Volume 14 Number 17 1986 Nucleic Acids Research

Molecular analysis of a deletion polymorphism in alpha satellite of human chromosome 17: evidence for homologous unequal crossing-over and subsequent fixation

John S.Waye and Huntington F.Willard*

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

Received 28 May 1986; Accepted 28 July 1986

ABSTRACT

The human alpha satellite DNA family is organized into chromosome-specific subsets characterized by distinct higher-order repeats based on a ~171 basepair monomer unit. On human chromosome 17, the predominant form of alpha satellite is a 16-monomer (16-mer) higher-order repeat present in 500-1000 copies per chromosome 17. In addition, less adundant 15-monomer and 14-monomer repeats are also found constitutively on chromosome 17. Polymorphisms in the form of different higher-order repeat lengths have been described for this subset, the most prominent polymorphism being a 13-monomer (13-mer) higher-order repeat present on ~35% of all chromosomes 17. To investigate the nature of this polymorphism, we have cloned, sequenced and compared the relevant regions of the 13-mer to the previously characterized 16-mer repeat. The results show that the repeats are virtually identical, with the principal difference being the exclusion of three monomers from the 13-mer repeat. We propose that the 13-mer is the product of an isolated homologous recombination event between two monomers of the 16-mer repeat. Sequence comparisons reveal the approximate site of recombination and flanking regions of homology. This recombination site corresponds to a position within the alphoid monomer which has been previously implicated in an independent homologous recombination event, suggesting that there may exist a preferred register for recombination in alphoid DNA. We suggest that these events are representative of an ongoing process capable of reorganizing the satellite subset of a given chromosome, thereby contributing to the establishment of chromosome-specific alpha satellite subsets.

INTRODUCTION

The genomes of most eukaryotes are comprised of unique sequence and repetitive DNA, the latter class being either interspersed with unique DNA or tandemly reiterated (1). A curious property of many repetitive DNA families is a high degree of homogeneity within species (reviewed in 2-6), thought to be the consequence of a concerted process of homogenization occurring both within and between chromosomes (7). Indeed, it has been suggested that periodic homogenization of tandemly repeated satellite DNA may have immediately preceded (and may be causally related to) active speciation (8). One mechanism thought to be involved in the evolution of such satellite DNA families is homologous unequal crossing-over between or within tandem arrays,

which can generate potentially novel repeat structures with altered periodicities (9). Subsequent fixation of a variant repeat might then lead to the apparent reorganization of these satellite sequences within a genome.

In the genomes of humans and other primates, the centromeric region of each chromosome is dominated by a tandemly repeated DNA family based on a monomer repeat length of ~171 basepairs (bp) (10-15). This diverse DNA family, alpha satellite, constitutes as much as 5% of the human genome and is organized as chromosome-specific subsets (16-19). Each subset is characterized by a distinct higher-order repeat(s) which has been independently established or fixed. Operationally defined, the higher-order repeat represents a recently amplified unit, identified by restriction endonuclease cleavage sites which occur once within the higher-order repeat (20,21). As such, independent copies of the higher-order repeat show only limited sequence divergence compared to homologies amongst monomers which constitute the higher-order repeat (22,23). For example, the human X chromosome higher-order repeat is a 2.0 kilobasepair (kb) BamHI repeat comprised of 12 diverged monomers arranged as two adjacent and related pentamer blocks plus an additional 2 monomers also related to monomers within the pentamer blocks (22). Independent copies of this higher-order repeat are >99% identical in sequence, whereas identities between monomers within the repeat range from 65-85% (22).

As part of our analysis of the human alpha satellite family, we have cloned and characterized representative higher-order repeats from several human chromosomes (22-25). Extensive sequence comparisons have led to the derivation of monomeric configurations for the different higher-order repeats. Based on these analyses, it is evident that different subsets may share common monomeric organizations and can be grouped accordingly. Most notably, the higher-order repeats of chromosomes 1, 11, 17 and X are each based, in part, on a conserved pentameric configuration (ref. 23 and our unpublished results). Although these contemporary subsets are characterized by distinct higher-order repeats, each has apparently evolved from the same ancestral pentamer. Homologous unequal crossing-over is a conceivable mechanism for the generation and fixation of subtly different higher-order repeats from such a progenitor pentameric repeat.

