Proc. Natl. Acad. Sci. USA
Vol. 86, pp. 202–206, January 1989
Genetics

Macromolecular organization of human centromeric regions reveals high-frequency, polymorphic macro DNA repeats

(α-satellite DNA/centromeres/restriction fragment length polymorphisms/pulsed-field gel electrophoresis)

ETHYLIN WANG JABS*, CORINTHA A. GOBLE, AND GARRY R. CUTTING

Genetics Unit, Department of Pediatrics, The Johns Hopkins University School of Medicine, Baltimore, MD 21205

Communicated by John W. Littlefield, September 9, 1988

ABSTRACT **To analyze the macromolecular organization of human centromeric regions, we used α-satellite, or alphoid, repetitive DNA sequences specific to the centromeres of human chromosomes 6 (D6Z1), X (XC), and Y (YC-2) and the technique of pulsed-field gel electrophoresis. Genomic DNA from 24 normal, unrelated individuals was digested and separated into fragments ranging from 23 kilobases (kb) to 2 megabases (Mb) in length. Digestion with 12 different restriction enzymes with 4- to 8-base-pair recognition sequences and hybridization with alphoid sequences revealed chromosome-specific hybridization patterns. Similarities in the organization of the centromeric regions of the three chromosomes included *Not* I, *Sfi* I, and *Sal* I fragments of >2 Mb and *Sau*3A1 and *Alu* I fragments of <150 kb. Each restriction enzyme with a 6-base-pair recognition sequence (*Ava* II, *Bam*HI, *Hin*dIII, *Hpa* I, *Pst* I, *Sal* I, *Sst* I, and *Xba* I) detected polymorphic DNA fragments of 50 kb to 2 Mb. Forty percent or more of the individuals screened revealed a unique hybridization pattern with these enzymes and at least one of the three chromosome-specific alphoid probes. Five individuals differed from one another in hybridization pattern for each of the three enzymes *Hin*dIII, *Hpa* I, and *Sst* I and for each of the three centromeric probes. All 24 individuals could be distinguished on the basis of unique hybridization patterns with only two enzymes and one chromosome-specific alphoid probe. Family studies showed that these polymorphisms are inherited. The high frequency of these macro restriction fragment length polymorphisms illustrates the high degree of variability of the centromeric region among normal individuals and demonstrates its usefulness for DNA fingerprinting and pericentromeric mapping by linkage analysis.**

Human centromeric regions contain repetitive satellite DNA composed of simple and complex tandem DNA repeats (1–3). A subset of satellite DNA is the alphoid, or α-satellite, DNA family, which is composed of tandem arrays of 170-base-pair (bp) repeats that are 60–95% homologous to each other (4, 5). These 170-bp monomers are organized into groups of pentameric units, consisting of five 170-bp repeats of varying homologies, on human chromosomes 1, 11, 17, and X (6); tetrameric units on chromosomes 13 and 21 (7); alternating tetrameric and dimeric units on chromosome 7 (8, 9); and dimeric units on chromosome 18 and other chromosomes (7, 10). On chromosome 6 there are groups of twenty 170-bp sequences with the central nine 170-bp monomers being inverted in nucleotide sequence and more homologous to one another than the monomers outside this inversion (4). These groups are organized into even larger sequences, of 1–6 kilobases (kb) in length, and repeated 100–5000 times (11). These larger repeats, referred to as macro DNA repeats, are chromosome-specific because they are >95% homologous to one another, and they are organized into tandem arrays estimated to be 0.5–10 megabases (Mb) long (4, 11).

By conventional gel electrophoresis, chromosome-specific polymorphisms have been detected and have been used in linkage analysis and gene mapping (12, 13). The variant large repeats result from different numbers of 170-bp monomers and point mutations (11). By molecular cytogenetic techniques, it has been shown that in normal individuals, the amount of chromosome-specific alphoid DNA may vary as much as 2- to 3-fold and directly correlates with the size of the relevant centromeric heterochromatic variant (14).

With the introduction of pulsed-field gel electrophoresis, which physically separates DNA fragments as large as 10^5–10^7 bp (15, 16), it is now possible to bridge the gap of our knowledge between the molecular and cytogenetic levels and analyze the macromolecular organization of alphoid DNA sequences that extend over several megabases. We have used this technique to look for macro DNA repeats and macro restriction fragment length polymorphisms (macro-RFLPs). In comparing the higher organization of the human centromeric regions of chromosomes 6, X, and Y, we have detected a high frequency of macro-RFLPs.

