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Homologous alpha satellite sequences on human acrocentric chromosomes with selectivity for chromosomes 13, 14 and 21: implications for recombination between nonhomologues and Robertsonian translocations

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ABSTRACT

We report a new subfamily of alpha satellite DNA (pTRA-2) which is found on all the human acrocentric chromosomes. The alphoid nature of the cloned DNA was established by partial sequencing. Southern analysis of restriction enzyme-digested DNA fragments from mouse/human hybrid cells containing only human chromosome 21 showed that the predominant higher-order repeating unit for pTRA-2 is a 3.9 kb structure. Analysis of a "consensus" in situ hybridisation profile derived from 13 normal individuals revealed the localisation of 73% of all centromeric autoradiographic grains over the five acrocentric chromosomes, with the following distribution: 20.4%, 21.5%, 17.1%, 7.3% and 6.5% on chromosomes 13, 14, 21, 15 and 22 respectively. An average of 1.4% of grains was found on the centromere of each of the remaining 19 nonacrocentric chromosomes. These results indicate the presence of a common subfamily of alpha satellite DNA on the five acrocentric chromosomes and suggest an evolutionary process consistent with recombination exchange of sequences between the nonhomologues. The results further suggests that such exchanges are more selective for chromosomes 13, 14 and 21 than for chromosomes 15 and 22. The possible role of centromeric alpha satellite DNA in the aetiology of 13q14q and 14q21q Robertsonian translocations involving the common and nonrandom association of chromosomes 13 and 14, and 14 and 21 is discussed.

INTRODUCTION

Alpha satellite DNA is a family of repetitive DNA that is localised at the centromeric region of all the human chromosomes (1-4). The fundamental repeated unit is a monomer of approximately 170 base pairs (bp) in length (5). These monomeric units are organised into long tandem arrays, which are sometimes interrupted by non-alphoid sequences (6). Several million base pairs of the alphoid DNA are estimated to be present on each chromosome, accounting altogether for at least a few per cent of the genome. Different subfamilies of alpha DNA have been identified that are characteristic of a single chromosome, or a small group of chromosomes, as defined by primary sequence, higher-order structure, and in situ hybridisation. These subfamilies include those that are present on the human chromosomes X (1,7,8), Y (4), 6 (3), 17 (6,9,10), 18 (11,12), 22 (13,14), and 13 and 21 (11,14).

Existence of chromosome-specific subfamilies suggests that exchange of sequences between nonhomologous chromosomes occurs very rarely. However, it is known (15-17) that one group of chromosomes, the acrocentrics (or nucleolus organising chromosomes) tend to undergo close association within the cell nucleoli during cell division. This could bring DNA

sequences on nonhomologous chromosomes into juxtaposition which could facilitate more frequent genetic exchanges leading to homogenisation of some sequences between the nonhomologues. It has also been proposed (18) that exchange between nonhomologous chromosomes may be responsible for the occurrence of Robertsonian translocations. These translocations, which generally involves recombination of whole acrocentric chromosome arms, are the most common chromosomal aberrations found in man, occurring with an incidence of 1:1,000 (19). Translocations between chromosomes 13 and 14, and between 14 and 21, have been observed to be much more frequent than other exchanges, together accounting for 80% of cases (18). Centromeric sequences with especially close homology between chromosomes 13, 14 and 21 might facilitate initial pairing, followed by exchange of long arms. We report here identification of a subfamily of centromeric alpha satellite DNA sequence which is shared by all the acrocentric chromosomes, but showing significantly greater selectivity for chromosomes 13, 14 and 21. The implications for recombination exchange between these chromosomes, and for Robertsonian translocations are discussed.

