicating that this part of the chAB4 unit apparently can vary between different members of the chAB4 family.

To include all the links, demonstrated in Figure 2a, we assume an inverted arrangement of probes N, R2, H1.4, HS6.4, TE1, and c8E2, surrounding variable structures composed of sequences recognized by probes 14E2, BE1, and 18E2, as demonstrated in Figure 2b. There are variants, where 18E2 and c8E2 or, alternatively, 14E2, G51E07, and c8E2 are deleted resulting in a direct connection of TE1 and BE1 in a single cosmid. All of these possible variants are also depicted in Figure 2b.

To strengthen the data on the structure of the chAB4 units and, additionally, to gain insight into the chromosomal surroundings of chAB4 we investigated four different yeast artificial chromosome (YAC) clones carrying chAB4-specific sequences in more detail. The YACs (781G5, 857B10, 52H5, 612B6) were kindly provided by the CEPH, Paris, France, and are described as part of a contig covering the long arm of chromosome 21 (Chumakov et al. 1992). The STS E341 used in the CEPH contig corresponds to the chAB4-specific probe H1.4., published in Assum et al. (1991). First, we investigated the YACs for the content of chAB4-specific sequences by Southern blot as well as PCR analysis. To this end we cloned all vector–insert–junction fragments used to establish the cosmid contig and sequenced them to identify primers for PCR (primer sequences in Table 1). Hybridization of YACs with chAB4-specific probes as well as PCR analysis revealed that all YACs are positive with all probes/STSs tested, with the exception of 612B6, which apparently contains only a part of the chAB4 repetition unit (data listed in Table 2). In addition, the Southern blot results obtained with probe H1.4 also revealed that YACs 857B10 and 781G5 comprise at least two chAB4 units each, because we were able to detect members of two different subfamilies of chAB4 (Assum et al. 1991) characterized by the presence or absence of a diagnostic *Eco*RI restriction site in both YACs.

To test whether the four YACs are chimeric or not, they were hybridized to normal metaphase spreads using fluorescence in situ hybridization (FISH).

All four YACs showed indentical hybridization signals in the heterochromatic region of chromosomes 1, 3, 4, 9, Y, and the pericentromeric region of the acrocentrics. In addition YACs 781G5 and 612B6 hybridized both to the euchromatic region of chromosome 1p, indicating their chimeric character.

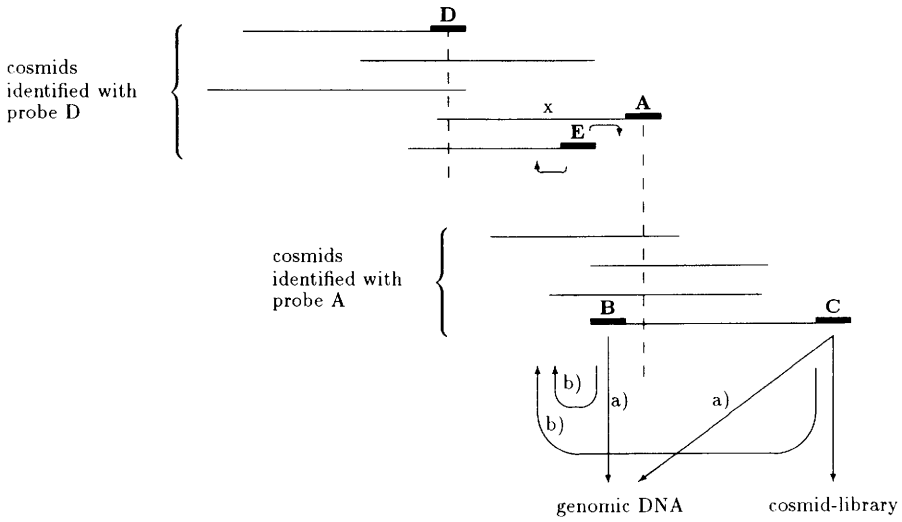

Figure 1 Strategy used to establish the cosmid–contig spanning the chAB4 region. (*A–E*) Vector–insert–junction fragments amplified to provide probes for chromosome walking. All junction fragments were hybridized to total human DNA to test the presence or absence of highly repetitive sequences (*a*), and to the chAB4-specific cosmids isolated in the previous step (*b*) to test for their ability to extend beyond the ends of the region already cloned. (*E*) Vector–insert–junction fragment used to verify the linkage between *A* and *D* originally established only on the basis of one single cosmid (x).