In this report, we describe the molecular relationship between different higher-order repeats found on chromosomes 17 of a proportion of the population. Because this chromosomal subset is characterized by a series of related higher-order repeats (23), some of which are polymorphic in human populations (24), it provides a unique opportunity to investigate the molecu-

lar events which have contributed to the generation of different higher-order repeats. Previously we have characterized the 16- and 15-monomer higher-order repeats found on all chromosomes 17 (23). In addition, we have noted repeat length polymorphisms ranging in lengths corresponding to 12-18 monomers (24). The most prominent polymorphic repeat unit is 13 monomers in length, present in approximately 500-1000 copies on at least ~35% of chromosomes 17 in different human populations. We have now cloned, sequenced and compared relevant regions of the 13-monomer repeat (13-mer) to the previously characterized 16-monomer repeat (16-mer). The results indicate that there is a precise three monomer deletion in the 13-mer relative to the 16-mer. The approximate boundaries of this polymorphic deletion have been determined and a probe corresponding to the deleted region has been constructed from the cloned 16-mer. Using this probe, we have shown that the 13-mer repeats of individuals representing different ethnic and racial groups share the same relative deletion. Thus, we propose that the 13-mer higher-order repeat has resulted from an isolated homologous unequal recombination event between two monomers of the 16-mer. The relatively uniform distribution of this polymorphism thoughout the human species supports the notion that the 13-mer was generated and fixed during human evolution at some point prior to the separation of the races. We suggest that such events constitute an ongoing process capable of introducing variability within a satellite family, and thus serve as a model for evolutionary mechanisms by which variant higher-order repeats would be isolated and fixed on individual chromosomes.

MATERIALS AND METHODS

Southern Blot Analysis

Methods for DNA preparation, restriction endonuclease digestion, electrophoresis, transfer to nitrocellulose, prehybridization and hybridization to radioactive DNA probes have been described (17,22,25). Filters were washed under high stringency conditions (final wash in 0.1 x SSC, 0.1 % SDS, 68° C) to minimize hybridization to alphoid sequences of heterologous chromosomes.

Hybridization Probes

Chromosome 17 alpha satellite genotypes were defined using a cloned copy of the 2.7 kb 16-mer higher-order repeat (p17H8) which has been described previously (23,24). Under conditions of high stringency (see above) this probe hybridizes not only to the cognate 16-mer repeat, but also to the other higher-order repeat lengths found on chromosome 17. The deletion probe (p17H8E10) used to assess the 13-mer repeats consists of a 270 bp subfragment

of pl7H8 which extends from the HindIII site (position 1614 in the 2.7 kb repeat) to position 1884. Position 1884 is an endpoint derived by progressive exonuclease III digestion of pl7H8 as described previously (23). This fragment corresponds to a portion of the region which is excluded from the 13-mer clone (pAR13) relative to the 16-mer clone (pl7H8).

Chromosome 17 Alpha Satellite Genotypes

The chromosome 17 alpha satellite genotypes and the frequencies of the polymorphic repeat lengths in a Caucasian population have been described (24). The deletion probe (pl7H8E10) was used to screen >100 individuals which were previously genotyped using the complete 16-mer probe (pl7H8). These individuals included members of the following racial and ethnic groups: Caucasian, American black, Chinese, Greek, Italian, Asian Indian, Lebanese, Mexican, Metis Indian, and Laotian.

Cloning of the 13-mer Repeat

The relevant portion of the 13-mer repeat was isolated from genomic DNA of an individual who was homozygous for the 13-mer higher-order repeat polymorphism. Ten micrograms of genomic DNA was cleaved with HindIII and EcoRI and size-fractionated by electrophoresis through 0.6% low melt agarose. The 2.2 kb fraction was isolated and mixed with 500 nanograms pSP65 vector (26) which had been digested with HindIII and EcoRI. The mixture was ligated in the gel and used to transform *E.coli* HB101. The resulting library, containing approximately 200 clones, was screened with the 16-mer insert from pl7H8.

Nucleotide Sequencing

The XbaI-EcoRI subfragment of pAR13 was subcloned into pSP65 and sequenced from each end using priming sites located to each side of the polylinker. The primers used were the M13 reverse primer (New England Biolabs) and a SP6 primer (Boehringer Mannheim) which is complementary to a region of the SP6 polymerase binding site of the vector. Double-stranded plasmid templates were sequenced using the dideoxy-termination method (27) as described by Korneluk et al. (28).