MATERIALS AND METHODS

Isolation, subcloning and partial sequencing of pTRA-2

pTRA-2 was isolated by screening a chromosome 21-specific library (ID code LL21NS02, Lawrence Livermore National Laboratory) using the E7 probe which contains alpha satellite DNA derived from chromosome 17 (6). The washing condition used was at a final of 2 x SSC (1 x SSC = 0.15M NaCl, 0.015M sodium citrate) at 65^{o}C. The 3.9 kb Hind III DNA insert from the original lambda charon 21A vector was excised and cloned into the Hind III site of pUC9 plasmid vector. Further subcloning of the seven fragments (S1-S7) used pUC19. For sequencing, S5 was cloned directly, or following cleavage, into M13 MP10, MP18 or MP19. Sequencing was carried out in both orientations by dideoxy chain termination method using Bresa kits.

In situ hybridisation

For in situ hybridisation, chromosomes were prepared from cultured lymphocytes, pretreated with RNase, denatured, and hybridised as described previously (6). The BudR incorporation method was used to band chromosomes after hybridisation to allow direct correlation of autoradiographic grains and chromosome bands (6). DNA probes were nick-translated with a mixture of ^{3}H-dATP, ^{3}H-dCTP and ^{3}H-dTTP (Amersham). Following hybridisation, slides were washed at 0.1 x SSC at 60^{o}C for 1 hr.

RESULTS

Isolation and partial sequencing of pTRA-2

The pTRA-2 clone is a 3.9 kb DNA which came from a chromosome-21 library as described in Materials and Methods. Since this clone was isolated using a chromosome-17 alpha

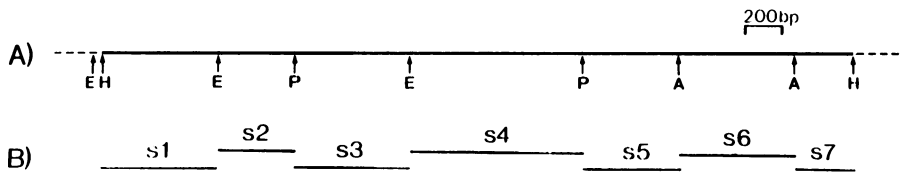

Fig. 1-A, partial restriction map for pTRA-2, where E = EcoRI, H = Hind III, P = Pst I, A = Alu I. The insert and vector sequences are denoted by a solid line and dotted line respectively. Note that the 5' EcoRI site shown on the map is found in the vector arm. Additional Alu I sites are present within the insert but these have not been mapped. B, subclones (S1-S7) of pTRA-2 insert.

satellite DNA probe (6) under reduced hybridisation stringency, it was presumed to be alphoid in nature. This was confirmed by direct sequencing of part of the clone. To facilitate sequence analysis (and subsequent Southern and in situ hybridisation experiments) this DNA was partially mapped (Fig. 1A) and subcloned into smaller fragments (S1-S7, Fig. 1B).

Restriction mapping indicated the presence of 2 sites for each of Hind III, EcoRI, and Pst I within the 3.9 kb cloned genomic fragment. No site was found for Nco I, Sca I and Pvu II, while Msp I gave a single site (not mapped) within the insert. The nucleotide sequence of one of the subclones S5, was determined. Direct comparison of the 462-bp DNA with the alpha satellite concensus sequence revealed two complete and one incomplete repeats of the basic 170 bp sequence which is characteristic (5) of alpha satellite DNA (Fig. 2). These monomeric units, designated S5/1, S5/2 and S5/3 shared 90.6%, 88.3% and 77.2% homology with the concensus sequence, and an average of approximately 70% and 80% homology with the human alpha -R1

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          61         71         81         91        101        111        121        131        141
CON1  CTTCCTTTTC ATAGAGCAGT TTTGAAACAC TCTTTTTGTA GAATCTGCAA GTGGATATTT GGACCGCTTT GAGGCCTACG GTGGAAACGG
 VAR     T    G              G                                                          T   T
S5/1  T..T.....G ..T.......  ..G......- .......... .......... .......... ...G...A.G AC.....ATA G......A..
  /2  T..T.....G T.T.......  ..GC.....G .......... .......... .........A ...GT..A.. AC.....ATA G......A..
  /3  T.CT.....G ..T..A....  ..T....T.. .....C.... .......A.. .-........ ...G.CG... .CA....AT. G..A...A..