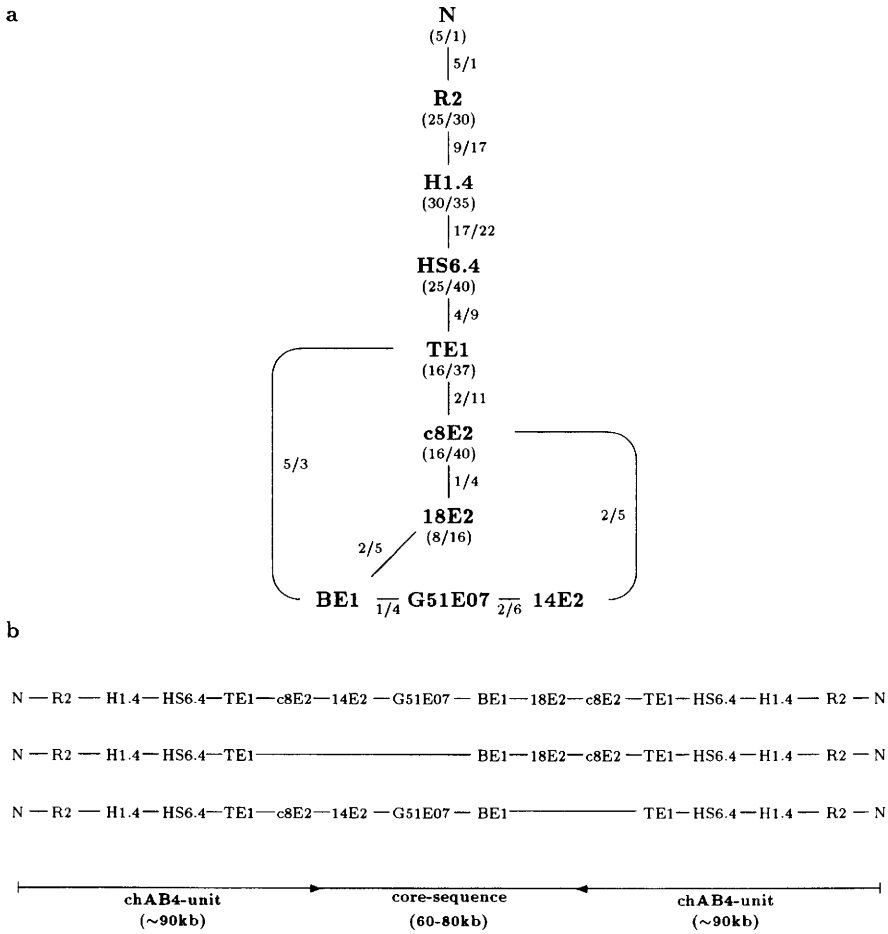

Figure 2 (*a*) Links between chAB4-specific probes. Numbers in parentheses indicate the numbers of positive clones detected with an individual probe in the chromosome (21/22)-specific library. Numbers without parentheses indicate the numbers of cosmid clones that establish the link between two probes in the chromosome 21/22-specific library. (*b*) Linear arrangement of chAB4-specific probes resulting in an inverted organization of chAB4 units. Indicated are variants of chAB4 units, where 18E2 and c8E2 or, alternatively, c8E2, 14E2, and G51EO7 are deleted.

The localization of chAB4 on chromosomes 4 and Y is in contrast to former results from chromosome in situ hybridization with ^{3}H-labeled probes, which assigned chAB4 only to chromosomes 1, 3, 9, and the acrocentrics (Assum et al. 1991). To exclude that the signals on chromosomes 4 and Y are attributable to common non-chAB4 sequences in the four YACs we performed FISH using a cosmid carrying only chAB4-specific sequences as a probe (Fig. 3). The results clearly confirmed the localization of chAB4 on chromosomes 1, 3, 4, 9, Y, and the acrocentrics. The same data could be obtained independently by STS analysis of chromosome-specific hybrid cell lines with chAB4-specific primers (see below).

To characterize the sequences surrounding chAB4 in more detail, we established vector–insert–junction fragments from the nonchimeric YACs 857B10 and 52H5 and hybridized them to total human DNA as well as to cosmids spanning the entire chAB4 region. YAC ends that do not hybridize to chAB4 and therefore represent flanking sequences were cloned, sequenced, and compared to the EMBL data base. We were able to amplify and sequence three of the four vector–insert–junction fragments. They showed strong homology to either α-satellite DNA (857B10), β-satellite DNA (857B10), or satellite III sequences (52H5) indicating that chAB4 is located in close vicinity to the centromere. Moreover, it can be deduced that YAC 857B10 covers one entire chAB4 cluster because both ends do not show homology to chAB4 sequences and therefore extend beyond the boundaries of the chAB4 region. From YAC 52H5 we could only amplify one junction fragment that shows homology to satellite III sequences. Although we cannot test the localization of the other end, it is very likely—regarding the length of the YAC (280 kb) and the hybridization results with chAB4-specific probes—that YAC 52H5 ends within the chAB4 region. The sequence composition of the four YACs was further elucidated by hybridizing them with various satellite-specific probes, especially with α satellites, β satellites, satellite III sequences, and a probe called p22Hom48.4 (Metzdorf et al. 1988) which, according to information from the Reference Library Database (RLDB), is also located within chAB4 between markers TE1 and c8E2. The results show that the YACs 781G5 and 857B10 contain markers of all

Table 1. Characterization of Cosmid Clones Identified with chAB4-specific Markers

probes/ STSs	cosmid clones, chromosome 21	cosmid-pocket	cosmid clones, chromosome 22		primersequences for STS-analysis
N					N-S:5'-CACTGTGTAT AATTAATGGCTTG-3' N-R:5'-ATACTCAAGC TTTGGATGTTATG-3'
R2	ICRFc102D01106,N,* ICRFc102E0655	- 81	ICRFc106H0431,N		R2-S:5'-CTGACCCATT CTGCTTCCCA-3' R2-R:5'-GCCTTATAAG ACCAAACCTATAA-3'
H1.4	ICRFc102F0764,R,* ICRFc102E0176,H ICRFc102E1178,H ICRFc102D12105,R ICRFc102G1089,H ICRFc102D0946,H	1 3 - - - -	ICRFc106D0956,R ICRFc106B1156,H ICRFc106A0834,R,H ICRFc106H1085,H ICRFc106G1273,H ICRFc106A1261,R	ICRFc106G1257,H ICRFc106D0689R,H ICRFc106D025,R,H ICRFc106D0959,R,H	H1.4-R:5'-TTCTTTGGAT CTGGGGTTTGC-3' H1.4-S:5'-CAGAAATTAG AAATCAATGAGACA-3'
HS6.4	ICRFc102A1062 ICRFc102G08121	2 2	ICRFc106B1032 ICRFc106A0250 ICRFc106B071 ICRFc106G0845,T,*		
TE1	ICRFc102H11101,C,P ICRFc102A0573 ICRFc102H0745,C,P ICRFc102H0351 ICRFc102B1087 ICRFc102B1099 ICRFc102G0391 ICRFc102H0473	2 2 2,4,14,33 2 3 26 1,20,94 3	ICRFc106E0440,H ICRFc106B0197,B,* ICRFc106G0279,P,C ICRFc106F0367,C ICRFc106F0360 ICRFc106D0448 ICRFc106B0447 ICRFc106A0341,C,P	ICRFc106E0889	TE1-R:5'-ACCAGGGACA CTCTTATGACT-3' TE1-S:5'-TTATGGTATC TGTGCAGCTGC-3'
BE1	ICRFc102B0357,18 ICRFc102F02122 ICRFc102B01111	- 2 3	ICRFc106D0782 ICRFc106C0776,14 ICRFc106D0814 ICRFc106H0785 ICRFc106E117,14	ICRFc106C0841 ICRFc106B1141 ICRFc106D015 ICRFc106A0589,14,*	BE1-R:5'-GGTATAACAC ATGCACAAATTAAA-3' BE1-S:5'-TTGTGTACAA GCTACATCACTG-3'
c8E2	ICRFc102D0688 ICRFc102E0158,14 ICRFc102E09122 ICRFc102G12121 ICRFc102F0761,T,P,* ICRFc102C0459 ICRFc102B08117,T	2,14 14 3 3,94 2,14 3,14,94,130 -	ICRFc106F102,T ICRFc106C0654,P,T ICRFc106A0267 ICRFc106B0533,P ICRFc106B0363,T ICRFc106F0545 ICRFc106F0465,18	ICRFc106E1011,T	c8-R:5'-AGCAAGCTTC AGAAGCAGACA-3' c8-S:5'-GTTGATCCAG GTTTCAGTTATAT-3'
14E2			ICRFc106A1270 ICRFc106B0740 ICRFc106D0990,18 ICRFc106C048 ICRFc106H0757	ICRFc106F0211,C,* ICRFc106C0639 ICRFc106C0275 ICRFc106A0141	14-R:5'-AATTTTTGAT GTGCATGTTATCTC-3' 14-S:5'-TGGAAAGTCA AGTATGTATGGAA-3'
18E2	ICRFc102E1046 ICRFc102A0852 ICRFc102C0797 ICRFc102C0685 ICRFc102A0468 ICRFc102D05102 ICRFc102D04102	- - - 1,82 2,9 - -	ICRFc106F0297,C ICRFc106A0146,C ICRFc106H0161,C ICRFc106H0585 ICRFc106H0930 ICRFc106F1092 ICRFc106B1270 ICRFc106D0669	ICRFc106D0485 ICRFc106C0585 ICRFc106A0265 ICRFc106B1039 ICRFc106A0672 ICRFc106D0675	18-R:5'-TGTAATTAGG ACTAATCAGATGAA-3' 18-S:5'-CTTACTCAGT CATTTTACCCTTT-3'