RESULTS

Chromosome 17 Higher-Order Repeats

We have previously characterized a prominent 16-mer higher-order repeat and a less abundant 15-mer repeat, both represented constitutively on human chromosome 17 (23). In addition, Mendelian segregating polymorphisms in the form of different higher-order repeat lengths have also been described (24).

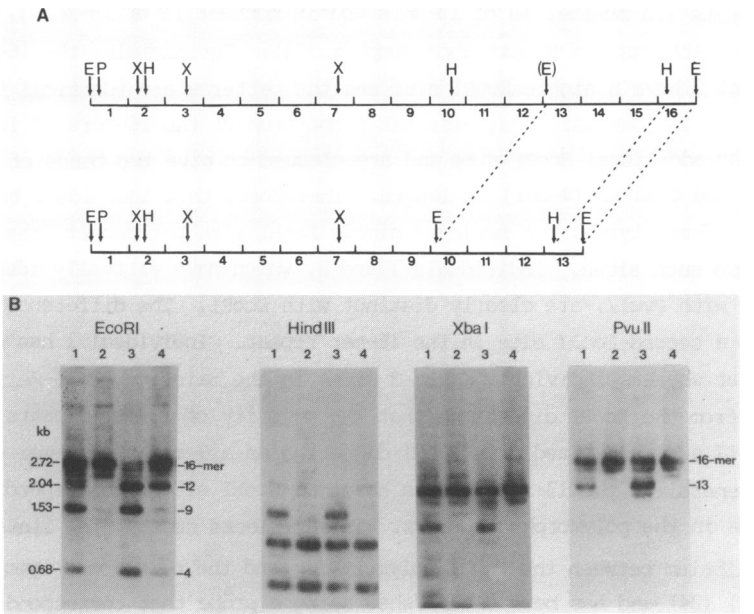

Figure 1. The restriction endonuclease maps of the 13 and 16 monomer higher-order repeats. (A) The approximate positions of EcoRI (E), HindIII (H), PvuII (P) and XbaI (X) sites within the 16-mer (upper line) and 13-mer (lower line) higher-order repeats are shown. The numbers refer to monomer designations used throughout the text. The EcoRI site in parentheses indicates that the site is polymorphic (24). (B) Southern blot analysis of genomic DNA from different individuals (1-4) analyzed with the indicated restriction endonucleases. Relevant band sizes and monomer lengths are indicated.

The most prominent polymorphism is noted by the presence of up to 500-1000 copies of a 13-mer repeat on ~35% of all human chromosomes 17 (24). Southern blot analysis of DNA from selected genotyped individuals was used to derive restriction maps for both the 16-mer and 13-mer higher-order repeats (Fig. 1A), the former being confirmed by sequence analysis of cloned 16-mers. Examples of Southern blot patterns which illustrate the comparative restriction maps are shown in Fig. 1B. PvuII, an enzyme that generally cleaves once per higher-order repeat (regardless of length), gives the clearest indication of the repeat lengths represented in a given individual. Thus, individuals 2 and 4 have the constitutive 16-mer/15-mer pattern whereas individuals 1 and 3 have, in addition to these repeats, a prominent 13-mer repeat and less abundant 14-mer and 12-mer lengths (Fig. 1B). Similarly, EcoRI cuts most repeats once, the cleavage site defining the first base of monomer 1 (23) (Fig. 1A). However, due to the presence of a second polymorphic EcoRI site in

some repeats (in monomer 10 of 13-mers and in monomer 13 of 16-mers), the resulting patterns can appear more complex (24). For example, the 16-mers of individual 2 have a single EcoRI site and the patterns are identical for EcoRI and PvuII. In contrast, a significant proportion of the 16-mers of individual 4 have the additional EcoRI site and are cleaved to give two bands of 2.0 kb (12-mer) and 0.68 kb (4-mer) in length. Therefore, this individual has two distinct 16-mer types, one having a single EcoRI site per repeat, the other having two such sites. Individuals 1 and 3, which have virtually identical patterns with PvuII, are clearly distinct with EcoRI. The difference again concerns a second EcoRI site in the 16-mer repeat. Individual 1 has one site per repeat whereas individual 3 has 2 sites in the majority of 16-mers. It is evident from the EcoRI digestions that the majority of 13-mer repeats observed with PvuII are recognized with EcoRI digestion as a 9-mer plus 4-mer combination. Generally, the 13-mer repeats have two EcoRI sites, corresponding to the sites in the polymorphic 16-mers. This reflects substantial linkage disequilibrium between the EcoRI polymorphism and the deletion polymorphism (13-mers) (24) and has been established using a probe that corresponds to monomers 13-16 of the 16-mer (Fig. 1A). This probe hybridizes preferentially to the 4-mer fragments derived from 13-mer, as well as the 16-mer, repeats (data not shown).

