

Long-range organization of tandem arrays of α satellite DNA at the centromeres of human chromosomes: High-frequency array-length polymorphism and meiotic stability

(pulsed-field gel electrophoresis/restriction fragment length polymorphism/human repetitive DNA/segregation)

RACHEL WEVRICK AND HUNTINGTON F. WILLARD*†

Department of Medical Genetics, University of Toronto, Toronto, ON M5S 1A8, Canada

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ABSTRACT The long-range organization of arrays of α satellite DNA at the centromeres of human chromosomes was investigated by pulsed-field gel electrophoresis techniques. Both restriction-site and array-length polymorphisms were detected in multiple individuals and their meiotic segregation was observed in three-generation families. Such variation was detected in all of the α satellite arrays examined (chromosomes 1, 3, 7, 10, 11, 16, 17, X, and Y) and thus appears to be a general feature of human centromeric DNA. The length of individual centromeric arrays was found to range from an average of ≈ 680 kilobases (kb) for the Y chromosome to ≈ 3000 kb for chromosome 11. Furthermore, individual arrays appear to be meiotically stable, since no changes in fragment lengths were observed. In total, we analyzed 84 meiotic events involving $\approx 191,000$ kb of α satellite DNA from six autosomal centromeres without any evidence for recombination within an array. High-frequency array length variation and the potential to detect meiotic recombination within them allow direct comparisons of genetic and physical distances in the region of the centromeres of human chromosomes. The generation of primary consensus physical maps of α satellite arrays is a first step in the characterization of the centromeric DNA of human chromosomes.

Centromeres control the disjunction of homologous chromosomes in the first meiotic reductional division, and of sister chromatids in the second meiotic division and in mitosis. Centromeric DNA forms a distinct site for interactions with the spindle apparatus via the kinetochore, a complex proteinaceous structure (1, 2). The properties of centromeric DNA are therefore expected to be somewhat different from those of the rest of the chromosome in terms of replication, transcription, and recombination. Meiotic recombination is reduced near the centromere in many organisms (3–6). In the budding yeast *Saccharomyces cerevisiae*, meiotic recombination is reduced near the centromere both in wild-type yeast and in strains in which the cloned centromere has been displaced to another position on the chromosome (4, 7). In the fission yeast *Schizosaccharomyces pombe*, the centromere of chromosome II is contained in a 60-kilobase (kb) fragment containing repetitive DNA, in which meiotic recombination is greatly reduced (5). Limited data based on mapping of chiasmata suggest that this may also be the case in human chromosomes (6).

The mammalian centromere is cytogenetically defined as the primary constriction in metaphase chromosomes. The human α satellite DNA family is the predominant class of DNA located at the centromere of each human chromosome and, in total, constitutes several percent of the genome (8). Multiple 171-base-pair (bp) monomer units of α satellite DNA

are organized into higher-order repeat units, which are tandemly arranged to form arrays comprising up to millions of base pairs of DNA at human centromeres. At a molecular level, distinct chromosomal subsets from at least 12 autosomes and the X and Y chromosomes have been identified and are distinguished by characteristic long-range periodicities revealed by restriction endonucleases that cleave once per tandemly arranged higher-order repeat unit (8). Mechanisms for the generation and maintenance of tandem arrays of repeated sequences have been postulated (9) and include recombinational events such as unequal sister-chromatid exchange and sequence conversion. Misalignment and recombination between higher-order α satellite repeat units (10, 11) can lead to sequence homogenization and to spreading and fixation of polymorphic variants (8). Further, such events are predicted to result in contractions and expansions in the overall length of the arrays of the chromosomes of chromatids involved and may lead to the generation of a distribution of centromeric array sizes in a population (8).