          151        161        171         10         20         30         40         50
CON1  AAATATCTTC ATATAAAAAC TAGACAGAAG CATTCTCAGA AACTTCTTTG TGATGTGTGC ATTCAACTCA CAGAGTTGAA C
 VAR  G T -                -
S5/1  A.A.A.A... .C......A. .......... ......G... .......... .......CT. .......... .......... .
  /2  A.A.A.A... .C......A. .......... ......G... .......... .......CT. .....-.... .........G T
  /3  A.A-A..... .C......GA A.A..TCTTT .TG
```

Fig. 2- Nucleotide sequence of subclone S5 and comparison with the concensus sequence. The concensus sequence (CON 1) was derived from a compilation (20) of approximately 140 individually-sequenced, published human alpha DNA monomers, where VAR represents a second equally abundant nucleotide present at the same position. A dot denotes a nucleotide similar to the concensus, whereas a hyphen denotes a deleted base. The three monomeric 171 bp-units for S5 are designated as S5/1, S5/2, and S5/3 (incomplete unit with only 123 bp). [We noted that the CON 1 sequence is virtually identical to a similarly derived concensus sequence recently reported by Willard and Waye (21)].

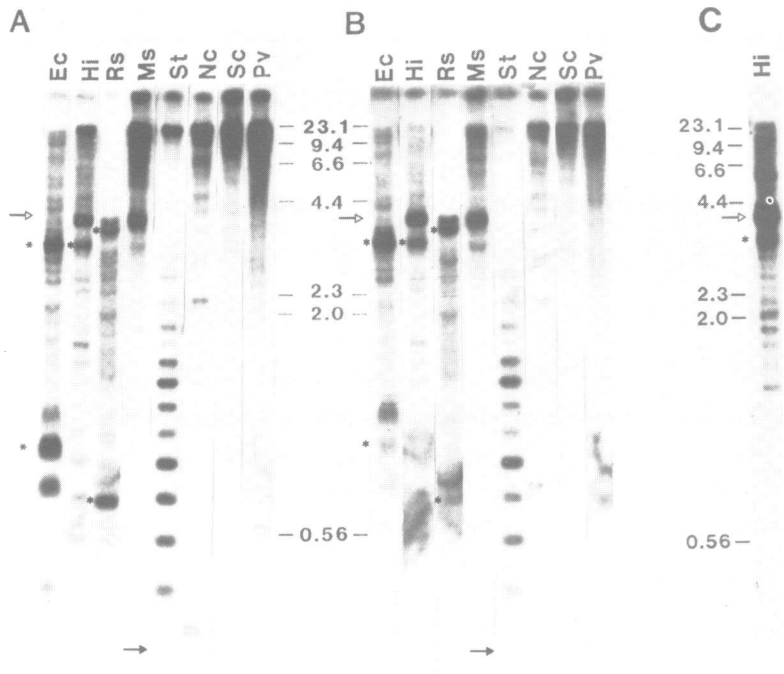


Fig. 3- Southern analysis. A and B, Total genomic DNA (5 μg) from somatic cell hybrid, WAVR 4d-F9-4A, digested with EcoRI (Ec), Hind III (Hi), Rsa I (Rs), Msp I (Ms), Stu I (St), Nco I (Nc), Sca I (Sc) and Pvu II (Pv). C, Genomic DNA from a normal human male digested with Hind III. The filters were probed with ^{32}P-labelled pTRA-2 clone, followed by washing at 2 x SSC (A) or 1 x SSC (C) at 65°C, and 0.02 x SSC at 60°C (B). All genomic tracks were digested to completion as evident from the ethidium bromide-stained gel (picture not shown). The solid arrow points to the position of the basic 170-bp alpha DNA monomeric band, which was more clearly visible on longer film exposure (not shown). The open arrow shows the position of the predominant 3.9 kb higher-order repeating structural unit. The asterisks indicate the 3 kb and 0.9 kb EcoRI-, 3 kb Hind III-, and 3.3 kb and 0.6 kb Rsa I- fragments (refer to text). Molecular weight markers were derived from Hind III digestion of lambda phage DNA.

(22) and the chromosome-X alpha (7) concensus sequences, respectively. These data therefore established the pTRA-2 clone as a member of the human alpha satellite DNA. Further evidence came from Southern analysis and in situ experiments below.

Genomic organisation of pTRA-2

The higher order genomic structure of the pTRA-2 sequence was studied using a somatic hybrid cell line, WAVR 4d-F9-4A, which carries only human chromosome 21 in a mouse background (23). We have independently confirmed the karyotype of this cell line and established that there were 0, 1, 2 and 3 copies of chromosome 21 in 12%, 72%, 15% and 1% respectively, of the present cell population (giving a mean copy number of one chromosome-21