MATERIALS AND METHODS

Isolation of Alphoid DNA from the Human Y Chromosome and Acrocentric Chromosomes. The flow-sorted, human chromosome Y library with *Hin*dIII DNA inserts (LLOYNS01) and the chromosome 15 library with *Eco*RI DNA inserts (LA15NS02), prepared and distributed by the Lawrence Livermore and Los Alamos National Laboratories (17), were screened at low stringency with an alphoid DNA probe, D6Z1, which is specific to chromosome 6 under high stringency (18). A 2.1-kb *Hin*dIII DNA fragment was obtained from the chromosome Y library and was cloned into pEMBL. This sequence hybridizes predominantly to the male-specific 6.0-kb *Eco*RI DNA repeat by Southern blotting (19, 20) and localizes to the centromere of the Y chromosome by *in situ* hybridization (data not shown). This probe is designated as YC-2 and by hybridization analysis is >95% homologous to the Y centromeric probe DYZ3 (19) and by nucleotide sequence analysis is >80% homologous to the partial sequence of DYZ3 (data not shown).

From the chromosome 15 library, we obtained a 3.05-kb *Eco*RI DNA fragment, which was cloned into pEMBL. This probe is designated 15C-3-1 and hybridizes by *in situ* hybridization predominantly to chromosome 15 and the other D-group chromosomes. Nucleotide sequence analysis revealed that a portion of the insert is alphoid DNA and another portion is composed of 5-bp simple repeats. DNA sequencing was performed by the the dideoxy chain-termination method on double-stranded templates with adenosine 5′-[α-[^{35}S]thio]-

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

Abbreviations: RFLP, restriction fragment length polymorphism; Mb, megabase(s).

*To whom reprint requests should be addressed.

triphosphate as the radioactive label and forward or reverse M13 primers (4).

DNA Purification. DNA from human lymphocytes was isolated as described (21). Recombinant plasmids p308 (D6Z1) (18), XC (4), and YC-2 were purified by the method of Maniatis *et al.* (22). The plasmid p308 is a 3-kb *Bam*HI DNA fragment inserted into pBR322. This sequence is repeated and specific to the centromeric region of chromosome 6. The plasmid XC is a 2-kb *Bam*HI DNA fragment inserted into pBR322. In the genome, the sequence is repetitive and specific to the centromeric region of chromosome X.

Conventional Gel Electrophoresis. Methods for DNA preparation, restriction endonuclease digestions, electrophoresis, transfer to hybridization transfer membrane (Nitroplus 2000, a nylon-based nitrocellulose manufactured by Micron Separations, Westboro, MA), prehybridization, hybridization with ^{32}P-labeled nick-translated DNA, stringency washes, and autoradiography have been described by Jabs *et al.* (21). All filters were washed under stringent conditions in 0.1× SET at 68°C (1× SET is 150 mM NaCl/5 mM EDTA/50 mM Tris, pH 7.8).

Preparation of High Molecular Weight DNA. Ten milliliters of EDTA-anticoagulated blood was layered onto an equal volume of Histopaque-1077 (Sigma) and then centrifuged at 400 × *g* for 30 min. The mononuclear cell layer was washed with 10 ml of Dulbecco's phosphate-buffered saline (PBS, GIBCO) and centrifuged at 250 × *g* for 15 min. The leukocytes were resuspended in PBS at a concentration of 0.5–2.0 × 10^7 cells per ml and warmed to 37–40°C. An equal volume of 2% agarose (low-melting-point agarose, Bethesda Research Laboratories) in 125 mM EDTA was warmed to 37–40°C and added. The mixture was pipetted into a plastic mold with 70-μl wells. Twenty-five to thirty agarose plugs were removed and shaken at 50°C for 24 hr in 20 ml of NDS (1% *N*-lauroylsarcosine/0.5 M EDTA, pH 9.4) containing proteinase K at 2 mg/ml. Before restriction digestion, the EDTA concentration was lowered and the proteinase K was inactivated by two 2-hr dialyses in TE (10 mM Tris, pH 7.4/0.1 mM EDTA) plus 0.1 M phenylmethylsulfonyl fluoride at room temperature, followed by three 2-hr dialyses against TE. Restriction digestion was performed overnight on each block in a total buffer volume of 300 μl (as specified by the enzyme supplier) with 10 units of enzyme per microgram of DNA to a maximum of 60 units of enzyme per block. The restriction enzyme was removed by incubating each block at 50°C in 1 ml of NDS for 1 hr and then in 300 μl of NDS with proteinase K at 2 mg/ml for 1 hr.

