A homologous subfamily of satellite III DNA on human chromosomes 14 and 22

K.H.Choo*, E.Earle and C.McQuillan

The Murdoch Institute for Research into Birth Defects, Royal Children's Hospital, Flemington Road, Parkville, 3052 Victoria, Australia

ABSTRACT

We describe a new subfamily of human satellite III DNA that is represented on two different acrocentric chromosomes. This DNA is composed of a tandemly repeated array of diverged 5-base-pair monomer units of the sequence GGAAT or GGAGT. These monomers are organised into a 1.37-kilobase higher-order structure that is itself tandemly reiterated. Using a panel of somatic cell hybrids containing specific human chromosomes, this higher-order structure is demonstrated on chromosomes 14 and 22, but not on the remaining acrocentric chromosomes. In situ hybridisation studies have localised the sequence to the proximal p-arm region of these chromosomes. Analysis by pulsed-field gel electrophoresis (PFGE) reveals that 70−110 copies of the higher-order structure are tandemly organised on a chromosome into a major domain which appears to be flanked on both sides by non-tandemly repeated genomic DNA. In addition, some of the satellite III sequences are interspersed over a number of other PFGE fragments. This study provides fundamental knowledge on the structure and evolution of the acrocentric chromosomes, and should extend our understanding of the complex process of interchromosomal interaction which may be responsible for Robertsonian translocation and meiotic nondisjunction involving these chromosomes.

INTRODUCTION

The five pairs of human acrocentric chromosomes [13−15, 21 and 22] all have a morphologically similar short arm which can be subdivided into the p11, p12 and p13 regions. These regions contain several families of tandemly repeated DNA (reviewed in Ref.1). The ribosomal genes within p12, and the DNA distal to this region, show close sequence homology amongst the five nonhomologous chromosomes, reflecting sequence homogenisation and concerted evolution through interchromosomal recombination (2−4). The demonstration that a number of other families of repetitive DNA also appear to be represented on the five acrocentric chromosomes suggests that

such interchromosomal homogenisation may be widespread throughout the short arms of these chromosomes (5−9). An exception is the 1.8-kilobase (kb) KpnI subfamily of satellite III DNA which has been shown to map specifically to the p11 region of chromosome 15 (10). In addition to these sequences on the short arm, the centromeres of the acrocentric chromosomes carry many different subfamilies of alpha satellite DNA, some of which are unique to a single chromosome (11−13), while others are shared by different subgroups of these chromosomes (14−18). These latter observations suggest a more complex pattern of evolution and interchromosomal homogenisation of the centromeric alpha DNA compared to sequences on the short arms (1). In order to understand these processes more fully, we have initiated an investigation of the molecular organisation of the short arms of these chromosomes. We report here a new sequence which maps to the proximal p-arm region of chromosomes 14 and 22, but not 13, 15 and 21. Sequence analysis established the DNA to be a subfamily of human satellite III and excluded the presence of an alphoid sequence previously reported on chromosomes 14 and 22 (16). The higher-order structure and long-range genomic organisation of this sequence are described.

MATERIALS AND METHODS

Isolation of Clone and Sequencing

pTRS-47 clone was isolated by screening a chromosome 14-specific EcoRI library using a satellite III probe pTR9-H2 (Vissel, Earle, Filby and Choo, in preparation) under a low hybridisation washing stringency of 6×SSC at 45°C (1×SSC is 0.15M NaCl, 0.01M sodium citrate). The DNA insert was excised from the lambda phage Charon 21A vector, gel purified, and cloned into the EcoRI site of pUC9 plasmid vector. For sequencing, the pTRS-47 DNA was subcloned into M13 mp18 and mp19 vectors. Sequencing was carried out by dideoxy chain termination method using USB Sequenase.

In situ Hybridisation, Somatic Cell Hybrids and PFGE Analysis

In situ hybridisation and BrdU-incorporation banding of metaphase chromosomes were performed as previously described

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              EcoRI
   1    GGAAT TCAAT  GGAAT ATAAC  GAAAT GGATA  GGATC AGAAC  GGAAC AGAGC  GGAGT GGAGT  TGAGT GGAGT      70

  71    GGATC GGAGT  GCAGT GGAAA  GGAAT GGAAT  AGAAT GGAAT  GGAAT GCAGT  GGAGT GGAAT  GGAAT GAAGT     140

 141    GGAAT GGAGT  TGAGT GGAGT  GGATC GGAGT  GCAGT GGAAA  GGAAT GGAAG  AGAAT GGAAT  GGAAT GGAAT     210
                                                      ▽      ▼
 211    GCAGT GGACT  GGAAT GGAAT  GGAGT GGAGT  GGAGT GCAGT  G---- GGAAT  CGAGT GGAGT  GGAGT GGAAT     280
                                                      ▽      ▼
 281    GGACT GGAAT  GGAAT GGATT  GGAGT GGAGT  GCAGT GGAAT  CGAGT GGAGT  GGAGT GGAAT  GGAGT AGAAT     350
                                                                  AluI
 351    GGAAT GGAGT  GGAGT GTAGT  GGAAT GGAAT  GGAAT GGTGA  ATGAA TGTCA GCTAA GATTG  TGCAA CTGCA     420
                                 XhoII                ▼
 421    TTCCA GTCTG  GGTGA CAAAG  TGAGA TCCAG  TCGAA GTAAA  GGAAT GGAAT  GGAAT AGAGT  AAAAT GGAAT     490