From the outset, it was apparent that the principal difference between the 13- and 16-mer repeats was a relative deletion in the 13-mer. The restriction maps derived using XbaI and HindIII (Fig. 1A) enabled us to position the deletion relative to the 16-mer. With XbaI, the largest fragment observed in digest of DNA from individuals homozygous for the 16-mer repeat was simply reduced in size (by three monomers) in digests of DNA from individuals possessing the 13-mer repeat. This placed the deletion downstream of the XbaI site in monomer 7 (Fig. 1A). In contrast, digestion of 13-mer repeats with HindIII resulted in the generation of a larger fragment than seen in 16-mers. This indicated that the deletion had eliminated the HindIII site of monomer 10 in the 16-mer. Thus, the data from genomic blotting experiments are consistent with there being a three monomer deletion in the 13-mer relative to the 16-mer, its position localized to the region of the 16-mer bound by the XbaI (monomer 7) and EcoRI (monomer 13) sites and encompassing the HindIII (monomer 10) site.

Cloning of the 13-mer Higher-Order Repeat

To optimize cloning efficiency, we employed DNA from an individual who was homozygous for the amplified 13-mer repeat (similar to individual 3, Fig.

1B). Genomic restriction maps had indicated the approximate region of the relative deletion (see above) and based on those results, a fragment length corresponding to the region of the 13-mer defined by the HindIII (monomer 2) and EcoRI (monomer 10) sites (see Fig. 1A) was isolated from genomic DNA and a plasmid library was constructed (see Materials and Methods). Of the ~200 clones obtained, one (pAR13) hybridized with the cloned 16-mer repeat and had the predicted restriction map for the 13-mer repeat.

Molecular Analysis of the 13-mer Repeat

Because the relative deletion in the 13-mer was thought to reside between the XbaI (monomer 7) and EcoRI (monomer 10) sites, detailed characterization centered around this subfragment of clone pAR13. The complete nucleotide sequence was determined and compared with the sequence previously obtained for the corresponding region of the 16-mer repeat (23). The 13-mer and 16-mer sequences were aligned by either the XbaI or EcoRI sites as shown in Fig. 2A. A most striking feature is the abrupt and symmetric homology breakpoint occurring at positions 1517 and 2029 of monomers 9 and 12 respectively. When the sequences were aligned by their XbaI sites, there are only 4 mismatched positions in the 463 bp preceding this point. However, in the 63 bp that follow, there are 12 mismatches. The converse relationship was evident when the homology comparisons were based on EcoRI alignment of the sequences. In this case, just 2 mismatches occur in the first 63 bp (from the EcoRI site) (one of these mismatches being due to the polymorphic EcoRI site present in pAR13 and absent in the 16-mer clone) while 111 mismatches occur in the 463 bp that follow. These data are consistent with there being a breakpoint in the 13-mer which represents the junction formed between positions 1516 and 2029 or 1517 and 2030 of the 16-mer (Fig. 2A). As represented in Fig. 2B, the deletion is precisely three monomers in length and is contiguous in nature. Homologous unequal crossing-over between monomers 9 and 12 of the 16-mer (with the putative point of strand exchange corresponding with the homology breakpoints) is a most probable mechanism for generation of this 13-mer (Fig. 2B).