per cell). Fig. 3 shows results of the analysis of total genomic DNA from this cell line following digestion with different restriction enzymes and Southern hybridisation using pTRA-2 as a probe. Digestion with Stu I revealed a ladder of bands based on an apparent increment of approximately 170 bp. A major 3.9-kb band was seen with Hind III, Rsa I and Msp I, suggesting that this is the predominant higher-order repeating unit for pTRA-2 on chromosome 21. With Rsa I, the detection of two additional strong bands at 3.3 kb and 0.6 kb (Fig. 3A) indicates the presence of two sites within a substantial portion of the 3.9 kb units. Digestion with EcoRI resulted in the disappearance of the 3.9 kb band and the appearance of two main bands at 3 kb and 0.9 kb, suggesting the presence of two sites within the unit. No Nco I, Sca I or Pvu II sites were detected as seen by hybridisation to high molecular weight DNA on the Southern filter. It is also evident from Fig. 3 that some sequence heterogeneity exist as seen by the presence of secondary bands (e.g. the 3 kb Hind III and some high molecular weight - Msp I bands) and the removal of some signals under high stringency washing conditions (e.g. the 0.9 kb EcoRI and 0.6 kb Rsa I bands).

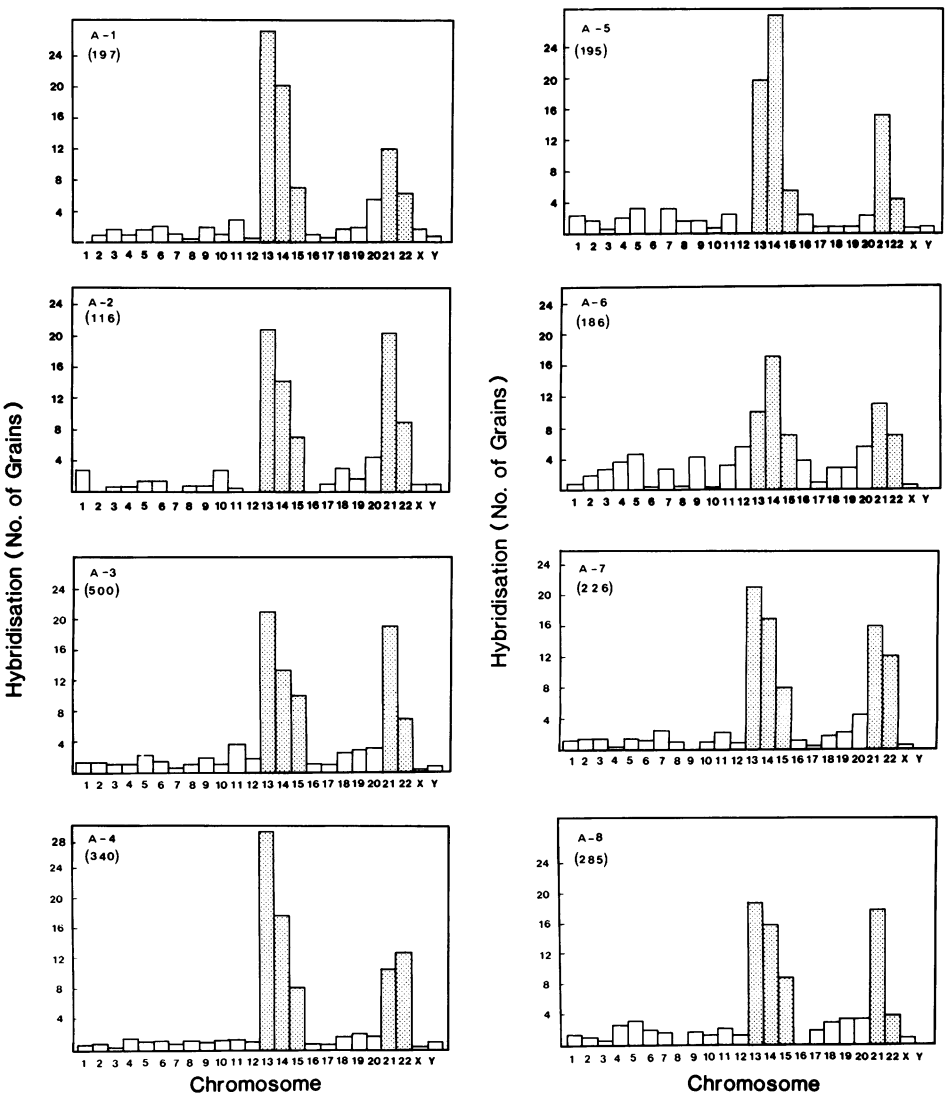
The restriction patterns for Hind III, EcoRI, Msp I, Nco I, Sca I and Pvu II shown in Fig. 3A and B therefore agree well with the map for the cloned pTRA-2 insert described above. This suggests that the cloned fragment is a true representative of its major component on human chromosome 21. When this fragment was used to probe restriction digests of total human genomic DNA, a more complex pattern was seen, an example of which is shown in Fig. 3C. This is understandable since the probe hybridises to a number of chromosomes in addition to chromosome 21 (see in situ results below). Despite this, digestion with Hind III (Fig. 3C) revealed a major band at 3.9 kb and a second band at 3 kb, corresponding to those seen in the hybrid cell line (Fig. 3A and B). This suggests that the organisation of the pTRA-2 sequence on the other chromosomes may be similar to that seen on chromosome 21. However, direct studies using somatic cell hybrids with specific human chromosomes would be necessary to clearly establish this.

In situ hybridisation