 491    GGAAT GGTGT  GGAGT GGAAT  GGAAT GGAGA  GGAAT GGAGT  GGAGT GGAGT  GGAGT GGAGT  GGAAT GGAGT     560

 561    GGAGT GGAAT  GGAGA GTGAT  GGAGA GGAAT  GGAAT GGAAT  GGAAT GGAAT  GGAGT GGAAT  GGAAT GGAAT     630

 631    GGAGT GGAAT  GGAAT GGAAT  GTAGA GGAGT  GGAGT GGATT  GGAGT GGAGT  GGAAT GGAGT  GGAAT AGAGT     700

 701    GAAAT TTAGT  GGAGT GTAAT  GGAGT GGAGT  GGAGT GGCAG  TTGAG TGGCA  TGGAT CAGGT  GCAGT GGAAT     770
                                                      ▽      ▼
 771    GGAAT GGAAT  GGAGT GGAGT  GGAGA GGAGT  GGAGT GGAAT  CGAAT GGAAT  GGCAT GGAGT  GGAGT GGAAT     840

 841    GGAGT GGATT  GGAAT TGAAT  GCAGT GGAAT  GGAAT GCAAT  GGAGT GGAGT  GGAGT GCAGT  GGAGT GGAGT     910

 911    GGAGG GGAAT  GGAAT GGAGT  GGAGT AAAAT  GGTTT GGAAT  GGAGT GGGGT  GGAAT GGAGT  GGGTT GGAAT     980

 981    GGAGT GGAGT  GGAGT AGAAC  GGAGT G-ATT  GGGGT GGAAT  GGAAT AGAGT  GGAAT GGAAT  GGAGT GGAGT    1050

1051    GGAGT AGAAC  GGAGT GATTG  G-AGT GGAAT  GGAAT ACAGT  AGAGT GGAAT  GCAGT GGAGT  GGAAT GGAAT    1120

1121    GGAGT GGAGT  GGCAT GGAAA  GGAAT GGAGA  GGAAT GGAAT  GGAAT GGAAT  GGAAT GGAAT  GGAAT GGAAT    1190

1191    GGAAT GGAAC  GGTGA AATAA  AATGT GAGTT  AAGAT TGTGC  -CACT GCATT  GCAGT CTGGG  GGAC- AGAGT    1260
                          ▼
1261    GAGAT ACAGT  C---- GAAAT  AAA-- GGAAT  GGAAG GGACT  GGAGT AGAAT  GGAAT GGAAT  TGAGT GGAGT    1330

1331    GGAAT GGAAT  GAAGT GGAGA  GGAAT GGAAT  GGAGT GGAAT  GCAAT GGAGG
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Figure 1. Nucleotide sequence of the pTRS-47 clone. The sequence has been organised to allow maximum alignment of the 5 bp repeat unit, where a dash (−) represents a hypothetical deletion that is not part of the sequence. The two predominant GGAAT and GGAGT monomers are indicated by double and single lines, respectively. Restriction sites for EcoRI, AluI and XhoII are shown. HinfI and TaqI sites are indicated by an open and solid triangle, respectively. If all the hypothetical deletions are removed from the sequence, the actual length of this clone is 1,366 nucleotides.

(19) with a final hybridisation washing stringency of 0.1 × SSC at 65°C. The hybrid cell lines used were CF34−2-3 (human chromosomes 13, 12 and 6p)(20), CP43 (human chromosome 14 only)(21), F4SC13C19 (human chromosomes 1, 14 and X)(22), FIR5R3 (human chromosomes 14, 18 and X)(23), HORL-Ia (human chromosomes 15 and X)(22), WAVR-4d-F9−4A (human chromosome 21 only)(24), PgMe25Nu (human chromosomes 22 and X)(25), HY137P (human chromosomes 8, 9 and 17) and HY75E1 (human chromosomes X, 5, 9 and 12)(M. Rocchi, personal communication). The karyotype of these hybrids was independently confirmed in our own cytogenetics laboratory prior to their use.

Pulsed field gel electrophoresis (PFGE) utilised a Bio-Rad CHEF apparatus at 12°C. Unless otherwise stated, all filter hybridisations were carried out at high stringency in phosphate buffer (0.5M sodium phosphate, pH 7.2, 1% bovine serum albumin, 7% SDS, and 1mM EDTA) at 75.5°C, followed by washing in 1 × SSC, 0.1% SDS at 82°C. This condition has

previously been shown to completely eliminate cross-hybridisation of different subfamilies of alphoid satellite DNA (13).