Highly informative polymorphisms detected by chromosome-specific α satellite probes should form the basis for a series of centromere-based genetic linkage maps of the human genome (12–16) and may also provide a set of molecular markers for investigating both the structure and the genetic behavior of centromeres (17). Recent analyses of arrays of α satellite (18–21) and other satellite DNAs (22–25) by pulsed-field gel electrophoresis techniques have demonstrated the potential of long-range mapping techniques for studies of the genomic organization of tandemly repeated DNA. In this study, we have used chromosome-specific α satellite probes and pulsed-field gel techniques to measure the sizes of α satellite arrays at the centromeres of several human chromosomes in different individuals. We have demonstrated array length polymorphism among different copies of each of the centromeres examined and a high degree of restriction fragment length polymorphism within each array. Such centromere polymorphisms have been used to estimate the meiotic stability of centromeric DNA by tracing individual restriction fragments contained in these megabase-sized arrays through successive meioses.

MATERIALS AND METHODS

Cell Lines and Probes. Lymphoblastoid cell lines used for the family analysis of restriction fragment length polymorphisms were obtained from the National Institute of General Medical Sciences Mutant Cell Repository (Camden, NJ). These cell lines were derived from 13 individuals in a three-generation family (K-1333). High molecular weight DNA

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Abbreviation: CHEF, contour-clamped homogeneous electric field.
*To whom reprint requests should be addressed.

†Present address: Department of Genetics, Stanford University, Stanford, CA 94305.

from these cell lines and other established cell lines, including somatic cell hybrids containing a limited number of human chromosomes (e.g., see ref. 26), was prepared as described below. Cloned α satellite probes specific for the following chromosomes were used: chromosome 1 [probe pSD1-1 (*D1Z5*), ref. 18]; 3 [probe p3-5 (*D3Z1*), ref. 21]; 7 [probes pMGB7 (*D7Z2*) and pa7t1 (*D7Z1*), ref. 27]; 10 [probe pa10RP8 (*D10Z1*), ref. 28]; 11 [probe pLC11A (*D11Z1*), ref. 29]; 16 [probe pSE16.2 (*D16Z2*); ref. 36]; 17 [probe p17H8 (*D17Z1*), ref. 26]; X [probe pBamX7 (*DXZ1*), ref. 30]; and Y [probes Y97 (*DYZ3*), ref. 31, or pYCS-1c (*DYZ3*), C. Sharp and H.F.W., unpublished work].

DNA Preparation and Electrophoresis. High molecular weight DNA was isolated from cultured lymphoblasts essentially as described (32). *S. cerevisiae* chromosome size markers were strain YNN265 (Bio-Rad). *Sch. pombe* chromosome size markers were prepared from strain 972 by the method of Vollrath and Davis (33). Contour-clamped homogeneous electric field (CHEF) gel electrophoresis (ref. 34; apparatus from Bio-Rad) was used in the mapping experiments. CHEF electrophoresis was performed in 0.5 \times TBE buffer (45 mM Tris/45 mM boric acid/1 mM EDTA, pH 7.6) at 15°C, as specified in the figure legends. After ethidium bromide staining and photography, pulsed-field gels were treated with 0.25 M HCl for 10 min prior to transfer of DNA. Methods of transfer to nylon filters, prehybridization, and chromosome-specific hybridization with 32 P-labeled α satellite probes have been described (26).

Choice of Restriction Enzymes. The repetitive nature of α satellite DNA requires the choice of appropriate restriction enzymes for each array under investigation. Enzymes chosen for these experiments, as for previous α satellite polymorphism studies (12, 28), were those that cut frequently in nonrepetitive DNA but infrequently within the α satellite array. These enzymes release a limited number of large fragments from the α satellite array and are thus useful not only for segregation analysis in families but also for array size estimates, since they yield fragments expected to contain a minimal amount of flanking nonrepetitive DNA. However, they may not exclude from these fragments other simple sequence DNAs, as may be the case with the two distinct α

satellite arrays on chromosome 7 (27) or with satellite III near the Y chromosome centromere (35).