In situ hybridisation to metaphase chromosomes was used to determine the distribution of pTRA-2 sequences on human chromosomes. Thirteen unrelated normal adult males and females were analysed. Under low stringency conditions, the probe hybridised extensively to the centromere of all the human chromosomes (data not shown). However, at high stringency (see Materials and Methods), preferential hybridisation to a small group of chromosomes was evident (Fig. 4). Despite the apparent variation in the grain-distribution profile between individuals (discussed below), chromosomes 13, 14 and 21 were consistently the three most heavily labelled chromosomes (except in JC, where chromosome 22 is slightly heavier than chromosome 21) (Fig. 4, A-1 to A-13). With most of the individuals, a slightly greater hybridisation to chromosomes 15 and 22 was also seen compared to the rest of the chromosomes.

The reproducibility of the hybridisation profiles in Fig. 4A was tested in two ways.

First, metaphase cells from the individual JM was hybridised with each of the seven subclones of pTRA-2, and the results were normalised and pooled (Fig. 4,B). This represented a total scoring of 747 grains and the distribution profile was closely similar to that seen when the complete pTRA-2 fragment was used (Fig. 4,A-6). In the second approach, we employed pTRA-2 as probe and constructed separate profiles using, as examples, individuals JC (Fig. 4, A-4) and MD (Fig. 4, A-12), based on approx. 100, 200 and >300 grains, and we have arrived at profiles which are not significantly different for each of the three values (data not shown) (see Discussion).



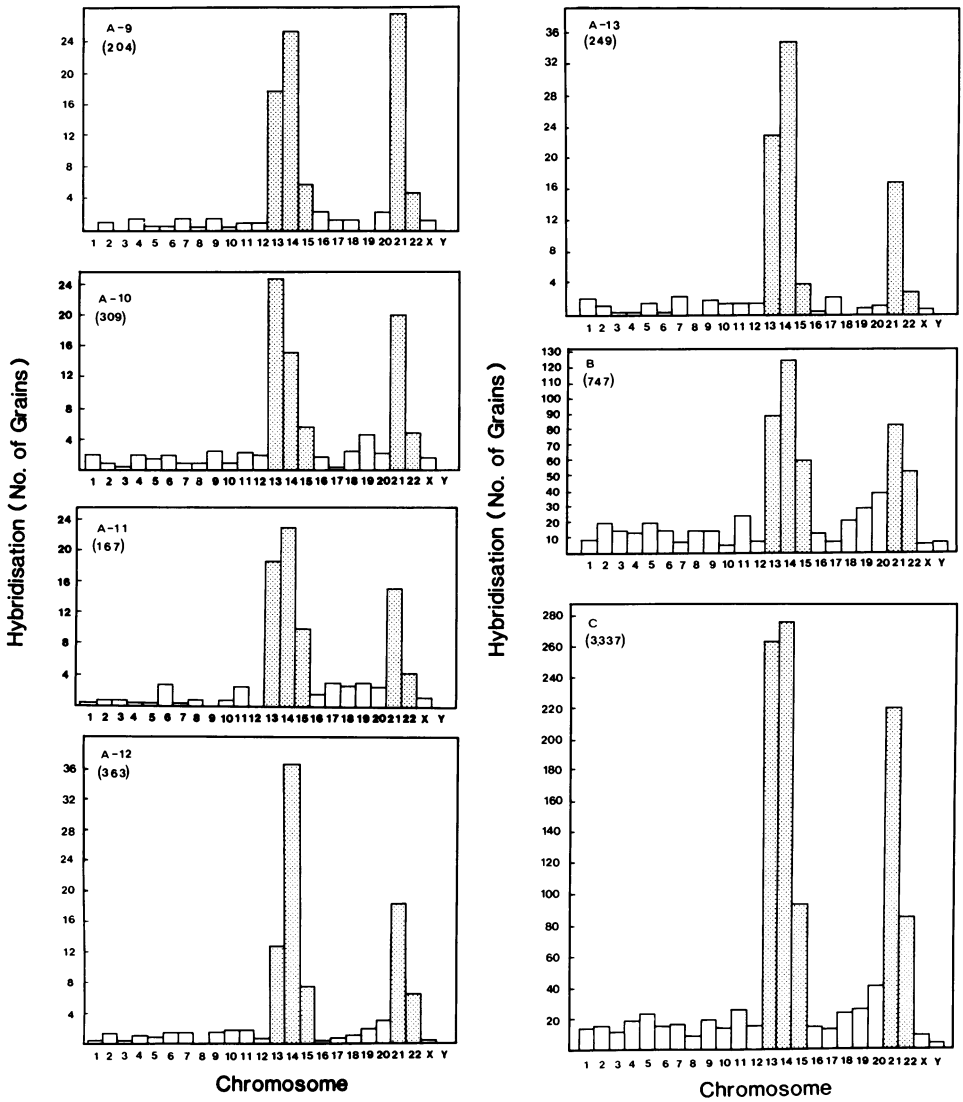


Fig. 4- Counts of grains on centromeres of human metaphase chromosomes following in situ hybridisation using pTRA-2 as probe. A-1 to A-13, male individuals DF, HD, DW, JC, PG, JMG, JM and female individuals VP, RMC, JR, RH, MD and DK, respectively. The number of grains counted is shown in brackets. In presenting the histograms (A-1 to A-13 and B), the results were normalised to 100 grains. B, sum of results using each of seven subclones, S1-S7, on JM, where approx. 100 grains were scored for each subclone. C, Sum of all results for A-1 to A-13 (using the normalised values). Results for the X- and Y-chromosomes have not been corrected for haploid dosage. The stippled area represents counts for each of the five acrocentric chromosomes.

TABLE 1 Distribution of autoradiographic grains on acrocentric chromosomes.

	CHROMOSOME							
	13	14	15	21	22	13+14+21	15+22	13+14+15+21+22
A-1	27	20	7	12	6	59	13	72
A-2	22	15	8	21	10	58	18	76
A-3	21	13	10	19	7	53	17	70
A-4	28	18	8	11	12	57	20	77
A-5	20	28	5	15	4	63	9	72
A-6	10	17	7	11	7	38	14	52
A-7	21	17	8	16	12	54	20	74
A-8	19	16	9	18	4	53	13	66
A-9	18	26	6	28	5	72	11	83
A-10	24	15	6	20	5	59	11	70
A-11	19	23	10	16	4	58	14	72
A-12	13	36	7	18	6	67	13	82
A-13	23	35	4	17	3	75	7	82
B	13	18	8	12	8	43	16	59