RESULTS

Isolation of a Human Satellite III Sequence

Two chromosome 14-specific libraries were screened at low stringency with a probe which, by direct sequencing, was shown to be satellite III DNA (see Materials and Methods). The HindIII library (ID code LL14NS02, Lawrence Livermore National Laboratory) yielded no positives in > 10^6 colonies screened. Many strong positives (~5% of all colonies) were detected in the EcoRI library (ID code LA14NS01, Los Alamos National Laboratory). One of these was designated pTRS-47 and characterised further. This clone has an insert size of approximately 1.4kb. Cross-hybridisation of this DNA with cloned alphoid sequences derived from chromosomes 14 (16),
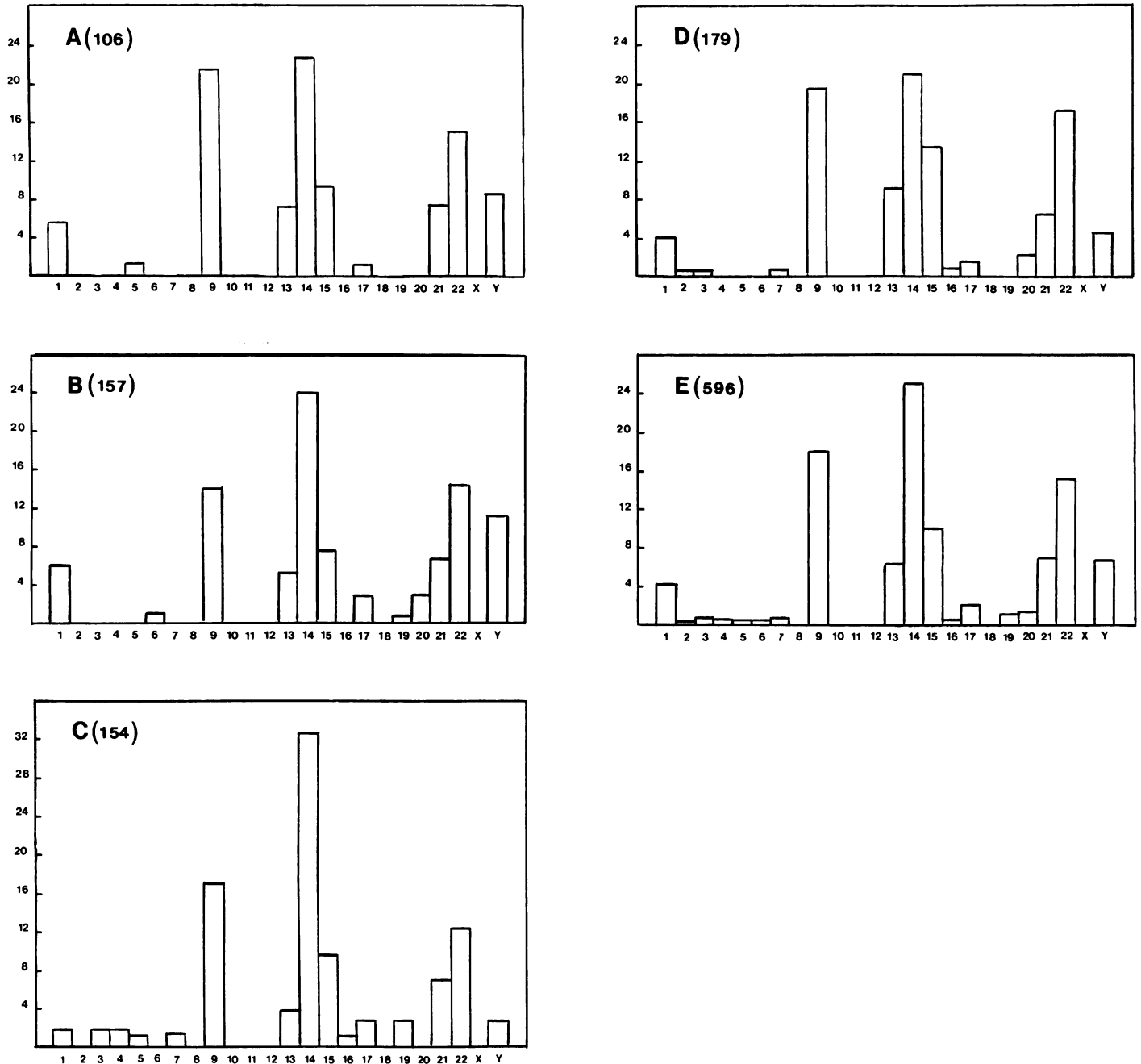
Figure 2. Count of grain (vertical axes) on human metaphase chromosomes (horizontal axes) following *in situ* hybridisation using pTRS-47 as a probe. A to D represent 4 unrelated normal male individuals. The actual numbers of autoradiographic grains counted are shown in parentheses, but these are normalised to 100 grains in the histograms. E is the sum of results (using the normalised values) for the 4 individuals. Counts for chromosomes 1, 9 and Y were found predominantly in the q12 heterochromatic band, whereas counts for chromosomes 13, 14, 15, 21 and 22 were located in the proximal short arm region. Slides were washed at a final stringency of 0.1×SSC at 65°C.

15 (13), 17 (26) and X (26) at low stringency did not reveal any detectable alphoid DNA within the insert (data not shown). This was subsequently confirmed by direct sequencing.