chAB4-specific markers used to identify chromosome 21- and 22-specific cosmid clones by hybridization of the libraries. Only clones investigated in more detail are shown. Letters indicate that an individual clone was also found to be positive with additional markers (N = N; H = HS6.4; T = TE1; B = BE1; 14 = 14E2; 18 = 18E2; C = c8E2; P = p22hom48.4). Cosmid pockets refer to Nizetic et al. (1994). Cosmids indicated by an asterisk were used to establish the minimal contig. Markers were also used to define primer sequences for STS analysis.

Table 2. Southern Blot/STS Analysis of YACs with chAB4-specific Probes/STS

	YAC			
Probe	781G5	52H5	612B6	857B10
N	+/+	+/+	+/+	+/+
R2	+/+	+/+	+/+	+/+
H1.4	+/+	+/+	+/+	+/+
HS6.4	+/+	+/+	+/+	+/+
TE1	+/+	+/+	+/+	+/+
c8E2	+/+	+/+	−/−	+/+
14E2	+/+	+/+	−/−	+/+
BE1	+/+	+/+	−/−	+/+
18E2	+/+	+/+	−/−	+/+

the satellite families tested, whereas 52H5 only contains satellite III sequences and p22Hom48.4 sequences; YAC 612B6 turned out to be negative with all sequences. Because YAC 857B10 obviously covers one complete chAB4 locus, carries at least two chAB4 copies, and is not chimeric, we choose this clone for restriction mapping using pulsed-field gel electrophoresis (PFGE) to confirm the chAB4 structure inferred from the cosmid contig.

First we looked for potential restriction sites of rare-cutting enzymes within the chAB4 region by digesting the cosmids representing one entire chAB4 unit. We found restriction sites for *Sfi*I within three cosmids, linking sequences c8E2–18E2, c8E2–14E2, and BE1–14E2, respectively, and one restriction site for *Not*I between 14E2 and BE1. Digestion of YAC 857B10 with *Sfi*I and *Not*I, separation of fragments by PFGE, and hybridization with chAB4-specific probes resulted in a restriction pattern that absolutely matches the chAB4 structure deduced from the cosmid–contig (depicted schematically in Fig. 4). *Sfi*I digestion yields four fragments of 40, 50, 160, and 200 kb. Probes R2, H1.4, HS6.4, and TE1 from the inverted part of the chAB4 unit all hybridize to two fragments, the 160-kb as well as the 200-kb fragment, whereas sequences comprising the core sequence only hybridize to one fragment, the 40-kb (14E2) and 50-kb (BE1, 18E2) fragments, respectively.

*Not*I digestion and hybridization with chAB4-specific probes results in two fragments of 200 and 250 kb length. Again, whereas R2, H1.4, HS6.4, and TE1 hybridize to both fragments, the sequences of the noninverted core region detect only one fragment. Only a discrepancy arises with probes c8E2 and N, which from the cosmid contig were predicted to be part of the inverted sequences of chAB4: c8E2 and N in both cases (*Sfi*I and *Not*I digestion) detect only one fragment, but this may be attributable to a deletion of the corresponding region within clone 857B10. Hybridization of satellite-specific probes to the PFGE filters resulted in the 200-kb fragment with α satellites and the 160-kb fragment with β satellites and satellite III sequences after *Sfi*I digestion indicating that the large *Sfi*I fragments flank the smaller 40- and 50-kb *Sfi*I fragments. *Not*I digestion yields the 200-kb fragment with α satellites and the 250-kb fragment with β and satellite III sequences (see Fig. 4). Because all results are in agreement with each other we propose the chAB4 units to be arranged as inverted repeats of ~90 kb encompassing a core sequence

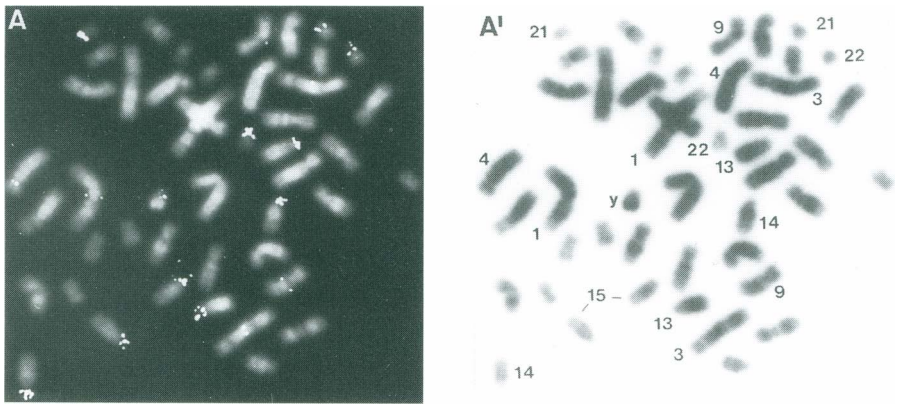

Figure 3 (*A*) Fluorescence in situ hybridization to normal male metaphase spreads using the chAB4-specific cosmid ICRFc102D01106 as a probe. This probe results in a multiple signal pattern of FITC fluorescence. In addition to strong signals in the centromeric region and short arms of all acrocentric chromosomes, specific signals were found in the centromeric regions of chromosomes 1, 3, 4, 9, and the nonheterochromatic region of the Y chromosome. Note that the signals on the nonacrocentric chromosomes are generally weak. (*A'*) Inverted DAPI image of *A* to visualize the banding pattern more clearly. The homologous chromosomes showing specific fluorescent signals are indicated by their numbers and the Y chromosome is indicated as well.