C	20.4	21.5	7.3	17.1	6.5	59	14	73

A-1 to A-13, B and C, as in Fig. 4. The values represent percentage of total grains found on the centromere of the chromosomes.

In order to contruct a "consensus" chromosomal profile for the pTRA-2 sequence, the results for the 7 males and 6 females studied were pooled. In the histogram shown in Fig. 4-C, a total of 3,337 grains were scored, representing a combined analysis of 216 metaphase cells. The results indicated that chromosomes 13, 14 and 21 were the most highly labelled chromosomes. The extent of labelling for chromosomes 13 and 14 was approx. similar, while that for chromosome 21 was slightly lower. In comparison, chromosomes 15 and 22 showed a greatly reduced signal, although they are also clearly significantly above the other background chromosomes. Table 1 provides a numerical summary of the relative distribution of autoradiographic grains on the five acrocentric chromosomes. As can be seen (Table 1, row C), 73% of the grains were found over these five chromosomes, with the following distribution: 20.4%, 21.5%, 17.1%, 7.3% and 6.5% on chromosomes 13, 14, 21, 15 and 22, respectively. As a group, chromosomes 13, 14 and 21 together account for 59%, while chromosomes 15 and 22 for 14% of the grains. An average of 1.4% of grains (range: 0.5% - 3.3%) was found on each of the remaining non-acrocentric chromosomes. These results represent localisation of greater than 80% of all the autoradiographic grains, with the remaining grains scattered randomly over the background.

DISCUSSION

We report the isolation and characterisation of a repetitive sequence (pTRA-2) that hybridises preferentially to the centromeres of human acrocentric chromosomes. This sequence has all the properties of alphoid DNA as defined by (i) cross-hybridisation to the centromeres of all the human chromosomes but showing a greater specificity for a smaller subset of

chromosomes; (ii) sequence-homology to the concensus and chromosome-X alphoid DNA sequences; (iii) formation of a Stu I ladder with an apparent multiplicity of 170 bp; (iv) organisation into a larger structure (3.9 kb) which forms the predominant higher-order amplification unit; and (v) its repetitive nature, where the number of copies of sequence closely homologous to pTRA-2 is estimated to be >100 on chromosomes 21 (data not shown).