Nucleotide Sequence Analysis

Figure 1 shows the complete nucleotide sequence for the pTRS-47 clone. The sequence is comprised essentially of a tandemly repeated array of diverged, 5-bp monomer units of the sequence 5'-GGAAT. Of the total of 276 monomers in this clone, 89 (or 32%) have completely preserved the consensus GGAAT

sequence. Interestingly, a second sequence, 5'-GGAGT, also has a relatively abundant representation within the clone, constituting 69 (or 25%) of the monomers. The clone contains three HinfI sites, all of the sequence GG(A/T)TC, as opposed to GG(G/C)TC. Based on these characteristics (10,27,28), we infer that pTRS-47 is a human satellite III DNA. An examination of the sequence has further revealed a single cleavage site for EcoRI, AluI and XhoII, multiple sites for TaqI and no sites for RsaI, AccI, AvaII, BamHI, HindIII, MspI, BglII, HaeIII, PvuII, PstI and KpnI. This information is relevant to the investigation of the
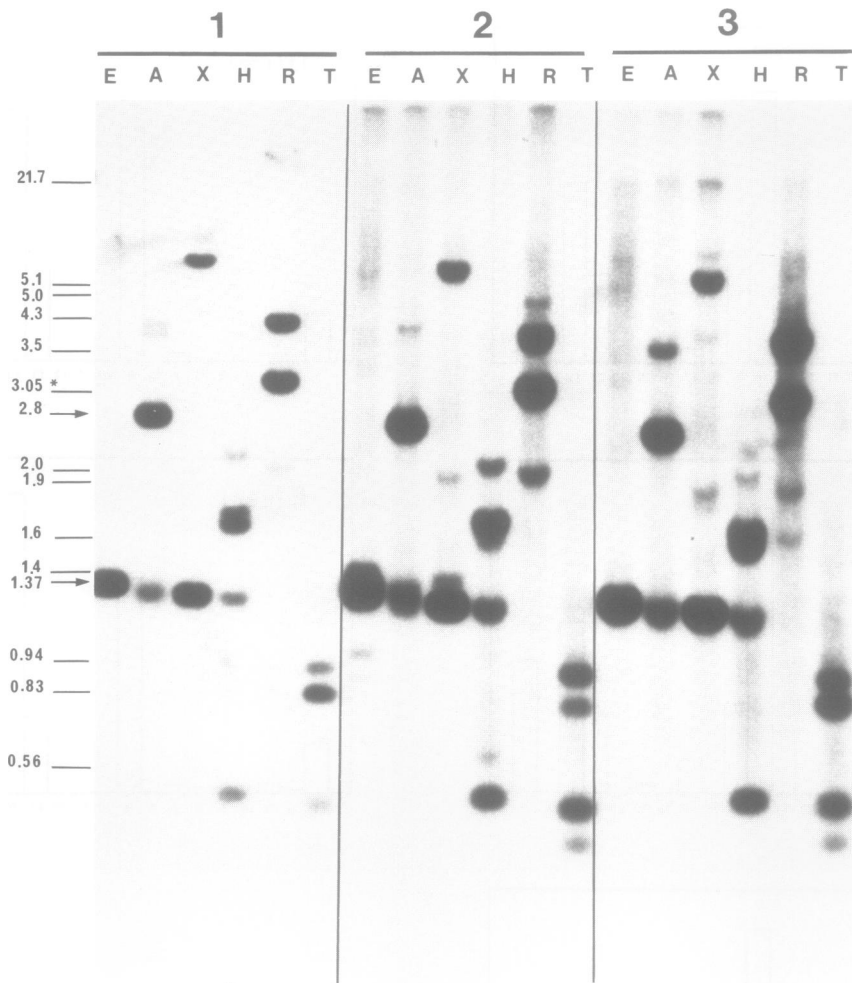
Figure 3. Determination of higher-order genomic structures using standard Southern blot analysis. 