RESULTS

Long-Range Polymorphisms in α Satellite DNA. Previous experiments suggested the presence of α satellite polymorphisms involving large DNA fragments (12, 18–21, 28). To investigate the frequency of these polymorphisms in a number of different α satellite arrays (from chromosomes 1, 3, 7, 10, 11, 16, 17, X, and Y), we screened DNA from multiple individuals with enzymes not expected to cut in the higher-order repeat unit in CHEF gels. A pattern of multiple hybridizing bands of varying, apparently random lengths was seen in most of the arrays investigated with multiple enzymes, with examples shown in Fig. 1 A and B for probes from the centromeres of chromosomes 11 and 16. For example, an α satellite probe for the centromere of chromosome 16 detected fragments ranging in size from 40 to 1200 kb in *Bgl* II-digested DNA from six unrelated individuals, with a different pattern in each individual examined (Fig. 1B). Probes associated with larger arrays may leave some fragments unresolved under these running conditions (e.g., Fig. 1B). DNA from a large three-generation family (K-1333) was analyzed by CHEF electrophoresis in order to investigate the segregation of restriction site and array length polymorphisms (Fig. 2); these data demonstrate Mendelian inheritance of the arrays and their associated polymorphisms.

Array Length Polymorphisms and Size Estimates. The smaller arrays, found in the centromeric DNA of the Y chromosome (Fig. 1C) and in domain I of chromosome 7 centromeric α satellite array (data not shown), yield only one or two hybridizing fragments respectively upon digestion with a number of enzymes, presumably representing the entirety of each array in a single fragment. The presence of only one band per chromosome allowed unambiguous assignment of bands to individual chromosomes, and, accordingly, size estimates for these arrays could be derived from the lengths of these single hybridizing bands. Size estimates obtained with different enzymes were generally consistent. However, since any such measurement introduces a possible error in the inclusion of some flanking DNA due to the