A number of chromosome-specific subfamilies of alpha satellite DNA in the human genome has earlier been reported (see Introduction, and reference 24 for a review). pTRA-2 is clearly different from any of these as seen from the size of its higher-order repeat unit and its chromosome distribution pattern. Devilee *et al.*, (11,25) and Jorgensen *et al.*, (14) have described a subfamily of alphoid DNA which is shared by chromosomes 13 and 21, while Jorgensen *et al.*, (14) and McDermid *et al.*, (13) have described another subfamily on chromosome 22. Alphoid DNA showing specificity for chromosomes 14 and 15 have not previously been described. Our results therefore indicate that pTRA-2 represents a new subfamily of the alpha satellite DNA. This study, together with those of Devilee *et al.*, (25), Jorgensen *et al.*, (14) and McDermid *et al.*, (13), further suggest the likely co-existence of more than one subfamily of alphoid DNA on at least chromosomes 13, 21 and 22.

The compilation of the in situ hybridisation results shown in Fig. 4C has involved the use of 13 unrelated individuals and the scoring of a large number of metaphase cells (total : 216) as well as autoradiographic grains (total : 3,337). It should therefore represent a reasonably accurate "concensus" chromosome - distribution profile for the pTRA-2 sequence. This concensus profile agrees well with those of the constituent profiles especially for chromosomes 13, 14 and 21, which, in every case (except JC, see Results), are the three most highly labelled chromosomes. With chromosomes 15 and 22, however, the peaks in individual cases are not always greater, or significantly greater, than the rest of the chromosomes. Despite this, the concensus profile in Fig. 4C (and Table 1C) clearly indicates that, within a larger, pooled population sample, both these chromosomes showed a greatly accentuated grain count over each of the remaining nonacrocentric chromosomes. Other workers have previously reported that individual subfamilies of alpha satellite DNA tend to show specificity for a cognate chromosome (see referance 24). It is therefore interesting that members of the pTRA-2 subfamily are found on five different chromosomes. The possible underlying mechanism for such an occurrence will be discussed below.

The significance of the differences seen in the in situ profiles between individuals is presently not clear. Control experiments (see Results) have indicated the reproducibility of these profiles. We therefore believe that the major differences between the individuals are due to genetic polymorphism (representing a composite score for each pair of homologous chromosomes since these cannot be distinguished in the in situ assay). High frequencies of DNA polymorphism have previously been described for other subfamilies of alphoid DNA (3,8,9). However, until the precise nature of the polymorphism for the pTRA-2 sequence is

defined, the "concensus" profile will mainly be used in the following discussion.