Because the 13- and 16-mer repeats are ~99% identical in sequence (disregarding the portion deleted in the 13-mer), the sensitivity of this analysis was maximized. The recombination occurred between two monomers (monomers 9 and 12 of the 16-mer) which are only ~75% identical in sequence (23), thereby facilitating recognition of the putative recombination junction. However, as highlighted in Fig. 2A, there are stretches of 15 and 19 perfectly matched nucleotides immediately 5'and 3', respectively, of the

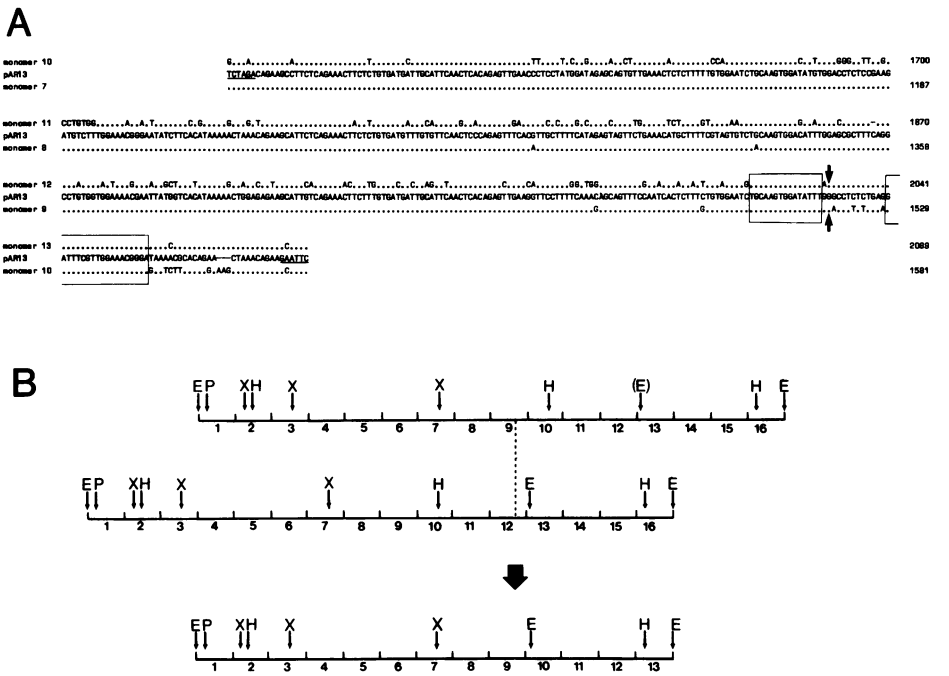

Figure 2. Nucleotide sequence of the 13-mer higher-order repeat. The sequence of the pAR13 region bound by the XbaI (monomer 7) and EcoRI (monomer 10) sites is given in part A. The sequence is presented as monomers according to the EcoRI register of the 16-mer higher-order repeat (23). Comparisons with the sequence of a 16-mer clone (p17H8, ref. 23) were based on either XbaI (sequence line below pAR13) or EcoRI (sequence line above pAR13) alignment. Positions which differ between the sequences are shown and periods indicate positions of sequence identity. Monomers (left) and base positions (right) in the 16-mer repeat sequence are indicated. Arrows show the putative sites of strand exchange between monomers 9 and 12 of the 16-mer. Regions of perfect match flanking these positions are shown within boxes. (B) Schematic representation of homologous unequal crossing-over between monomers 9 and 12 of two misaligned 16-mer repeats. The putative point of strand exchange is shown by the broken line.

putative exchange points in monomers 9 and 12 of the 16-mer. Therefore, with minimal disruption of the patterns of homology between the 13-mer and 16-mers, the putative junction boundaries could be shifted in the 5' direction up to 15 bp (requiring one additional mismatch) or, less likely, in the 3' direction up to 30 bp (requiring an additional four mismatches). The possible significance of recombination occurring at this position within the monomers will be discussed later.