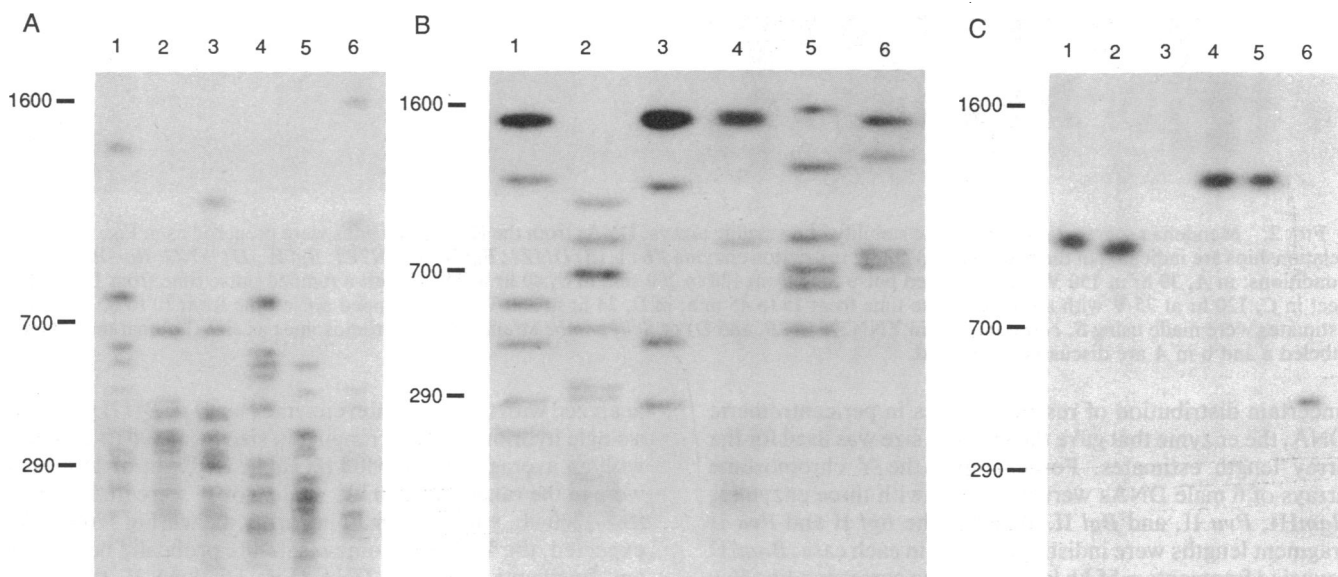


FIG. 1. The extent of polymorphism in α satellite DNA, shown in digests of six human genomic DNAs. DNAs were digested, fragments were separated by CHEF gel electrophoresis and transferred to nylon filters, and the resulting Southern blots were probed. (A) Locus *D11Z1*; restriction enzyme *Pvu* II. (B) *D16Z2*; *Bgl* II. (C) *DYZ3*; *Pvu* II. In A and C, lanes 2 and 3 represent a male and his mother. All other samples are from unrelated individuals. CHEF electrophoresis was at 200 V with a pulse time of 60 sec for 15 hr followed by 90 sec for 8 hr. Size estimates were made using chromosomes from *S. cerevisiae* strain YNN295 as markers (marker sizes in kilobase pairs at left).