Pulsed-Field Gel Electrophoresis. DNA from agarose blocks was electrophoresed through a 1% agarose gel (10 × 10 cm) in TBE (0.45 M Tris/0.045 M boric acid/0.001 M EDTA, pH 8.0), using an orthogonal-field alternating gel electrophoresis (OFAGE) apparatus or a contour clamped homogeneous electric field (CHEF) gel apparatus as described (23). Concatamers of 48.5-kb λ DNA and intact *Saccharomyces cervisiae* chromosomes of 218, 282, 358, 445, 556, 610, 642, 761, 800, 834, 940, 970, 1115, 1580, and 2500 kb served as size markers (23). To resolve DNA fragments of 23–500 kb, electrophoretic conditions were 30-sec pulse times at 170 V for 19.5 hr; to resolve DNA of 250 kb to 2 Mb, conditions were 45-sec pulse times at 240 V for 18 hr. The OFAGE apparatus was used to screen for the presence and size range of hybridizing bands. Electrophoresis by the CHEF method, which produced linear DNA lanes, was used for accurate sizing of bands to within 10% (23).

Transfer and Hybridization. Transfer of large DNA fragments was enhanced by staining the gel with ethidium bromide (1 μg/ml) for 20–30 min and nicking the DNA by exposing it to a UV (302 nm) source for 10 min. The DNA was denatured, neutralized, transferred, and hybridized as described for conventional gels (21). All hybridized filters were washed under stringent conditions (0.1× SET, 68°C). Autoradiography required exposures up to 1 week. Digestions were interpreted as complete if the autoradiograph yielded hybridizing bands that were reproducible and consistent among different samples from the same individual on different filters, if the same filters were reprobed with another alphoid or single-copy probe and yielded consistent bands between unrelated individuals, or if the hybridizing bands were inherited within a family. Filters were washed with water for 30 min at 80°C prior to rehybridization with another probe. Removal of the previous signal was confirmed by autoradiography.

RESULTS

Comparison of the Macro Restriction Patterns at the Centromeric Regions of Different Human Chromosomes. High molecular weight DNA from 24 normal individuals (23 Caucasians and 1 Oriental) were digested with *Alu* I, *Ava* II, *Bam*HI, *Hin*dIII, *Hpa* I, *Not* I, *Pst* I, *Sal* I, *Sau*3A1, *Sfi* I, *Sst* I, or *Xba* I. These restriction enzymes were chosen to determine the frequency and pattern of 4- to 8-bp recognition sequences at the centromeric region. These digests usually produced multiple discrete bands or smears of unresolved bands of hybridization with each of the chromosome-specific alphoid probes. The size ranges of the centromeric DNA restriction fragments produced by a given enzyme were similar for different chromosomes among different individuals (Fig. 1, Table 1). The restriction enzymes *Alu* I and *Sau*3A1, which recognize 4-bp sequences, digested DNA containing alphoid sequences into fragments of <150 kb on chromosomes 6, X, and Y. The enzymes *Ava* I, *Bam*HI, *Hin*dIII, *Hpa* I, *Pst* I, *Sst* I, and *Xba* I, which recognize 6-bp sequences, digested DNA containing alphoid DNA into fragments of ≥50 kb. The infrequently cutting enzymes, including *Sal* I, *Not* I, and *Sfi* I, which recognize 6- and 8-bp sequences, produced very few centromeric DNA fragments smaller than 2 Mb. The hybridization patterns for *Alu* I digests were the same for the 6, X, and Y chromosomes