1, CP43 (a somatic hybrid cell line carrying only human chromosome 14); 2 and 3, a normal human male and female individual, respectively. Restriction enzymes used were EcoRI (E), AluI (A), XhoII (X), HinfI (H), RsaI (R) and TaqI (T). The arrows point to the 1.37 kb higher-order repeating unit and its putative 2.8 kb dimer. Mol. wt. markers and arrows pointing to the 1.37 and 2.8 kb bands were Lamda phage DNA cleaved with EcoRI and HindIII. Asterisk indicates a mol. wt. band derived from a '1 kb DNA ladder' (BRL).
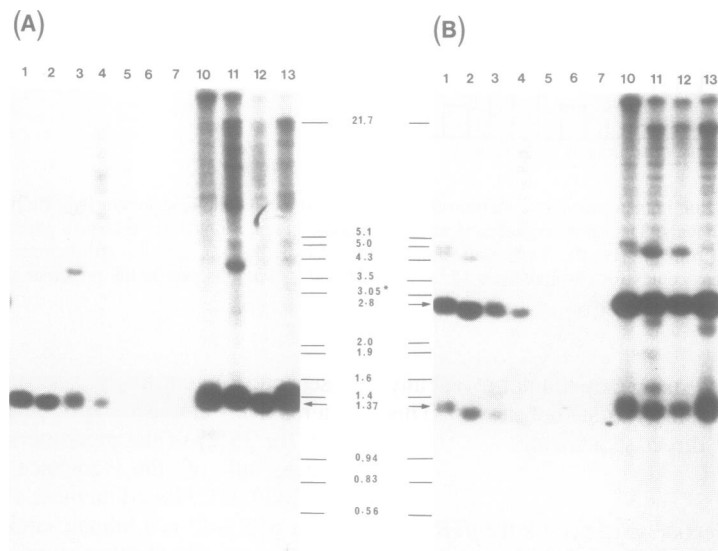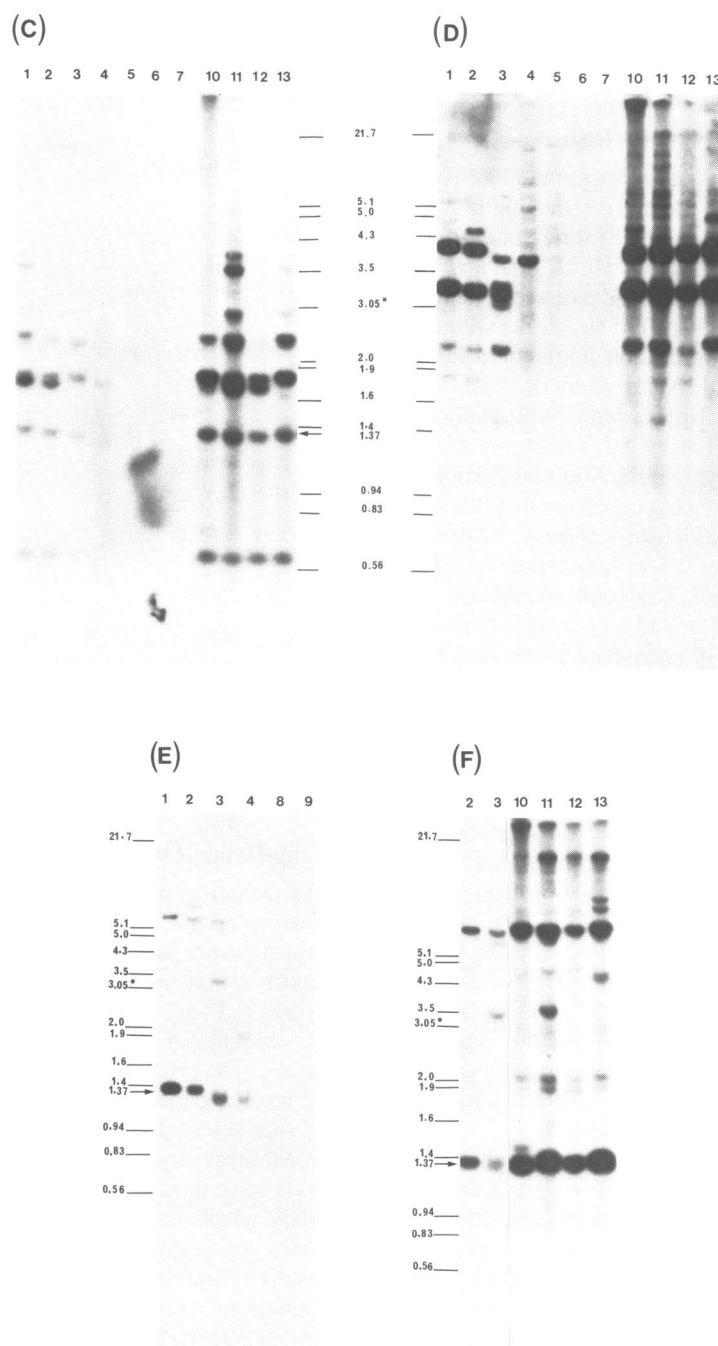
Figure 4. Comparison of higher-order genomic structures on different chromosomes. 1−9 are different somatic cell hybrids: 1, CP43 (human chromosome 14 only); 2, F4SC13C19 (human chromosomes 1, 14 and X); 3, FIR5R3 (human chromosomes 14, 18 and X); 4, PgMe25Nu (human chromosomes 22 and X); 5, CF34-2-3 (human chromosomes 13, 12 and 6p); 6, HORL-Ia (human chromosomes 15 and X); 7, WAVR-4d-F9-4a (human chromosomes 21 only); 8, HY137P (human chromosomes 8, 9 and 17); and 9, HY75E1 (human chromosomes 5, 9, 12 and X). 10−13 are total genomic DNA from two normal human males (10 and 13) and two normal human females (11 and 12). Restriction enzymes used were: A, EcoRI; B, AluI; C, HinfI; D, RsaI; E and F, XhoII. Mol. wt. markers were as in Fig.3.