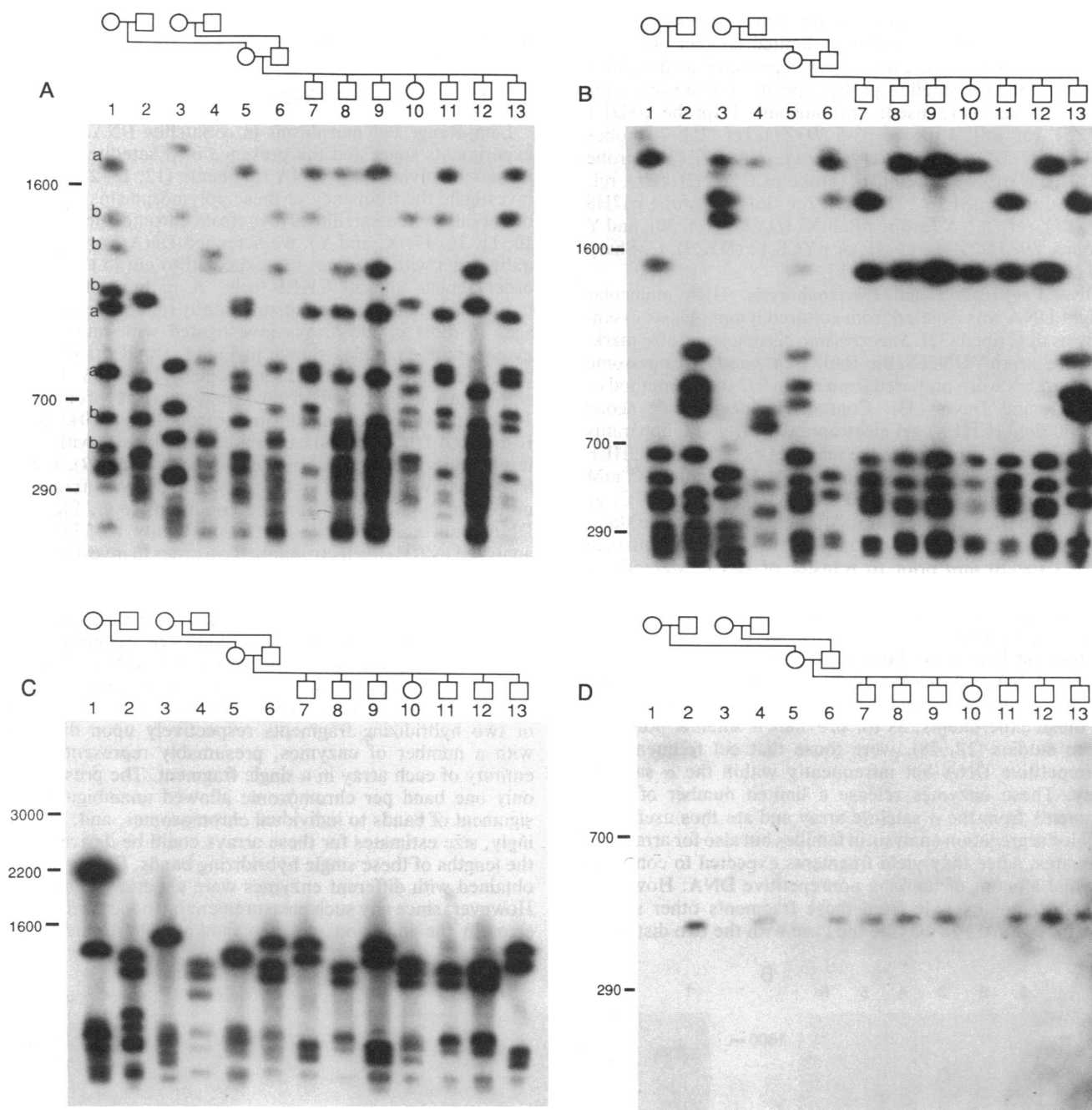


FIG. 2. Mendelian segregation and meiotic stability of α satellite arrays. DNAs from the K-1333 cell lines were prepared as in Fig. 1. Family relationships are indicated in the pedigree. (A) *D7Z1*; restriction enzyme *Xba* I. (B) *D11Z1*; *Bgl* II. (C) *D17Z1*; *Bgl* II. (D) *DYZ3*; *Bam*HI. CHEF conditions: in A, 39 hr at 150 V with a ramped pulse time from 120 to 200 sec; in B, 40 hr at 150 V with a ramped pulse time from 120 to 200 sec; in C, 120 hr at 75 V with a ramped pulse time from 15 to 45 min; in D, 24 hr at 200 V with a ramped pulse time from 10 to 80 sec. Size estimates were made using *S. cerevisiae* strain YNN295 (A, B, and D) or *Sch. pombe* strain 972 (C) chromosomes as size (kb) markers. Bands labeled a and b in A are discussed in the text.

uncertain distribution of restriction sites in pericentromeric DNA, the enzyme that gave the smallest size was used for the array length estimates. For example, the Y chromosome arrays of 6 male DNAs were examined with three enzymes, *Bam*HI, *Pvu* II, and *Bgl* II. Whereas the *Bgl* II and *Pvu* II fragment lengths were indistinguishable in each case, *Bam*HI revealed fragments ≈ 55 kb larger than the corresponding *Pvu* II or *Bgl* II fragment. These results are consistent with those of Tyler-Smith and Brown (19), who analyzed two Y chromosome arrays in somatic cell hybrids in a similar manner. The Y chromosome α satellite arrays measured appeared to fall into two size groups. DNA from each of 15 males

analyzed with the restriction enzyme *Pvu* II or *Bgl* II revealed a single hybridizing band ranging in size from 300 to 1200 kb, with an average size of 680 kb (Table 1). Eight of 15 arrays were in the range 300–380 kb, while 6 of 15 were in the range 890–1200 kb, with the remaining array measuring 570 kb. As expected, the Y chromosome α satellite probe did not detect any fragments in female DNA (Fig. 1C, lane 3), thus confirming the chromosome-specific nature of these probes and the conditions of hybridization.

In contrast to the relatively simple cases above, multiple bands from larger autosomal arrays could only be separated and assigned to one of the two homologues by analysis of

Table 1. Estimates of α satellite array size

Chromosome (locus)	No. of samples	Mean size, kb	Range, kb
1 (<i>D1Z5</i>)	8	915	440–1510
7-I (<i>D7Z2</i>)	21	265	100–550
7-II (<i>D7Z1</i>)	8	2580	1530–3810
10 (<i>D10Z1</i>)	8	2110	1390–2515
11 (<i>D11Z1</i>)	8	2970	1960–4760
16 (<i>D16Z2</i>)	8	1290	430–1805
17 (<i>D17Z1</i>)	9	2365	1165–3710
Y (<i>DYZ3</i>)	15	680	300–1200