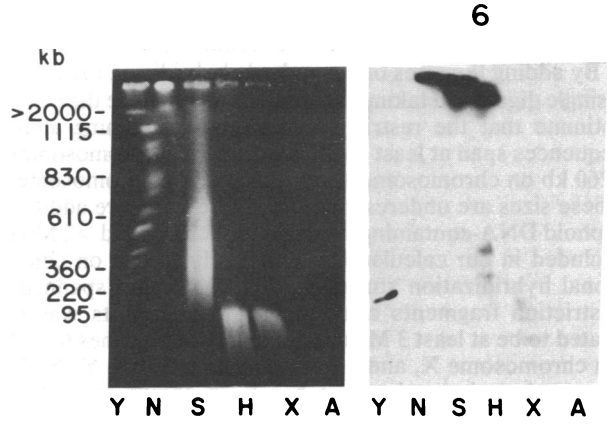

FIG. 1. Genomic and alphoid DNA fragments resolved by pulsed-field gel electrophoresis. Genomic DNA was digested with *Not* I (N), *Sal* I (S), *Hin*dIII (H), *Xba* I (X), or *Alu* I (A), electrophoresed, stained with ethidium bromide (*Left*), and transferred. The filter was probed with the chromosome 6 alphoid repeat (*Right*). The yeast marker (Y, strain YP291) is in the leftmost lane. Note the multiple hybridizing bands and different hybridization patterns for different enzymes. *Not* I and *Sal* I cut infrequently in the genome and also in alphoid DNA, as shown by the absence of hybridizing fragments of <2 Mb in lanes N and S. *Xba* I and *Hin*dIII, with 6-bp recognition sites, cut more frequently in the genome and in alphoid DNA. *Alu* I cuts very frequently in genomic and alphoid DNA, producing fragments of <95 kb.

Table 1. Macro restriction patterns of human alphoid DNA detected by using probes specific to centromeric regions of chromosomes (Chr.) 6, X, and Y

Enzyme	Size of alphoid DNA fragments,* kb			No. of different patterns/ no. of individuals analyzed		
	Chr. 6	Chr. X	Chr. Y	Chr. 6	Chr. X	Chr. Y
4 bp†						
Alu I	<150	<150	<150	1/5	1/5	1/5
*Sau*3A1	<150	<150	<150	1/5	1/5	1/5
6 bp†						
Ava II	>100	‡	<300, >400 (P)	1/5	—	5/5
*Bam*HI	‡	‡	>1050 (P)	—	—	3/5
*Hin*dIII	<715 (P)	<840 (P)	<1580 (P)	5/8	8/8	8/8
Hpa I	<1580 (P)	<800 (P)	<800 (P)	5/5	5/5	5/5
Pst I	<1115 (P)	<775 (P)	<50, >50	19/23	10/23	1/19
Sst I	<800 (P)	<500 (P)	<800 (P)	5/5	5/5	5/5
Xba I	100, 47, 30, 27	<100, 250–600, >700, <948 (P)	<100	1/19	5/5	1/14
Sal I	>2000	>2000, <2000 (P)	>2000	1/5	3/5	1/5
8 bp†						
Sfi I	>2000	>2000, <2000	>2000	1/5	1/5	1/5
Not I	>2000	>2000	>2000	1/12	1/12	1/12

*The majority of the hybridizing bands are of the size listed first. Fewer hybridizing bands are of the size(s) listed after the first comma. Even fewer hybridizing bands are of the size listed after the second comma. (P) designates presence of polymorphic bands in different individuals.
†Length of recognition sequence of restriction enzyme.
‡No hybridizing bands were detected between 23 kb and 2 Mb. In the case of *Bam*HI, restriction sites occur at ≈3-kb intervals in alphoid repeats on chromosome 6 and at ≈2-kb intervals in alphoid repeats on chromosome X. Therefore, all the hybridizing bands are <23 kb.

among different individuals; the *Not* I hybridizing bands were also indistinguishable between chromosomes among different individuals. All other enzymes yielded chromosome-specific hybridization patterns with the chromosome-specific alphoid probes.

Double digests with *Not* I and each of the enzymes recognizing 6-bp sequences yielded hybridization patterns that were not different from those produced by digestion with each of the 6-bp-specific enzymes alone (data not shown). This suggests that the *Not* I sites are outside the more frequently occurring enzyme sites at the human centromeric regions. Double digests with two enzymes other than *Not* I reduced the size of some bands but not others. The large number of hybridizing fragments precluded the accurate mapping of these restriction sites relative to one another.

By adding the sizes of the multiple hybridizing bands from a single digest and taking the largest sum of all the digests, we estimate that the restriction fragments containing alphoid sequences span at least 2320 kb on a pair of chromosomes 6, 1260 kb on chromosome X, and 1050 kb on chromosome Y. These sizes are underestimates because there are additional alphoid DNA-containing fragments of <23 kb and >2 Mb not included in our calculations. Previously, based on conventional hybridization studies (4, 11), the region spanned by restriction fragments containing alphoid repeats was estimated to be at least 3 Mb on a pair of chromosomes 6, 10 Mb on chromosome X, and 0.5 Mb on chromosome Y. By both electrophoretic methods, it has been shown that alphoid DNA is contained within different chromosomal regions of 1–10 Mb.