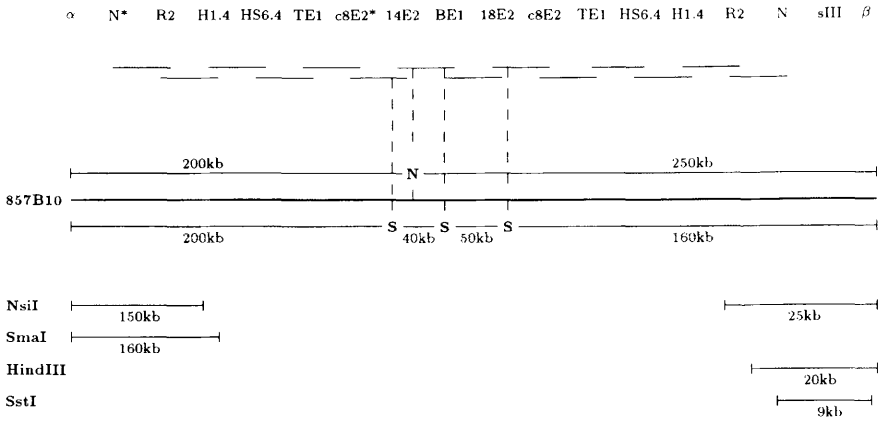

Figure 4 (*Top*) Cosmids representing one entire chAB4 unit are demonstrated. Restriction sites for *Sfi*I and *Not*I were identified by cutting the cosmids with these enzymes. (*Middle*) Restriction map of YAC 857B10 obtained after digestion of the YAC clone with *Sfi*I and *Not*I, separation of the fragments with PFGE and hybridization with chAB4-specific probes. The data inferred from the cosmid–contig absolutely match the restriction pattern of YAC 857B10 with one discrepancy: N and c8E2, which from the cosmid–contig was predicted to be part of the inverted sequences of chAB4, most probably was deleted from one chAB4 unit (N*, c8E2*) in YAC 857B10. In addition, smaller restriction fragments containing the chAB4-specific sequences N or R2 together with satellite-specific sequences are indicated at the *bottom*. (*N*) *Not*I, (*S*) *Sfi*I; (α) α satellites; (sIII) satellite III sequences, (β) β satellites.

of 60–80 kb. If the sequences of the chAB4 units are organized in the manner predicted from the cosmid contig and from the YAC mapping data, N and R2 should be the sequences closest to the satellite sequences at the ends of the YAC insert (cf. Fig. 4). To confirm this, we looked for frequently cutting restriction enzymes that do not cut within satellite sequences, digested YAC 857B10 with these enzymes (e.g., *Nsi*I, *Sma*I, *Hin*dIII, *Sst*I), separated fragments by PFGE, and hybridized with probes specific for α satellites, β satellites, and satellite III sequences, R2 and N, respectively. We were able to detect fragments that hybridize with R2 and α satellites on the one hand after digestion with *Nsi*I and *Sma*I, and N, satellite III, and β satellites on the other after cutting with *Nsi*I, *Hin*dIII, and *Sst*I (depicted schematically in Fig. 4).

In summary, these data show that in YAC 857B10 the following order of sequences could be determined: α satellites–chAB4 sequences–satellite III sequences–β satellites. To assign this structure to individual human chromosomes, we used the α-satellite-specific part of YAC 857B10 to perform FISH to normal metaphase spreads. This resulted in a strong signal only on chromosome 22, as shown in Figure 5. Therefore, the structure of YAC clone 857B10 (shown in Fig. 4) also represents a fine-structure map of the pericentromeric region of chromosome 22.

To address the question whether chAB4 consists of the same basic structure on other chromosomes, we performed STS analysis with DNA from a panel of chromosome-specific hybrid cell lines. As summarized in Table 3, we found all chAB4-carrying chromosomes to be positive with all STSs with the exception of BE1, which cannot be amplified from DNA of chromosome 3. Beside signals on chromosome 2 and 18 with STS 14E2 and 18E2 we can detect chAB4-specific sequences in the chromosome 20-specific hybrid cell line with all STSs tested. However, these signals are most probably attributable to a contamination of the hybrid cell line with DNA from

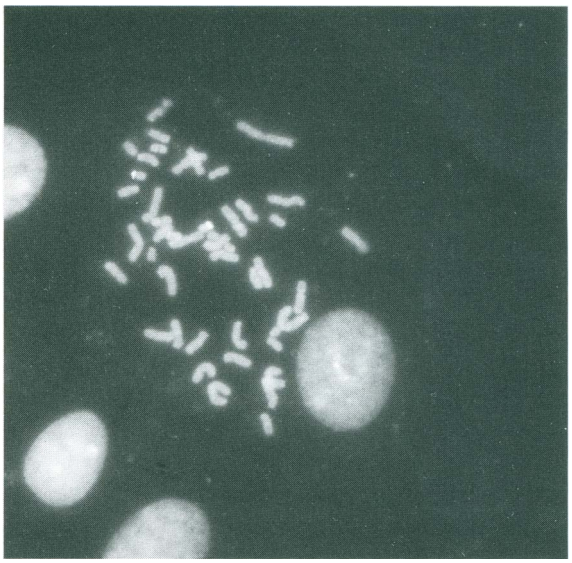

Figure 5 FISH to normal male metaphase spreads using an α satellite-specific probe derived from YAC 857B10. Hybridization under conditions of high stringency resulted in a specific signal in the centromeric region of chromosome 22.