their meiotic segregation in a large family. The segregation of bands was consistent with the presence of a single large array per chromosome, and thus bands that always cosegregated could be assigned to the same centromeric array. For example, the probe *pat1* (domain II, chromosome 7) recognized a large set of hybridizing bands in each member of a three-generation family (Fig. 2A). In an *Xba* I digest of DNAs from this family, all the hybridizing bands in the third generation could be traced from the parents and the grandparents, with no rearrangements or recombination observed. The sum of the sizes of cosegregating bands (such as the bands labeled "a" in Fig. 2A, lane 1) in each individual grandparent (Fig. 2, lanes 1–4) provided four of the eight size estimates, while bands in the grandparents that did not segregate to the parents (Fig. 2, lanes 5 and 6) were assumed to make up the remaining arrays, and their sum was used for an additional four estimates (such as the bands labeled "b" in Fig. 2A, lane 1). Any bands that appeared to be doublets were scored as such. As well, up to three different pulsed-field gels were run under different conditions, with the same restriction enzyme, to resolve and measure all the bands belonging to a particular array (data not shown). The possible exclusion of relatively small fragments, although a source of underestimation, is expected because of their size to have little effect on the overall estimates. Based on these considerations and on such analysis with α satellite probes from the centromeric DNA of chromosomes 1, 7, 10, 11, 16, and 17 (Fig. 2 and data not shown), we obtained size estimates and ranges for the centromeric arrays shown in Table 1. We noted a 2- to 4-fold variation in α satellite array size between homologous chromosomes. This involved a difference of up to 3000 kb between the largest and smallest arrays measured on homologous chromosomes (e.g., chromosomes 11).

Meiotic and Mitotic Stability of α Satellite Arrays. Southern blots of DNA from a large three-generation family were inspected for evidence of recombinational events. All the α satellite fragments could be resolved and measured for each of the arrays investigated and were analyzed for changes in length due to possible meiotic or mitotic recombinational events. No such events were observed, indicating the absence of major structural rearrangements or recombinations in any of the α satellite arrays. An estimate of the total physical distance involved was derived by tracing the segregation of the four grandparental arrays for each chromosome and by multiplying the size of each array by the number of meioses through which it had been transmitted. For example, for chromosome 17 (Fig. 2C), the 2070-kb array from the maternal grandmother in lane 1 segregated through the mother to four children, for a total of 8280 kb transmitted with no meiotic recombination observed. A similar analysis was performed for the three other segregating grandparental arrays, leading to a figure of $\approx 37,000$ kb of chromosome 17 α satellite transmitted through this family. These data, and data from a similar analysis of α satellite arrays from the centromeres of chromosomes 1, 7, 10, 11, and 16, are summarized in Table 2. In addition, since all centromeric fragments could be traced back to a previous generation, it is

Table 2. Meiotic stability of α satellite arrays

Autosome	Sizes of transmitted arrays,* kb	Total transmitted, kb	Crossovers observed
1	1855; 1510; 680; 965	14,500	0
7 [†]	3890; 2380; 2710; 1960	40,930	0
10	2515; 1390; 2405; 2240	31,700	0
11	3485; 4760; 3020; 2425	44,430	0
16	875; 1805; 1320; 1665	22,330	0
17	2070; 2600; 2150; 3710	37,370	0
	(Total)	191,260	0

*In the order maternal grandmother, maternal grandfather, paternal grandmother, paternal grandfather as in the pedigree in Fig. 2.

[†]The two arrays on chromosome 7 (7-I and 7-II in Table 1) were combined for this calculation.

clear that no major rearrangements or recombinational events have occurred during propagation of the lymphoblast cell lines in culture. Thus there is no evidence for unusual mitotic events in these autosomal arrays or in the Y centromere array (Fig. 2D).