However, there may be other repeats within these large DNA fragments containing alphoid DNA. We have obtained a human DNA fragment of 3.05 kb (15C-3-1) with a region of simple repeats and alphoid sequences. Sequence data reveals that within a region of DNA there are alphoid repeats (lowercase letters) and 5-bp simple repeats (uppercase letters): ttcctttatagaattgtattttagatctgtcttcgttagactctttgaagaaacactacac...AAGGG-AGTGG-AAGTG-AGTGG-AGTGG-AATGC-AGTGT-ACTGG-AATGG-AGTGGG-ATTG-ATTG-ATTGG-AATGG-AGTGG-AGTGG-AGTGC-AGCAG-AGTGT-AGTGA-AATGG-AATGG.

Macro-RFLPs at the Centromeric Regions of Specific Chromosomes. Variant DNA fragments ranging from 50 kb to 2 Mb in size were identified by each restriction enzyme with a 6-bp recognition sequence (*Ava* II, *Bam*HI, *Hin*dIII, *Hpa* I, *Pst* I, *Sal* I, *Sst* I, and *Xba* I) and at least one of the chromosome-specific alphoid probes (Table 1, Fig. 2). More than 40% of all the individuals tested showed polymorphic macro DNA repeats for each of the restriction enzymes with a 6-bp recognition site and for one or more of the alphoid probes. The striking feature of the variant hybridization patterns was that 5 of 5 individuals had different patterns from each other for three of the eight enzymes recognizing 6-bp sequences (*Hin*dIII, *Hpa* I, and *Sst* I) at each of the three centromeric regions. The enzyme *Pst* I generated 19 different patterns among 23 individuals whose DNA was probed with the chromosome 6 alphoid repeat and 10 different patterns among 23 individuals whose DNA was probed with the X chromosome alphoid repeat. *Pst* I polymorphisms were not detected with the Y chromosome probe. The other 6-bp restriction enzymes (*Ava* II, *Bam*HI, *Sal* I, and *Xba* I) detected 3–5 different hybridization patterns among the DNAs of 5 individuals screened with an alphoid DNA probe from either the X or Y chromosome. All 24 individuals analyzed could be distinguished from one another by using the enzymes *Pst* I and *Hin*dIII and the alphoid probe from chromosome 6.

Five unrelated families consisting of parents and one to five offspring were studied to determine whether these macro-RFLPs were inherited. The DNA fragments of the offspring appeared to be derived from one or both parents (Fig. 3). We did not detect any *de novo* macro DNA repeats in 26 meiotic events.

DISCUSSION

We have examined the macroorganization of human centromeric regions by pulsed-field gel electrophoresis. In some respects, centromeric organization is similar to that of the rest of the genome. The enzymes with 4- or 6-bp recognition sites, which cut frequently within the genome, also frequently cut the DNA at the centromeric region of chromosomes 6, X, and Y (4, 5, 19) (Fig. 1). Enzymes with 8-bp recognition sites

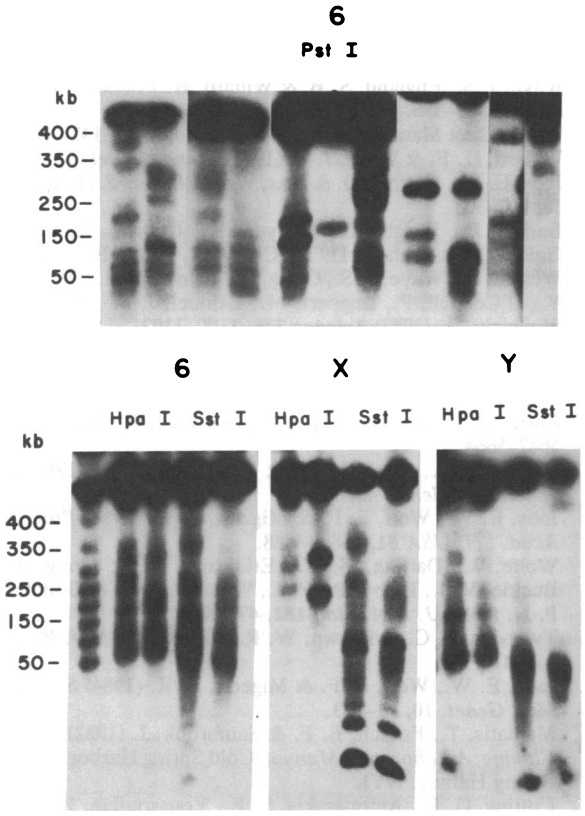

FIG. 2. Macro-RFLPs. (*Upper*) Genomic DNA of 11 different individuals was digested with *Pst* I and probed with the chromosome 6 alphoid probe. Note that the hybridization patterns are different for each individual. (*Lower*) *Hpa* I and *Sst* I digestions of two different genomic DNAs yield variant fragments at all three centromeric regions (chromosomes 6, X, and Y). Leftmost lane, size markers (λ concatamers).

such as *Not* I and *Sfi* I, which cut infrequently within genomic DNA, also yield few fragments containing alphoid DNA that are <2 Mb long.