higher-order and long range organisation of this sequence (see below).

Chromosomal Distribution of pTRS-47 Sequences

In situ hybridisation to metaphase chromosomes was used to determine the distribution of the pTRS-47 sequence in the human genome. Four unrelated normal males were analysed. As shown in Fig.2A-D, significant hybridisation was observed on the heterochromatic q12 band of chromosomes 1, 9, Y and the proximal short-arm region of the acrocentric chromosomes. Amongst the acrocentric chromosomes, discernible preferential hybridisation to chromosomes 14 and 22 was seen in all four individuals and in the pooled data (Fig.2A-E). Despite the considerable variation in the autoradiographic grain-distribution profile between the individuals, these results suggested the possible identification of a satellite III subfamily that is selective for chromosomes 14 and 22. Somatic cell hybrids carrying human chromosomes were used to assess this possibility (see below).

Attempts to raise the stringency of in situ hybridisation to elicit greater specificity of the probe were unsuccessful as these only led to a non-specific removal of signals from the slides.

Higher-Order Structure of the Cloned Repeat

A somatic cell hybrid (CP43) carrying chromosome 14 as its only human complement was used to determine the higher-order structure of the pTRS-47 sequence. Of the 28 different restriction enzymes tested, only EcoRI, AluI, XhoII, HinfI, RsaI and TaqI cleaved within the repeated sequence to give discrete bands on the gel (Fig.3 − 1). The enzymes which did not cleave within the sequence (as seen by a large hybridisation fragment at the top of the gel tracks) were AccI, ApaI, AvaII, BamHI, BglII, DraI, EcoRV, HaeIII, HindII, HindIII, KpnI, MspI, NcoI, NsiI, PstI, PvuII, SacI, ScaI, SpnI, SspI, StuI and XbaI (picture not shown). As indicated in Fig.3 − 1, EcoRI, AluI, XhoII and HinfI all produced a common band of 1.37 kb, suggesting that this is the higher-order repeat unit for the pTRS-47 sequence. With AluI, the strong band at approximately 2.8 kb corresponded in size to a likely dimeric structure of the 1.37 kb unit. In addition, both HinfI and TaqI cleaved within this unit to give smaller fragments, although, with HinfI, a doublet of prominant bands at 1.6 and 1.7 kb was also seen. With RsaI, two bands which were significantly larger than 1.37 kb were observed. The authenticity of this higher-order structure was confirmed by the demonstration that it was the same as those found in total human genome (Figs.3 − 2 and 3 − 3). These results were consistent with the sequence map of pTRS-47 which indicated the presence of a single site for EcoRI, AluI and XhoII, multiple sites for HinfI and TaqI, and no sites for the rest of the enzymes tested including RsaI (Fig.1), thereby establishing the pTRS-47 clone as a true representative of this subfamily of satellite III DNA. The observation of multiple HinfI sites within the pTRS-47 sequence (Fig.1), and of HinfI bands greater than 1.37 kb (Fig.3) can be explained by the highly polymorphic nature of the HinfI sites in satellite III DNA because of the relative ease of gaining a HinfI site (GANTC) by a single-base substitution based on a tandemly repeated sequence of [G(GAAT G)GAAT]n.

Additional somatic cell hybrids containing each of the five acrocentric chromosomes and chromosome 9 were analysed to determine the nature of the DNA that showed hybridisation to the pTRS-47 probe in the in situ hybridisation experiments (Fig.2). As shown in Fig.4A-F, the three independent hybrids carrying human chromosome 14 and one hybrid carrying human chromosome 22, all gave the 1.37 kb higher-order structure band with EcoRI, AluI, HinfI and XhoII. In addition, all four hybrids showed common bands for the 2.8 kb AluI dimer (Fig.4B), the 1.8 and 2.3 kb HinfI fragments (Fig.4C), the 4.3 kb RsaI fragment (Fig.4D), and the major TaqI fragments (not shown). A number of polymorphic bands were detected in the hybrid cells (Fig.4, lanes 1 − 4) and in the genomic DNA of four unrelated normal human individuals (Fig.4, lanes 10 − 13).

Analysis of hybrids containing human chromosome 9, 13, 15 or 21 with EcoRı, AluI, XhoII, HinfI, RsaI and TaqI did not reveal any hybridisation bands corresponding to any of the higher-order structures for chromosomes 14 and 22 (Fig.4, lanes 5 − 9; and data not shown). The autoradiographic signals seen on chromosomes 9, 13, 15 and 21 in the in situ hybridisation experiments could therefore be best attributed to cross-hybridisation to other satellite III subfamilies on these chromosomes due to the lower stringency used (see Discussion).
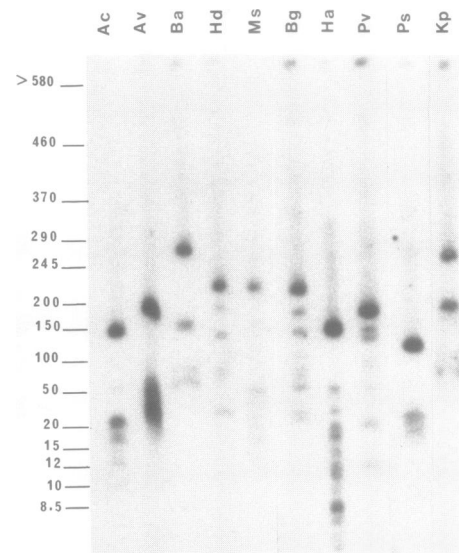

Figure 5. PFGE analysis of a hybrid cell line (CP43) carrying only human chromosome 14. Restriction enzymes used were AccI (Ac), AvaII (Av), BamHI (Ba), HindIII (Hd), MspI (Ms), BglII (Bg), HaeIII (Ha), PvuII (Pv), PstI (Ps), and KpnI (Kp). Asterisk denotes the major band seen with each enzyme. The mol. wt. markers were a mixture of S. cerevisiae chromosomes (Biorad), Lambda DNA ladder (Pharmacia) and HMW DNA markers (BRL).