DISCUSSION

We have used the polymorphic variation in α satellite DNA to investigate the long-range organization of centromeric α satellite arrays, of which many subsets have been characterized at a molecular level. In this study, we used pulsed-field gel restriction analysis to demonstrate variation in array length and Mendelian inheritance of sequence polymorphisms in α satellite arrays in multiple centromeric regions.

As analyzed in conventional gels, digestion of genomic DNA with enzymes that are not likely to cut within higher-order repeat units releases a small number of polymorphic fragments that can be scored as dominantly inherited traits (12, 28). In the course of this study, up to four completely informative restriction enzymes that cleave only rarely in the higher-order repeat unit were used to analyze the segregation of polymorphisms in family studies. The unique pattern of bands in each individual indicates that these sites are likely to be due to sequence variation (11) rather than to any long-range structure imposed upon these arrays. Chromosome-specific α satellite has now been described for at least 14 human chromosomes (e.g., refs. 8 and 28). By use of these probes and proper conditions, many families could now be typed for segregation of these centromeric arrays, thereby anchoring existing linkage maps for these chromosomes at the centromere (e.g., ref. 14).

Chromosome-specific probes for α satellite repeats have been used in this study to obtain size estimates for many of these tandem arrays. The arrays vary in size between homologous chromosomes in the population, consistent with theories of evolution of tandem arrays by recombinational mechanisms (e.g., ref. 9). The distribution of these array length polymorphisms in a larger sample could contribute to estimates of the rates of evolution of the arrays in a population, although better estimates of the frequency of unequal recombinational events (37) and the physical constraints on misalignment are required. Some data are available as to the extent of such mispairing in relatively short hypervariable (minisatellite) regions of the human genome (38, 39) and in the ribosomal RNA genes in yeast (40), although it is unclear what the equivalent parameter would be in human α satellite arrays. An interesting observation was made of the Y chromosome array length polymorphisms, which appeared to fall into two groups based on size. We have not yet attempted to characterize these groups for other distinguishing polymorphisms. This apparently bimodal distribution may be unique to the Y chromosome and may reflect a modified mechanism (or rate) of evolution of these long tandem arrays of DNA on

this chromosome. A mitotic recombination event creating two unequally sized arrays early in the evolution of this DNA family, combined with the absence of meiotic recombination for the Y centromere, is one possible explanation for this observation.

The investigation of meiotic segregation of multiple α satellite arrays (e.g., Fig. 2) provided no evidence of meiotic rearrangements or recombinational events in the meioses examined, suggesting that α satellite arrays are, to a first approximation, meiotically stable. This included 14 informative meioses per autosomal centromere examined, representing the segregation of about 191,000 kb of α satellite through 84 meiotic events (Table 2). The observation of no recombinational events in 191,000 kb suggests a frequency (based on 95% confidence limits) of 0–1.5% meiotic recombination per megabase of α satellite DNA. This compares with the average genome estimate of 1% recombination per megabase of DNA (41). Further analyses are required to determine more precisely the rate of recombination and whether recombination rates are suppressed at human centromeres as is the case in other organisms (3–6). However, it is clear from the data obtained thus far that α satellite arrays are not highly recombinogenic as has been described for some minisatellite loci (38).

The unique features of α satellite arrays and their analysis by pulsed-field gel electrophoresis provide a system for attempting to correlate genetic and physical distances. As such, these data should be of general significance for analysis of genetic linkage in the human genome, in addition to their specific relevance to centromere structure and behavior.

In *S. cerevisiae* and *Sch. pombe*, the first step in attempts to characterize DNA sequences responsible for centromere activity was to generate a primary map of the centromeric region. In *S. cerevisiae*, this required chromosome "walking" from genes flanking the centromere and eventually led to the discovery and cloning of essential centromere sequences (4, 42). In *Sch. pombe*, physical maps of the centromeric regions (5, 43) identified a region of repeated DNA elements not found elsewhere in the genome. Recent data have indicated that these repetitive DNA regions are associated with centromere activity (44). It is reasonable to expect that similar mapping of human centromeric sequences will eventually lead to the identification of the functional sequences themselves.

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