We have also noted differences between the human centromeric regions and the rest of the human genome. One distinctive feature of the centromeric regions is that there are 50-kb to 2-Mb blocks of DNA containing alphoid sequences that lack enzyme sites that generally allow digestion of genomic DNA into smaller fragments. The majority of genomic DNA is digested by 4-bp-recognition enzymes into fragment lengths averaging <1 kb and by 6-bp restriction enzymes into an average fragment length of 5 kb. These same enzymes digest alphoid DNA into fragments that can be resolved by conventional electrophoresis, but there are additional fragments that can only be detected by pulsed-field gel electrophoresis. The *Alu* I alphoid DNA-containing fragments are >0.3 and <150 kb in size; and the *Hin*dIII alphoid DNA-containing fragments are >6 kb with the largest fragments detected at ≈2 Mb. In addition, the less frequently cutting enzymes *Not* I, *Sal* I, and *Sfi* I digest DNA from the Duchenne muscular dystrophy gene on chromosome X (24, 25) and the major histocompatibility complex on chromosome 6 (26, 27) into fragments of <1.5 Mb. By comparison, these enzymes did not produce alphoid DNA fragments of <2 Mb. The restriction enzyme sites of *Not* I and *Sfi* I, which commonly occur in G+C-rich regions or at CpG-rich HTF ("*Hpa* tiny fragment") islands associated with transcribed genes (28), may be infrequent in alphoid DNA because alphoid sequences are relatively A+T-rich, transcriptionally inactive, and/or modified by methylation (4).

The most striking characteristics of the human centromeric regions are their chromosome-specific macroorganization and high frequency of macro-RFLPs. Others have detected macro-RFLPs associated with a given chromosome-specific alphoid probe. Tyler-Smith and Brown (20) studied two different human Y chromosomes in somatic cell hybrids. In each case, there was a single major block of alphoid DNA of 475 kb and 575 kb. The enzyme *Ava* II detected two clusters of sites within one block but not the other. We also detected *Ava* II variant macro DNA repeats of 475, 570, or 1000 kb at the Y centromeric region of three different individuals (Table 1). Using the enzymes *Pvu* II, *Bcl* I, *Bam*HI, and *Sal* I, Tyler-Smith and Brown (20) detected fragments of >800 kb. With each of these enzymes, we detected fragments of >940 kb with the YC-2 probe in the genome of the one individual tested. The enzymes *Pvu* II and *Bcl* I may prove to be associated with high-frequency macro-RFLPs at the Y centromeric region. By using a chromosome 1 alphoid centromeric probe, variant *Bgl* II fragments of <450 kb have been detected in four individuals (29).

We have detected the high frequency of macro-RFLPs at the human centromeric region by using five or more different individuals, several 6-bp restriction enzymes, and three different alphoid probes. Perhaps these enzymes detect high-frequency macro-RFLPs because their recognition sites occur outside a variable number of tandemly arranged large