Long-Range Organisation of the pTRS-47 Repeats

The technique of PFGE was used to determine the long-range genomic organisation of the pTRS-47 sequence. Several enzymes shown earlier not to cleave within the repeating array of this sequence were used. As shown in Fig.5, with the chromosome-14 hybrid (CP43), these enzymes yielded one major band within the size range of 150 − 300 kb as well as a number of other weaker bands. [The presence of significantly larger bands was excluded by resolving the DNA over a PFGE size range of 200 − 2,000 kb (not shown)]. These results suggested that the predominant repeat array was less than 300 kb long and that this array was likely to be flanked on both sides by other non-tandemly repeated DNA which has a more normal distribution of the common restriction enzyme sites. This possibility was supported by the results of double restriction enzyme cleavage (Fig.6 − 1a) which showed the reduction (or 'trimming') of the array to a minimum size of about 150 kb. The detection of other weaker bands in Fig.5 further suggests that some pTRS-47 sequences are interspersed on other PFGE fragments over a relatively large domain on this chromosome.

Next, we compared the above long-range organisation with those of the other two chromosome 14 and one chromosome 22 in hybrids F4SC13C19, FIR5R3, and PgMe25Nu. Fig.6 shows that, with the six enzymes used, the major band in the chromosome 14 in CP43 and F4SC3C19 gave a very similar PFGE band pattern. This band was, however, consistently smaller in the chromosome 14 in FIR5R3 in a manner which suggested the loss of about 50 kb of sequences within the repeating array in comparison to that of CP43 and F4SC13C19. The long-range structure of the chromosome 22 in PgMe25Nu is slightly less well defined since no single major band was seen. However, the detection of one to several moderately strong bands over the size range of 20 − 150 kb suggests that the predominant tandem array
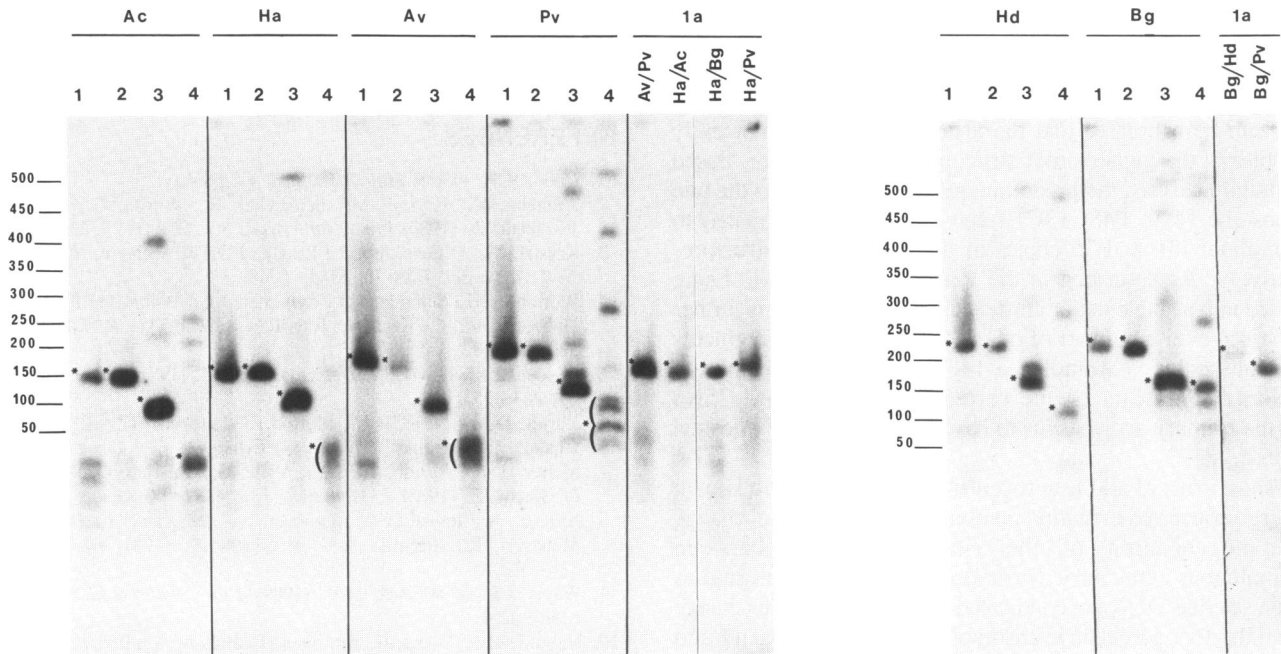
Figure 6. PFGE analysis of hybrid cell lines: 1, CP43 (human chromosome 14 only); 2, F4SC13C19 (human chromosomes 1, 14 and X); 3, FIR5R3 (human chromosomes 14, 18 and X); and 4, PgMe25Nu (human chromosomes 22 and X). 1a designates CP43 DNA which has undergone double restriction enzyme cleavage. Asterisk denotes the major band(s) seen in each gel track. Restriction enzymes used were as in Fig.5. Mol. wt. markers were Lambda DNA ladder.

seen in the chromosome 14 hybrids has been altered, lost, or reduced to smaller arrays, on this chromosome 22. Further, the presence of a number of other weaker bands of higher mol. wt. indicates that the pTRS-47 DNA is also interspersed over a number of other PFGE fragments covering a relatively large domain on this chromosome 22.