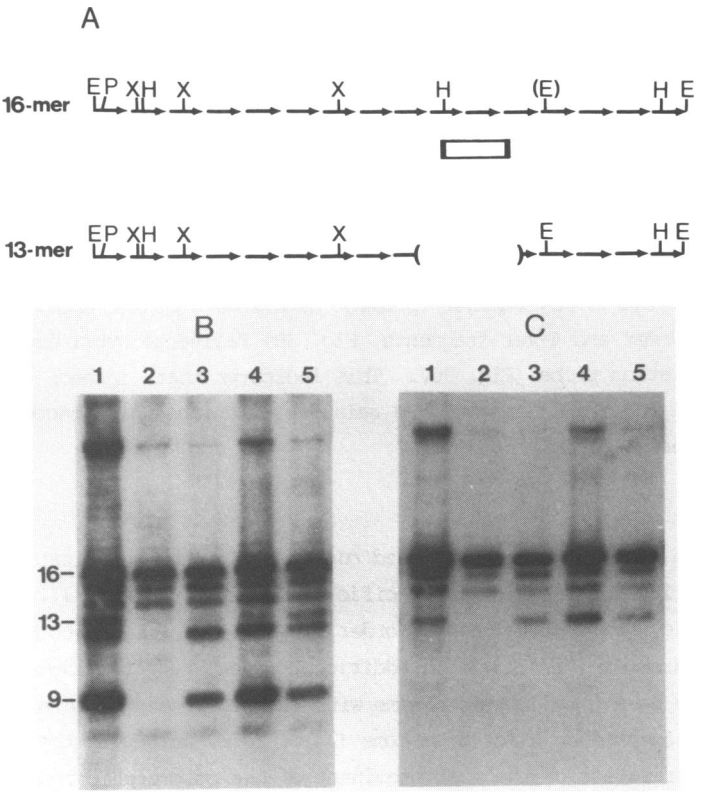

Figure 3. Characterization of independent 13-mer repeats using the deletion probe. (A) Schematic representation of the 16-mer and 13-mer repeats aligned for maximum homology. The region excluded from 13-mer clone (pAR13) is shown in parentheses and the region of the 16-mer which comprises the deletion probe (p17H8E10) is indicated by the closed rectangle. HindIII (H), PvuII (P), XbaI (X) and EcoRI (E) sites are shown. (B,C) The DNA from five individuals (1-5) digested with EcoRI and probed with the complete 16-mer probe (p17H8, panel B). Following autoradiography, the probe was melted off and the blot was rehybridized using the deletion probe (p17H8E10, panel C). The 16-, 13-, and 9-mer EcoRI fragments are indicated.

Universality of the 13-mer Higher-Order Repeat

As a result of extensive alpha satellite haplotype analyses, we estimate that a 13-mer higher-order repeat is present on at least ~35% of the chromosomes 17 in different human populations (ref. 24 and our unpublished results). However, it is conceivable that 13-mer repeats in different individuals may be the products of independent recombination events occurring in the same general region of the 16-mer. Using genomic restriction analy-

ses, the location of the 0.5 kb deletion can be placed only between the XbaI (monomer 7) and EcoRI (monomer 13) sites of the 16-mer, a region spanning >1 kb. In an effort to assess the extent to which the deletion of pAR13 is representative of 13-mers in general, we constructed a probe which corresponds to the region of the 16-mer which has been excluded from clone pAR13 (Fig. 3A, see Materials and Methods). EcoRI-digested DNA from unrelated individuals (N>100) of various racial and ethnic backgrounds (see Materials and Methods) were evaluated using the complete 16-mer probe and this deletion probe. As anticipated, the 13-mer repeats (present either as a 13-mer EcoRI fragment or as the two 9-mer and 4-mer fragments, Fig. 3B) failed to hybridize appreciably with the deletion probe (Fig. 3C). This indicates that, in each of these individuals, the 13-mer repeats had relative deletions which encompassed the length of the deletion probe.

DISCUSSION

In this report, we have extended our molecular characterization of the alpha satellite subset which is specific for human chromosome 17. In a previous study, we examined higher-order repeat lengths found to be constitutive for chromosome 17 (23). In addition to these repeats (15-mer and 16-mers), we have noted polymorphisms with respect to the different repeat lengths represented on this chromosome (24). Here, we report the isolation and characterization of the most prominent of the polymorphic repeats, a 13-mer higher-order repeat represented on ~35% of the human chromosomes 17 at copy numbers as high as 500-1000 per chromosome.

The characterization of related higher-order repeats from an individual chromosomal subset provides the opportunity to delineate the events involved in the as yet to be fully understood evolution of these tandem arrays. The 13-mer repeat is particularly informative because it is homologous to the constitutive 16-mer and it is only represented on a proportion of human chromosomes 17. These observations indicate that the generation of an amplified 13-mer is a relatively recent event in terms of alpha satellite evolution. Sequence comparisons between the 13- and 16-mer clones have substantiated the close relationship of these repeats. They are ~99% identical in sequence, differing principally by the exclusion of three monomers from the 13-mer.