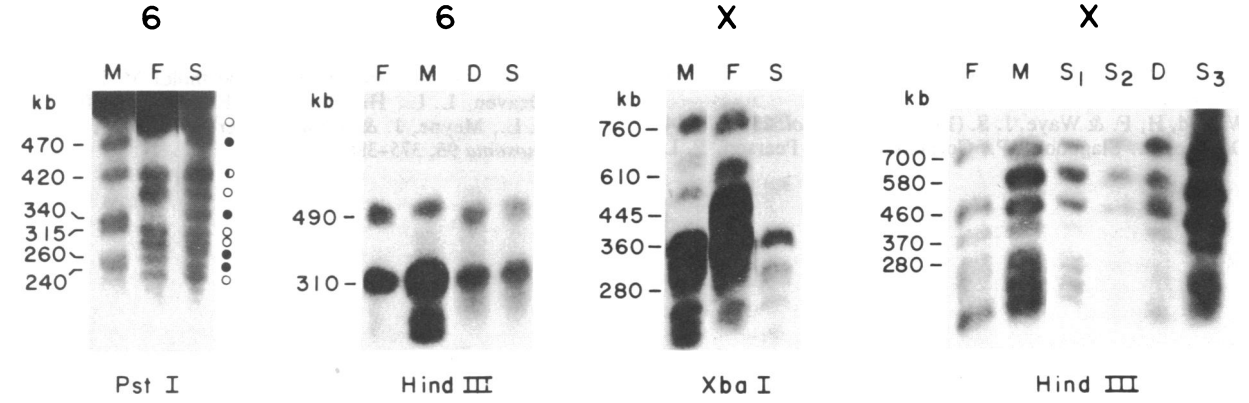

FIG. 3. Inheritance of alphoid macro-RFLPs in four families, as detected by hybridization with the chromosome 6 (*Left*) or X (*Right*) alphoid probe. At far left, genomic DNA samples from mother (M), father (F), and son (S) were digested with *Pst* I and probed with the chromosome 6 alphoid repeat. Open circles denote bands inherited by the son from the father, and the closed circles denote bands inherited from the mother. Note that these *Pst* I macro-RFLPs are different from those detected in other individuals in Fig. 2. The other families also show that sons (S) or daughters (D) have inherited bands from their parents. In the two families on the right the sons (S and S_1–S_3) have inherited all their X chromosome alphoid DNA fragments from their mothers, which is consistent with X-linked inheritance.

alphoid repeats. This phenomenon may be analogous to the variable number of tandem repeats of minisatellite DNA as described by Jeffreys *et al.* (30), but on a megabase scale. Surprisingly, enzymes that are known to cut within alphoid repeats of <1 to 6 kb also yield macro-RFLPs. This suggests that there are blocks of variant repeats of >50 kb that lack these restriction sites. Unequal chromatid exchange, which has been suggested as a mechanism involved in the evolution of alphoid sequences, could account for the occurrence of macro-RFLPs by exchange of large blocks of DNA, as could the more commonly postulated mechanisms of smaller exchanges and point mutations (14).

Other families of repeats may occur within these large blocks of DNA and may be involved in the variability of the macro restriction fragment length. Based on restriction mapping data, it has been suggested that other repeats may be contiguous to the tandem arrays of alphoid repeats (31, 32). We have isolated a region with alphoid DNA and simple repeats. Similar 5-bp simple repeats have been found at the heterochromatin of chromosome 15 (33), and simple repeats at the centromeric region of chromosome 9 have been shown by pulsed-field gel electrophoresis to span a 7- to 10-Mb region (34).

It is likely that chromosome-specific alphoid probes may be used to distinguish most centromeric regions of specific chromosomes in a given individual, as well as from those of another individual. Each individual's polymorphic macro DNA repeat may prove to be so infrequent in the population that it may be useful for identification of individuals by DNA fingerprinting. Also, because these patterns are inherited and new fragments have not been observed to arise frequently after meiosis, chromosome-specific alphoid DNA probes used in conjunction with pulsed-field electrophoresis may be useful for paternity testing, linkage analysis, and mapping of the human centromeric region.

Individual variation within the human centromeric regions begins at the nucleotide level, followed by point mutations, differences in the number of repeats, macro-RFLPs, and ultimately C-band heteromorphisms. The high degree of variability at the centromeric regions suggests that the function of these sequences is not dependent on strict organization of repeats at the centromeric regions.

We thank H. H. Kazazian, Jr. for his helpful discussion and critique of the manuscript. This research was supported by National Institutes of Health Grant HD22819 to E.W.J.