DISCUSSION

Although the results of the *in situ* hybridisation experiments gave initial indication of a preferential hybridisation of the pTRS-47 sequence to chromosomes 14 and 22, the relatively high level of hybridisation detected on the remaining acrocentric chromosomes and on chromosomes 1, 9 and Y, did not allow precise definition of the genomic distribution of this sequence. Since we have previously shown that the *in situ* hybridisation stringency used would permit the cross-hybridisation of different subfamilies of satellite DNA (17,18), somatic cell hybrids carrying specific human chromosomes were used to refine the chromosomal distribution profile. The latter analysis allowed the use of a very high stringency of hybridisation (15), and the results clearly indicated the presence of a common higher-order structure for pTRS-47 on chromosomes 14 and 22, but not on 9, 13, 15 and 21. Since some the hybrids showing negative hybridisation to pTRS-47 also carried additional human chromosomes, we were able to directly confirm the absence of this satellite III subfamily on chromosomes 5, 8, 12, 17 and X. It can therefore be concluded that the signals seen on chromosomes 9, 13, 15 and 21 in the *in situ* experiments were due to cross-hybridisation of different subfamilies of satellite III DNA on these chromosomes. This is consistent, for example, with the detection of the KpnI subfamily (10) of satellite III on chromosome 15 by pTRS-47 under slightly reduced stringency in Southern analysis (data not

shown). The detection of a particularly high level of *in situ* hybridisation to chromosome 9 is probably related to the large 9q12 heterochromatic region that is known to contain satellite III DNA specific for this chromosome (29). Although we were unable to study chromosomes 1 and Y in isolation under very high stringency condition, we predict that the *in situ* hybridisation signals detected were the result of cross-hybridisation of the different subfamilies of satellite III DNA known to be present and specific for these chromosomes (30,31), as were the case with chromosomes 9, 13, 15 and 21.

We have demonstrated that pTRS-47 has a higher-order repeating structure of 1.37 kb. This higher-order structure appears to be well established and conserved within the population since it is detected in all four independent hybrids containing a chromosome 14 or chromosome 22, and in four unrelated (and racially different) normal human individuals tested. As with other families of tandemly repeated DNA, the pTRS-47 sequence shows quantitative and qualitative polymorphic variations in both its higher-order structure and long-range genomic organisation. Such variations are believed to be the result of unequal crossing over between two homologous arrays (32).

Tandemly repeated DNA arrays, by their very nature, are generally devoid of a wide range of restriction enzyme sites. This contrasts with non-tandemly repeated genomic DNA which shows an average of one cleavage site in several hundred bases for a common 'four-base cutter', and one in several kilobases for a common 'six-base cutter'. Based on these assumptions and using PFGE in conjunction with single and double restriction enzyme digestions, we have shown that the bulk of the pTRS-47 sequence on the chromosome 14 of CP43 is organised into a large tandemly repeated domain of approximately 150 kb in size. The results also suggested that this domain is likely to be flanked on both

sides by other non-tandemly repeated genomic DNA, although we cannot exclude the presence of short stretches of other simple sequences within this DNA. If we assume that the 150 kb array-length is composed entirely of the 1.37 kb pTRS-47 sequence, then it can be estimated that the array contains approximately 110 copies of the higher-order structure of this sequence. Based on a similar analysis, the predominant pTRS-47 array on the two chromosome-14 in F4SC13C19 and FIR5R3 are estimated to contain about 110 and 75 copies of the higher-order structure, respectively. As described in the Results, in addition to being organised into a single major cluster, the pTRS-47 DNA is further interspersed over a number of other PFGE restriction fragments on each of the three chromosome-14. This latter feature appears particularly significant on the chromosome 22 in PgMe25Nu where the primary array seems to have been substantially deleted or altered.

The short arms of all the acrocentric chromosomes are known to undergo concerted evolution involving a rapid rate of exchange and homogenisation of the ribosomal genes between nonhomologous acrocentric chromosomes (2,3). Recent studies on alpha satellite DNA have indicated that such a broad exchange between the five acrocentric chromosomes does not extend into the centromeric region (1). It is less clear, however, how other sequences, especially those in the proximal p-arm region, may be involved in these exchanges and homogenisation activities. Previously a major alphoid subfamily (alpha XT) specific for chromosomes 14 and 22 has been reported (16). The identification of a common subfamily of satellite III on these two chromosomes, but not on 13, 15 and 21, in the present study, suggests that sequences in the proximal p-arm region has a pattern of evolution more akin to that of the centromeric alpha satellite DNA than that found in the p12 ribosomal DNA. Further, it would be reasonable to predict that the domain containing the pTRS-47 satellite III DNA is likely to be located in close proximity to that of the alpha XT DNA, as this would facilitate the evolution and maintenance of both types of DNA on two different chromosomes. Proof for this should come from direct long-range mapping of these sequences.

The human acrocentric chromosomes are very prone to nondisjunction and translocation (33,34). It is possible that these errors are intimately related to the peculiar properties of the short arms of these chromosomes. Recent studies on the pericentric alpha DNA have revealed a number of common domains which are shared by different subgroups of these chromosomes (reviewed in Ref.1). These studies have offered molecular evidence in support of a process of physical interaction and exchange between different nonhomologous acrocentric chromosomes in a manner which may explain the predisposition of these chromosomes to nondisjunction and Robertsonian translocation (1). The present study provides a basic knowledge of the structure of the acrocentric short arms and should extend our understanding of the mechanism of interaction between these chromosomes.

ACKNOWLEDGEMENTS

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