Because the repeats are highly homologous, the deletion junctions could be accurately defined and homology constraints could be assessed. We have determined the approximate junctions and have noted a stretch of 15 identical

nucleotides immediately 5' of the putative junction. In addition, 11 bp 3' of the junction, there is a 19 bp region of identity (Fig. 2A). At present, the significance of limited stretches of identity at the recombination site is unclear due to the fact that similar stretches of homology generally exist even between monomers of higher-order repeats from different chromosomes. Thus, while it is tempting to speculate that the these regions were involved in the unequal recombination event, establishment of precise homology requirements will be possible only when similar analyses are made of additional, independent recombination events.

We have been able to define the molecular events involved in the generation of a copy of the 13-mer higher-order repeat. The overall significance of these results is dependent on whether this is representative of a unique event from which the 13-mer higher-order repeats of all chromosomes 17 have resulted. To evaluate this, we constructed a probe from the 16-mer which allows us to accurately determine the position of the relative deletion in 13-mers of different individuals. Although the probe includes only 270 bp of the three monomer deletion in pAR13 (see Fig. 3A), we are able to conclude that all the 13-mers examined are characterized by deletions which, within an uncertainty of ~240 bp, appear identical. These data are consistent with the suggestion that the 13-mer repeats have resulted from a single, isolated recombination event occurring at some point prior to the divergence of the major races of man. However, we cannot at present rule out the possibility that small differences exist between 13-mer repeats of different individuals or that the same three monomer deletion has arisen independently multiple times.

Within limits of approximately 15 bp, we have been able to define the position within monomers which served as the point of exchange in a recombination event. As shown in Fig. 2A, the putative recombination site lies 13-29 bp upstream of the monomer register defined by the EcoRI cleavage site. Previously, we have described a monomer triplication (monomers 13, 14, 15) during evolution of the 16-mer repeat of chromosome 17 (23). Interestingly, the recombination site implicated in the monomer triplication coincides (given the level of uncertainty noted above) with the site identified in this study. This could be coincidental, or it may indicate that there exists a preferred register for recombination between alphoid monomers. This region is of interest because it is believed to be the domain which serves as the spacer region between uniformly phased nucleosomes in alpha satellite chromatin (29-33). If so, one might expect that this region would be readily accessable

to enzymes involved in recombination. In fact, a mammalian site-specific endonuclease has been purified (34,35) which cleaves African green monkey alpha satellite DNA at a position within this putative nucleosome spacer region (36). A preferred recombination register, therefore, could be dependent on the nucleosome phase as well as structural features of the nucleosome spacer sequences, a model originally put forth by Maio and coworkers (10). Again, analysis of independent recombination events will be necessary to establish the specificity, if any, governing recombination between alphoid repeats.

Homologous unequal crossing-over between higher-order repeats can result in the periodic genesis of distinct repeat units (9). In addition, unequal crossing-over has been suggested as a mechanism for the amplification or fixation of variant repeats within a tandem array (37-39). The example presented here serves to illustrate how unequal crossing-over and subsequent fixation can result in an apparent restructuring of the satellite sequences on a given chromosome during human evolution. With regard to the evolution of chromosome-specific subsets, two identical subsets could evolve into distinct, yet related subsets if these events were restricted to a particular chromosome. In this respect, the results presented in this report may be relevant to understanding the evolution of chromosome-specific subsets of tandemly repeated satellite DNAs.

ACKNOWLEDGMENTS

This work was supported by grants from the March of Dimes Birth Defects Foundation, the Hospital for Sick Children Foundation, and the Medical Research Council of Canada. H.F.W. is a research scholar of the Medical Research Council of Canada and J.S.W. is supported by an Ontario Graduate Scholarship.