1. Manuelidis, L. (1978) *Chromosoma* **66**, 1–21.
2. Brutlag, D. L. (1980) *Annu. Rev. Genet.* **14**, 121–144.
3. Singer, M. F. (1982) *Int. Rev. Cytol.* **76**, 67–112.
4. Jabs, E. W. & Persico, M. G. (1987) *Am. J. Hum. Genet.* **41**, 374–390.
5. Waye, J. S. & Willard, H. F. (1985) *Nucleic Acids Res.* **13**, 2731–2743.
6. Willard, H. F. & Waye, J. S. (1987) *J. Mol. Evol.* **25**, 207–214.
7. Devilee, P., Slagboom, P., Cornelisse, C. & Pearson, P. L. (1986) *Nucleic Acids Res.* **14**, 2059–2073.
8. Jorgensen, A. L., Bostock, C. J. & Bak, A. L. (1986) *J. Mol. Biol.* **187**, 185–196.
9. Waye, J. S., England, S. B. & Willard, H. F. (1987) *Mol. Cell. Biol.* **7**, 349–356.
10. Wu, J. C. & Manuelidis, L. (1980) *J. Mol. Biol.* **142**, 363–386.
11. Willard, H. F. & Waye, J. S. (1987) *Trends Genet.* **3**, 192–198.
12. Jabs, E. W., Meyers, D. & Bias, W. (1986) *Am. J. Hum. Genet.* **38**, 297–308.
13. Barker, D., Wright, E., Nguyen, K., Cannon, I., Fain, P., Goldgar, D., Bishop, D. T., Carey, J., Baty, B., Kivlin, J., Willard, H., Waye, J. S., Greig, G., Leinwand, L., Nakamura, Y., O'Connell, P., Leppert, M., Lalouel, J. M., White, R. & Skolnick, M. (1987) *Science* **236**, 1100–1102.
14. Jabs, E. W. & Carpenter, N. (1988) *Am. J. Hum. Genet.* **43**, 69–74.
15. Schwartz, D. C. & Cantor, C. R. (1984) *Cell* **37**, 67–75.
16. Carle, G. F. & Olson, M. V. (1984) *Nucleic Acids Res.* **12**, 5647–5664.
17. Fuscoe, J. C., Clark, L. M. & Van Dilla, M. A. (1986) *Cytogenet. Cell Genet.* **43**, 79–86.
18. Jabs, E. W., Wolf, S. F. & Migeon, B. R. (1984) *Proc. Natl. Acad. Sci. USA* **81**, 4884–4888.
19. Wolfe, J., Darling, S. M., Erickson, R. P., Craig, I. W., Buckle, V. J., Rigby, P. W. J., Willard, H. F. & Goodfellow, P. N. (1985) *J. Mol. Biol.* **182**, 477–485.
20. Tyler-Smith, C. & Brown, W. R. A. (1987) *J. Mol. Biol.* **195**, 457–470.
21. Jabs, E. W., Wolf, S. F. & Migeon, B. R. (1984) *Somat. Cell Mol. Genet.* **10**, 93–103.
22. Maniatis, T., Fritsch, E. F. & Sambrook, J. (1982) *Molecular Cloning: A Laboratory Manual* (Cold Spring Harbor Lab., Cold Spring Harbor, NY).
23. Cutting, G. R., Antonarakis, S. E., Youssoufian, H. & Kazazian, H. H. (1988) *Mol. Biol. Med.*, in press.
24. van Ommen, G. J. B., Verkerk, J. M. H., Hofker, M. H., Monaco, A. P., Kunkel, L. M., Ray, P., Worton, R., Wieringa, B., Bakker, E. & Pearson, P. L. (1986) *Cell* **47**, 499–504.
25. Kenwrick, S., Patterson, M., Speer, A., Fischbeck, K. & Davies, K. (1987) *Cell* **48**, 351–357.
26. Lawrence, S. K., Smith, C. L., Srivastava, R., Cantor, C. R. & Weissman, S. M. (1987) *Science* **235**, 1387–1390.
27. Carroll, M. C., Katzman, P., Alicot, E. M., Koller, B. H., Geraghty, D. E., Orr, H. T., Strominger, J. L. & Spies, T. (1987) *Proc. Natl. Acad. Sci. USA* **84**, 8535–8539.
28. Brown, W. R. A. & Bird, A. P. (1986) *Nature (London)* **322**, 477–481.
29. Waye, J. S., Durfy, S. J., Pinkel, D., Kenwrick, S., Patterson, M., Davies, K. E. & Willard, H. F. (1987) *Genomics* **1**, 43–51.
30. Jeffreys, A. J., Wilson, V. & Thein, S. L. (1985) *Nature (London)* **314**, 67–73.
31. McCutchan, T., Hsu, H., Thayer, R. E. & Singer, M. F. (1982) *J. Mol. Biol.* **157**, 195–211.
32. Potter, S. & Jones, R. (1983) *Nucleic Acids Res.* **11**, 3137–3153.
33. Higgins, M. J., Wang, H., Shtromas, I., Haliotis, T., Roder, J. C., Holden, J. & White, B. N. (1985) *Chromosoma* **93**, 77–86.
34. Moyzis, R. K., Albright, K. L., Bartholdi, M. F., Cram, L. S., Deaven, L. L., Hildebrand, C. E., Joste, N. E., Longmire, J. L., Meyne, J. & Schwarzacher-Robinson, T. (1987) *Chromosoma* **95**, 375–386.