Table 3. Analysis of Chromosome-specific Hybrid-cell Lines with chAB4-specific STS

	STS							
Chromosome[a]	N	R2	H1.4	TE1	c8E2	14E2	BE1	18E2
1	+	+	+	+	+	+	+	+
2	–	–	–	–	–	+	–	+
3	+	+	+	+	+	+	+	+
4	+	+	+	+	+	+	+	+
5	–	–	–	–	–	–	–	–
6	–	–	–	–	–	–	–	–
7	–	–	–	–	–	–	–	–
8	–	–	–	–	–	–	–	–
9	+	+	+	+	+	+	+	+
10	–	–	–	–	–	–	–	–
11	–	–	–	–	–	–	–	–
12	–	–	–	–	–	–	–	–
13	+	+	+	+	+	+	+	+
14	+	+	+	+	+	+	+	+
15	+	+	+	+	+	+	+	+
16	–	–	–	–	–	–	–	–
17	–	–	–	–	–	–	–	–
18	–	–	–	–	–	+	–	–
19	–	–	–	–	–	–	–	–
20	+	+	+	+	+	+	+	+
21	+	+	+	+	+	+	+	+
22	+	+	+	+	+	+	+	+
X	–	–	–	–	–	–	–	–
Y	+	+	+	+	+	+	+	+

[a] Chromosomes, which by FISH proved to carry chAB4 units, are indicated by boldface numbers.

chromosome 4 (according to information from the supplier; Drwinga et al. 1993). Taken together, these data indicate that the basic structure of chAB4, which we identified on chromosomes 21 and 22, is of great similarity among all chAB4-carrying chromosomes.

DISCUSSION

To clone a complete repetition unit of the long-range repetitive DNA family chAB4, 26 clones from a chromosome 21-specific and 44 clones from a chromosome 22-specific library were included into contigs.

At one end the contigs from both chromosomes break down within a similar region, most probably because of the existence of a genomic structure not stably clonable in cosmids. A number of overlaps found between cosmids from the other end of the contigs are not consistent with a simple linear arrangement of the cloned sequences. The assumption that this complicated structure may be a spurious result of the integration of chimeric cosmids into the contig can be ruled out by the fact that every marker used to establish the contig could also be found on chAB4-positive YAC clones from the CEPH library and in human/rodent cell hybrids with chAB4-carrying chromosomes. Moreover, 23 of the chromosome 21-specific cosmid clones are included in the cosmid pocket map published by Nizetic et al. (1994), and 20 of them are assigned to a small region on 21p, defined by two overlapping YAC clones (pocket P1, P2, and P3 in Nizetic et al. 1994). The simplest way to resolve the complicated structure shown in Figure 2a is to assume that chAB4 is organized as an inverted repeated sequence of at least 90 kb with variable, 60- to 80-kb-long nonduplicated core sequences inserted between two repeats oriented head to head. This structure was confirmed by restriction mapping of YAC clone 857B10. The chAB4 struc-

ture resembles that of some other genomically or somatically amplified sequences, for example, the structures of the mouse major urinary protein genes (Bishop et al. 1985), the mouse *sry* locus (Gubbay et al. 1992), the human α-amylase multigene family, (Groot et al. 1990), and somatically amplified adenylate deaminase genes in a CHO cell line (Hyrien et al. 1988). The major structural difference between chAB4 and the other families is the length of the nonduplicated core sequences. These sequences usually are between a few hundred and several base pairs in length, whereas the nonduplicated core sequence of chAB4 is at least 60 kb long. This is an important difference because such a long nonduplicated sequence can hardly be formed by the inversion–amplification model of Hyrien et al. (1988), which normally is used to describe the creation of such an inverted duplication and the amplification of the duplicated structure in one step. Therefore, we have to assume that either the chAB4 structure was not generated by processes described by this model or that the large nonduplicated chAB4 core sequence was formed by an asymmetric deletion of a larger inverted duplicated structure.

Another difference between chAB4 and other multisequence families is that chAB4 sequences are distributed to at least 10 different chromosomal loci in the human genome. Because we do find markers from the inverted part of chAB4 and from the nonduplicated core sequence on all chAB4-carrying chromosomes, we believe that the whole inverted structure was distributed to different loci. Whether chAB4 was translocated alone or together with heterochromatic sequences surrounding chAB4 is still unknown. The translocation of chAB4 alone could best be explained by the reintegration of an extrachromosomal intermediate, which, at least in theory, could result from recombination within a possible stem–loop structure. A similar process is discussed for the duplication and translocation of the human U1 small nuclear RNA (snRNA)–$tRNA^{Asn}$ gene cluster (Bernstein et al. 1985; Buckland 1989). However, it is also possible that chAB4 was translocated passively through an exchange of larger heterochromatic blocks because members of DNA families like β satellites or satellite III can both be found on the short arms of all acrocentrics as well as within heterochromatic blocks on nonacrocentric chromosomes. The results obtained with YAC 857B10 (see below) clearly show that chAB4 is located between α satellites on the one hand and β satellites and satellite III sequences on the other. The whole structure is derived from chromosome 22 as the alphoid sequences (α-satellite sequences) from 857B10 are located on this chromosome only. There is some confusion about the localization of chAB4 sequences in the literature. chAB4 sequences are included in two different YAC contigs from chromosome 21, published by Chumakov et al. (1992) from the CEPH and by Nizetic et al. (1994) from the ICRF. The CEPH contig maps chAB4 in the pericentromeric region of 21q. We analyzed four YACs from this region (781G5, 875B10, 52H5, and 612B6) in greater detail and were able to show that in addition to the CEPH marker E341, which is derived from the partial chAB4 sequence published in Assum et al. (1991), marker G51E07 is also located within chAB4. Therefore, chAB4 is directly linked to the 21q-specific marker D21S215. This link is established by several YAC clones, but all of them carry rearranged sequences with one exception. If this link is considered correct, at least one copy of chAB4 is located on 21q. However, as demonstrated for YAC 857B10 the clones that we analyzed, as well as the other ones assigned to this chromosomal region by the CEPH contig, do not necessarily originate from chromosome 21 or 21q, because none of the CEPH markers proximal of D21S215 are specific for chromosome 21.

In contrast to these results, the ICRF contig places chAB4 in the pericentromeric region of 21p. None of the chAB4-specific cosmid clones that are included in our contig are assigned to a YAC clone from 21q. The results of our FISH experiments place chAB4 on 21p, but we cannot exclude a second locus on 21q11.1. Therefore, it seems possible that chAB4 is located on both sides of the centromere on chromosome 21 and perhaps likewise on other acrocentric chromosomes.

The sequences cloned in YAC 857B10 most probably are derived from 22p, as they contain β-satellite sequences, and on the acrocentrics β-satellites seem to be located only on the short arms (Greig and Willard 1992; Assum et al. 1993). Therefore chAB4 is the non-satellite sequence located closest to the centromere at least on chromosome 22p, where it separates centromeric heterochromatic sequences from the constitutive heterochromatic sequences of the short arm of this chromosome. Whether this is also true for other acrocentric chromosomes is still unknown but seems to be possible because the

short arms of all the acrocentrics are structured very similarly.