*To whom correspondence should be addressed

REFERENCES

1. Singer, M.F. (1982) Int. Rev. Cytol. 76, 67-111.
2. Fedoroff, N.V. (1979) Cell 16, 697-710.
3. Kedes, L.H. (1979) Ann. Rev. Biochem. 48, 838-870.
4. Baltimore, D. (1981) Cell 24, 592-594.
5. Jeffreys, A. (1982) in Genome Evolution (Dover, G.A. and Flavell, R.B., eds.) Academic Press, London. pp. 157-176.
6. Dover, G.A., Brown, S.D.M., Coen, E.S., Dallas, J., Strachan, T. and Trick, M. (1982) in Genome Evolution (Dover, G.A. and Flavell, R.B.,

eds.) Academic Press, London. pp. 343-374.
7. Dover, G.A. (1982) Nature 299, 111-116.
8. Gillespie, D., Donehower, L. and Strayer, D. (1982) in Genome Evolution (Dover, G.A. and Flavell, R.B., eds.) Academic Press, London, pp. 113-133.
9. Southern, E.M. (1975) J. Mol. Biol. 94, 51-69.
10. Maio, J.J., Brown, F.L. and Musich, P.R. (1977) J. Mol. Biol. 117, 637-656.
11. Donehower, L. and Gillespie, D. (1979) J. Mol. Biol. 134, 805-834.
12. Manuelidis, L. and Wu, J.C. (1978) Nature 276, 92-94.
13. Rosenberg, H., Singer, M.F. and Rosenberg, M. (1978) Science 200, 394-402.
14. Rubin, C.M., Deininger, P.L., Houck, C.M. and Schmid, C.W. (1980) J. Mol. Biol. 136, 151-167.
15. Wu, J.C. and Manuelidis, L. (1980) J. Mol. Biol. 142, 363-386.
16. Maio, J.J., Brown, F.L. and Musich, P.R. (1981) Chromosoma 83, 103-125.
17. Willard, H.F. (1985) Amer. J. Hum. Genet. 37, 524-532.
18. Mitchell, A.R., Gosden, J.R. and D.A. Miller (1985) Chromosoma 92, 524-532.
19. Jorgensen, A.L., Bostock, C.J. and Bak, A.L. (1986) J. Mol. Biol. 187, 185-196.
20. Gillespie, D. (1977) Science 196, 889-891.
21. Brutlag, D.L. (1980) Ann. Rev. Genet. 14, 121-144.
22. Waye, J.S. and Willard, H.F. (1985) Nucl. Acids Res. 13, 2731-2743.
23. Waye, J.S. and Willard, H.F. (1986) Mol. Cell. Biol. (in press).
24. Willard, H.F., Waye, J.S., Skolnick, M.H., Schwartz, C.E., Powers, V.E. and England, S.E. (1986) Proc. Natl. Acad. Sci. USA (in press).
25. Willard, H.F., Smith, K.D. and Sutherland, J. (1983) Nucl. Acids Res. 11, 2017-2033.
26. Melton, D.A., Krieg, P.A., Rebagliati, M.R., Maniatis, T., Zinn, K. and Green, M.R. (1984) Nucl. Acids Res. 12, 7035-7056.
27. Sanger, F., Nicklen, S. and Coulson, A.R. (1977) Proc. Natl. Acad. Sci. USA 74, 5463-5467.
28. Korneluk, R.G., Quan, F. and Gravel, R.A. (1985) Gene 40, 317-323.
29. Musich, P.R., Maio, J.J. and Brown, F.L. (1977) J. Mol. Biol. 170, 657-677.
30. Musich, P.R., Brown, F.L. and Maio, J.J. (1982) Proc. Natl. Acad. Sci. USA 79, 118-122.
31. Wu, K., Strauss, F. and Varshavsky, A. (1983) J. Mol. Biol. 170, 93-117.
32. Zhang, X.Y., Fittler, F. and Horz, W. (1983) Nucl. Acids Res. 11, 4287-4305.
33. Strauss, F. and Varshavsky, A. (1984) Cell 37, 889-901.
34. Brown, F.L., Musich, P.R. and Maio, J.J. (1978) Nucl. Acids Res. 5, 1093-1107.
35. McKenna, W.G., Maio, J.J. and Brown, F.L. (1981) J. Biol. Chem. 256, 6435-6443.
36. McKenna, W.G., Brown, F.L., Musich, P.R. and Maio, J.J. (1982) J. Mol. Biol. 154, 379-384.
37. Smith, G.P. (1973) Cold Spring Harbor Symp. Quant. Biol. 38, 507-514.
38. Tartof, K.D. (1973) Cold Spring Harbor Symp. Quant. Biol. 38, 491-500.
39. Smith, G.P. (1976) Science 191, 528-535.