Fine-structure maps exist for the centromeres of various human chromosomes (Cooper et al. 1992, 1993; Trowell et al. 1993), but little is known about the sequences surrounding the alphoid and satellite III sequences located directly within the centromere.

On the short arms of the acrocentric chromosomes the region between α-satellite sequences and β-satellite sequences was shown to be involved in most of the recombination processes leading to dicentric Robertsonian translocation chromosomes (Gravolt et al. 1992). Perhaps these recombinations are facilitated by the inverted duplicated structure of chAB4 located exactly within this region. Moreover, if chAB4 is also located on the long arm of the acrocentrics, recombination events in chAB4 could also lead to monocentric Robertsonian translocation chromosomes. Because chAB4, or rather the region in which chAB4 is located, was shown to be a very unstable structure in an evolutionary sense (Assum et al. 1994), the frequent occurrence of Robertsonian translocations may well be another aspect of the dynamics exerted by this repetitive DNA family.

METHODS

YAC DNA, cosmid DNA, and DNA from Chromosome-specific Hybrid Cell Lines

Yeast strains containing YACs 781G5, 857B10, 52H5, and 612B6 were kindly provided by the CEPH. The YACs are part of a contig covering the long arm of chromosome 21, and they are positive with the chAB4-specific STS E341. For DNA isolation 200 ml of SD medium (0.7% yeast nitrogen base without amino acids, 2% glucose, 55 mg/liter of adenine and tyrosine, 14 grams/liter of casamino acids) were inoculated with a single colony from an agar plate and grown at 30°C until saturation (2–3 days). Cells were centrifuged for 5 min at 3000*g*, washed with 50 mM EDTA (pH 8), resuspended in 12 ml of YRB medium [1 M Sorbitol, 10 mM Tris/HCl (pH 7.5), 20 mM EDTA, 14 mM β-mercaptoethanol], and incubated with 5000 units of Lyticase (Sigma) for at least 30 min at 37°C. After an additional centrifugation spheroplasts were resuspended in 15 ml of SE buffer. Further steps were performed as described elsewere (Miller et al. 1988)

Cosmid clones were identified by hybridization of filter replicas from chromosome 21- and chromosome 22-specific libraries, provided by the ICRF (Nizetic et al. 1991). Hybridization was performed as recommended by the supplier of the filters. Cosmid DNA was prepared using the Qiagen plasmid midi kit (100).

DNA samples from a panel of somatic cell hybrids, each containing a single human chromosome, were obtained from the Corriell Cell Repository (mapping panel 2; Drwinga et al. 1993).

Isolation of Vector–insert–junction Fragments from YACs and Cosmids

Different methods for end sequence rescue were used. In the first, yeast DNA (1.5 μg) was digested with either *Pst*I or *Pvu*II, the resulting fragments were tailed with dTTP (for 10 min at 37°C) in a final volume of 10 μl, employing the terminal deoxynucleotidyl transferase (GIBCO, BRL). Five microliters of the tailing reaction was used to perform PCR: First-strand synthesis with primers TTL–*Pst*I or TTL–*Pvu*II, respectively, was carried out under low stringency conditions (see below). After addition of vector-specific primers YAC–SN (left arm) or YAC–RN (right arm), a PCR reaction was performed under high stringency conditions (see below).

The second method to generate end sequences is referred to as inverse PCR. Yeast DNA was digested with *Taq*I, and products were religated under conditions that enhance intramolecular ligation and amplified under high stringency conditions with vector-specific primers YAC–SN and YAC–SJ, which are oriented in opposite directions.

For less complex templates like cosmid DNA, a third method for isolating end sequences was applied: 500 ng of cosmid DNA was used as template to anneal an unspecific primer (EGAL-N) via random hexamers at its 3′ end under low stringency conditions (see below). After extension for 2 min a PCR reaction was performed under high stringency conditions using primers specific to the cosmid vector (Law4-1 or Law4-2, respectively) in combination with primer EGAL, which is identical with the 5′ region of primer EGAL-N.

High stringency conditions: Initial denaturation was at 95°C for 1min followed by 30 cycles of 92°C for 1 min, 55°C for 1 min, 72°C for 1 min. Low stringency conditions: Denaturation was at 95°C for 1 min, annealing at 20°C for 1 min, 30°C for 1 min, 40°C for 1 min, extension at 72°C for 2 min.

Sequences of PCR-primers used to amplify vector–insert–junction fragments are as follows:

YAC-SN	5′-CTTTAATTTATCACTACGGAATTC-3′
YAC-RN	5′-CGATCTCAAGATTACGGAATTC-3′
YAC-SJ	5′-ATAACATAACACATATACAATTGAAA-3′
TTL-Pst	5′-TGAATTCGATCG$(A)_{12}$TGCA-3′
TTL-Pvu	5′-TGAATTCGATC$(A)_{15}$CT-3′
EGAL	5′-CTAGGCACTCCTTAGTGGTATC-3′
EGAL-N	5′-CTAGGCACTCCTTAGTGGTATCNNNNNN-3′
Law4-1	5′-TAGGGAGACCGGAAGCTTAGG-3′
Law4-2	5′-CATACACATACGATTTAGGTGACACTATAG-3′

The PCR products representing vector–insert–junction fragments from cosmid clones were either used directly as probes for hybridization experiments or cloned into pUC18 vectors (Pharmacia Sure Clone Ligation kit in combination with BRL T4 DNA ligase) and sequenced to determine PCR primers for STS analysis (see also Results).

Probes

Beside vector–insert–junction fragments used for hybridization experiments the following probes were employed: the satellite III probe was PCR-amplified with primer sequences derived from a satellite III-containing clone pTRS-47 (satellite III-S: 5′-TAGGATCAGAACGGAACAGAG-3′; satellite III-R: 5′-TGCAATGCAGTGGCACAATC-3′, Choo et al. 1990); as an α-satellite probe we used clone L1.26, specific to chromosome 13 and 21 (Devilee et al. 1986); as a β-satellite probe, clone JoG6 (Assum et al. 1993) was employed; p22hom48.4, a 48-bp repetitive element, was kindly provided by N. Blin, (Metzdorf et al. 1988). chAB4-specific probes other than vector–insert–junction fragments, represent subcloned fragments already published in Assum et al. (1991).

Sequencing

Sequencing reactions were performed using the double-stranded DNA cycle sequencing system from BRL, Life Technologies, combined with digoxigenated primers (pUC M13 sequence primer, pUC M13 reverse primer) from MWG Biotech. Digoxigenated fragments were separated using the Direct Blotting Electrophoresis system from GATC (Konstanz, Germany), and were detected employing the DIG Nucleic Acid Detection Kit from Boehringer Mannheim.

FISH

The chAB4-specific cosmid ICRFc102D01106 as well as the α-satellite sequences of YAC 857B10, subcloned into a pUC 18 vector, were labeled with biotin-11–dUTP, and chromosomal in situ hybridization was performed as described elsewhere (Lichter et al. 1990). Hybridization was performed under high stringency (60% formamide). Following a final stringent washing step with 0.3 × SSC at 60°C, the labeled probe was detected via FITC conjugated to avidin. Identification of the chromosomes was achieved by counterstaining with DAPI. Emitted fluorescence of DAPI and FITC were detected by a conventional epifluorescence microscope, and images were captured by a cooled CCD camera. Separately recorded digital images were processed and overlayed electronically using the public domain software NIH Image (Rasband 1994).

PFGE

Preparation of high-molecular-weight DNA in agarose blocks was performed as described elsewere (Schwartz and Cantor 1984; Carle and Olson 1985; Snell and Wilkins 1986). For the separation of large DNA fragments on agarose gels by contour-clamped homogenous electric field electrophoresis (CHEF), we used the LKB 2015 Pulsaphor system: 1.2% agarose gels were run at 10.7 V/cm in 0.5 × TBE buffer at 10°C with a pulse time of 9 sec and 13.5 sec for 7.5 hr, respectively. For the separation of DNA fragments <50 kb we used the following conditions: 1.2% agarose gels were run at 16.6 V/cm in 0.15 × TBE buffer at 10°C with a pulse time of 0.3 sec and 0.6 sec for 1 hr, respectively, and 1.0 sec for 3 hr.

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REFERENCES

Assum, G., B. Böckle, T. Fink, U. Dmochewitz, and W. Krone. 1989. Restriction analysis of chromosomal sequences homologous to single-copy-fragments cloned from small polydisperse circular DNA (spc-DNA). *Hum. Genet.* **82:** 249–254.

Assum, G., T. Fink, C. Klett, B. Lengl, M. Schanbacher, S. Uhl, and G. Wöhr. 1991. A new multisequence family in human. *Genomics* **11:** 397–409.

Assum, G., T. Fink, T. Steinbeisser, and K. Fisel. 1993. Analysis of human extrachromosomal DNA elements originating from different β-satellite subfamilies. *Hum. Genet.* **91:** 489–495.

Assum, G., C. Gartmann, W. Schempp, and G. Wöhr. 1994. Evolution of the chAB4 multisequence family in primates. *Genomics* **21:** 34–41.

Bernstein, L.B., T. Manser, and A.M. Weiner. 1985. Human U1 small nuclear RNA genes: Extensive conservation of flanking sequences suggests cycles of gene amplification and transposition. *Mol. Cell. Biol.* **5:** 2159–2171.

Bishop, J.O., G.G. Selman, J. Hickman, L. Black, R.D.P. Saunders, and A.J. Clark. 1985. The 45-kb unit of major urinary protein gene organization is a gigantic imperfect palindrome. *Mol. Cell. Biol.* **5:** 1591–1600.

Buckland, R.A. 1989. Genomic organization of human asparagin transfer RNA genes: Localization to the U1 RNA gene and class I pseudogene repeat units. *Am. J. Hum. Genet.* **45:** 283–295.

Carle, G.F. and M.V. Olson. 1985. An electrophoretic karyotype for yeast. *Proc. Natl. Acad. Sci.* **82:** 3756–3760.

Choo, K.H., E. Earle, and C. McQuillan. 1990. A homologous subfamily of satellite III DNA on human chromosomes 14 and 22. *Nucleic Acids Res.* **18:** 5641–5648.

Choo, K.H., B. Vissel, A. Nagy, E. Earle, and P. Kalitsis. 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.

Chumakov, I., P. Rigault, S. Guillou, P. Ougen, A. Billaut, G. Guasconi, P. Gervy, I. LeGall, P. Soularue, L. Grinas,

L. Bougueleret, C. Bellanné-Chantelot, B. Lacroix, E. Barillot, P. Gesnouin, S. Pook, G. Vaysseix, G. Frelat, A. Schmitz, JL. Sambucy, A. Bosch, X. Estivill, J. Weissenbach, A. Vignal, H. Riethman, D. Cox, D. Patterson, K. Gardiner, M. Hattori, Y. Sakaki, H. Ichikawa, M. Ohki, D. Le Paslier, R. Heilig, St. Antonarakis, and D. Cohen. 1992. Continuum of overlapping clones spanning the entire human chromosome 21q. *Nature* **359:** 380–387.

Cooper, K.F., R.B. Fisher, and C. Tyler-Smith. 1992. Structure of the pericentric long arm region of the human Y chromosome. *J. Mol. Biol.* **228:** 421–432.

———. 1993. Structure of the sequences adjacent to the centromeric alphoid satellite DNA array on the human Y chromosome. *J. Mol. Biol.* **230:** 787–799.

Devilee, P., P. Slagboom, E. Bakker, H.P. Scholl, H.D. Hager, A.F.G. Stevenson, and C.J. Cornelisse. 1986. Two subunits of human alphoid repetitive DNA shows distinct preferential localisation in the pericentric regions of chromosomes 13, 18 and 21. *Cytogenet. Cell. Genet.* **41:** 193–201.

Dover, G. 1982. Molecular drive: A cohesive mode of species evolution. *Nature* **299:** 111–117.

Drwinga, H.L., L.H. Toji, C.H. Kim, A.E. Greene, and R.A. Mulivor. 1993. NIGMS human/rodent somatic cell hybrid mapping panels 1 and 2. *Genomics* **16:** 311–314.

Eckert, W.A., C. Plass, A. Weith, W. Traut, and H. Winking. 1991. Transcripts from amplified sequences of an inherited homogeneously staining region in chromosome 1 of the house mouse (Mus musculus). *Mol. Cell. Biol.* **11:** 2229–2235.

Ehrlich, S.D. 1989. Illegitimate recombination in bacteria. In *Mobile DNA* (ed. D.E. Berg and M.M. Howe), pp. 799–832. American Society of Microbiology, Washington D.C.

Fowler, J.C.S., L.A. Burgoyne, E.G. Baker, M.L. Ringenbergs, and D.F. Callen. 1989. Human satellite III DNA: Genomic location and sequence homogeneity of the TaqI-deficient polymorphic sequences. *Chromosoma* **98:** 266–272.

Gravolt, C.H., U. Friedrich, M. Caprani, and A.L. Jorgensen. 1992. Breakpoints in Robertsonian translocations are localized to satellite III DNA by fluorescence in situ hybridization. *Genomics* **14:** 924–930.

Greener, A. 1990. E. coli SURE strain: Clone "unclonable" DNA. *Strategies* **3:** 5–6.

Greig, G.M. and H.F. Willard. 1992. β Satellite DNA: Characterization and localization of two subfamilies from the distal and proximal short arms of the human acrocentric chromosomes. *Genomics* **12:** 573–580.

Groot, P.C., W.H. Mager, N.V. Henriquez, J.C. Pronk, F. Arwert, R.J. Planta, A.W. Eriksson, and R.R. Frants. 1990. Evolution of the human α-amylase multigene family through unequal, homologous, and inter- and intrachromosomal crossover. *Genomics* **8:** 97–105.

Gubbay, J., N. Vivian, A. Economou, D. Jackson, P. Goodfellow, and R. Lovell-Badge. 1992. Inverted repeat structure of the Sry locus in mice. *Proc. Natl. Acad. Sci.* **89:** 7953–7957.

Hyrien, O., M. Debatisse, G. Buttin, and B.R. de Saint Vincent. 1988. The multicopy appearance of a large inverted duplication and the sequence at the inversion joint suggest a new model for gene amplification. *EMBO J.* **7:** 407–417.

Koide, T., M. Ishiura, N. Hazumi, T. Shiroishi, Y. Okada, and T. Uchida. 1990. Amplification of a long sequence that includes a processed pseudogene for elongation factor 2 in the mouse. *Genomics* **6:** 80–88.

Krystal, M., P. D'Eustachio, F.H. Ruddle, and N. Arnheim. 1981. Human nucleolus organizers on nonhomologous chromosomes can share the same ribosomal gene variants. *Proc. Natl. Acad. Sci.* **78:** 5744–5748.

Lichter, P., C.C. Tang, G. Hermanson, G.A. Evans, D. Housman, and D.C. Ward. 1990. High resolution mapping of human chromosome 11 by in situ hybridization with cosmid clones. *Science* **247:** 64–69.

Looney, J.E. and J.L. Hamlin. 1987. Isolation of the amplified dihydrofolate reductase domain from methotrexate-resistant Chinese hamster ovary cells. *Mol. Cell. Biol.* **7:** 569–577.

Metzdorf, R., E. Göttert, and N. Blin. 1988. A novel centromeric repetitive DNA from human chromosome 22. *Chromosoma* **97:** 154–158.

Miller, S.A., D.D. Dykes, and H.F. Polesky. 1988. A simple salting out procedure for extracting DNA from human nucleated cells. *Nucleic Acids Res.* **16:** 1215.

Nizetic, D., G. Zehetner, A.P. Monaco, L. Gellen, B.D. Young, and H. Lehrach. 1991. Construction, arraying, and high-density screening of large insert libraries of human chromosomes X and 21: Their potential use as reference libraries. *Proc. Natl. Acad. Sci.* **88:** 3233–3237.

Nizetic, D., L. Gellen, R.M.J. Hamvas, R. Mott, A. Grigoriev, R. Vatcheva, G. Zehetner, M.L. Yaspo, A. Dutriaux, C. Lopes, J.M. Delabar, C. Van Broeckhoven, M.C. Potier, and H. Lehrach. 1994. An integrated YAC-overlap and "cosmid-pocket" map of the human chromosome 21. *Hum. Mol. Genet.* **3:** 759–770

Nonet, G.H., S.M. Carroll, M.L. DeRose, and G.M. Wahl. 1993. Molecular dissection of an extrachromosomal amplicon reveals a circular structure consisting of an imperfect inverted duplication. *Genomics* **15:** 543–558.

Purmann, L., C. Plass, M. Grüneberg, H. Winking, and

W. Traut. 1992. A long-range repeat cluster in chromosome 1 of the house mouse, Mus musculus, and its relation to a germline homogeneously staining region. *Genomic* **12:** 80–88.

Rasband, W. 1994, program NIH Image, written by Rasband,W. at the US National Institute of Health, available from Internet by anonymous ftp from zippy-nimh.nih.gov.

Schwartz, D.C. and C.R. Cantor. 1984. Separation of yeast chromosome-sized DNAs by pulsed field gradient gel electrophoresis. *Cell* **37:** 67–75.

Shen, S., J.L. Slightom, and O. Smithies. 1981. A history of the human fetal globin gene duplication. *Cell* **26:** 191–203.

Snell, R.G. and R.J. Wilkins. 1986. Separation of chromosomal DNA molecules from C. albicans by pulsed field gel electrophoresis. *Nucleic Acids Res.* **14:** 4401–4406.

Sylvester, J.E., D.A. Whiteman, R. Podolsky, J.M. Pozsgay, J. Respess, and R.D. Schmickel. 1986. The human ribosomal RNA genes: Structure and organization of the complete repeating unit. *Hum. Genet.* **73:** 193–198.

Trowell, H.E., A. Nagy, B. Vissel, and K.H.A. Choo. 1993. Long-range analyses of the centromeric regions of human chromosomes 13, 14 and 21: Identification of a narrow domain containing two key centromeric DNA elements. *Hum. Mol. Genet.* **2:** 1639–1649.

Urabe, K., A. Kimura, F. Harada, T. Iwanaga, and T. Sasazuki. 1990. Gene conversion in steroid 21-hydroxylase genes. *Am. J. Hum. Genet.* **46:** 1178–1186.

Vissel, B., A. Nagy, and K.H.A. Choo. 1992. A satellite III sequence shared by human chromosomes 13, 14, and 21 that is contiguous with α-satellite DNA. *Cytogenet. Cell. Genet.* **61:** 81–86.

Windle, B.E. and G.M. Wahl. 1992. Molecular dissection of mammalian gene amplification: New mechanistic insights revealed by analyses of very early events. *Mut. Res.* **276:** 199–224.

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