A number of models (14) can be proposed to explain the existence of the same subfamily of alpha satellite DNA on different nonhomologous chromosomes. A model based on the transfer of a portion of a subfamily between nonhomologous chromosomes by recombination exchange seems to best fit the available data (14,24,26). It has long been known (15,16,17,27) that the acrocentric chromosomes tend to undergo a nose-to-nose association or "fusion" in meiosis I. During this period, the chromosomal DNA undergoes homologous exchange and postreplicative repair. Exchange of sister chromatids has been observed to take place between newly synthesised chromatid strands on the same chromosome (28). It is therefore reasonable to assume that, during this stage, recombinational exchange between nonhomologous chromosomes can also take place, while any DNA sequences common to these chromosomes are in juxtaposition. Genetic exchange among ribosomal genes on nonhomologous chromosomes has been observed in man and apes (17). The occurrence of a relatively recent exchange involving a subfamily of alpha satellite DNA on human chromosomes 13 and 21 has also been suggested (14,25). Based on the results of the present study, it is therefore reasonable to propose recombination exchange between nonhomologous chromosomes as the evolutionary process responsible for the "homogenisation" of the pTRA-2 subfamily of alphoid DNA on the different acrocentric chromosomes. Such a process would appear to be more selective for chromosomes 13, 14 and 21 when compared to chromosomes 15 and 22 as seen from the different degree of homology for pTRA-2 between the two groups. In light of our own results and those of Devilee *et al.*, (25) and Jorgensen *et al.*, (14), a corollary postulation is that homogenisation can occur independently between each of two independent subfamilies of alphoid DNA on the same chromosome. The observation of major differences in chromosome distribution patterns (Fig. 4,A-1 to A-13) between some of the individuals further suggests that exchange or "cross-talk" between the different acrocentric chromosomes may occur relatively frequently in a fluid population as part of an ongoing evolution process.

To date, there is a lack of evidence in the literature to explain the highly nonrandom participation of chromosomes 13, 14 and 21 in Robertsonian translocation (see Introduction). A principal model which has been proposed was based on the assumption that these three chromosomes have in common a homologous segment, which is inverted in chromosome 14 relative to 13 and 21 (18,27). Such a homologous segment is expected to be large or composed of long stretches of repetitive sequences as these could enhance the initial pairing event. A recombination exchange within the inverted segment between chromosomes 13 and 14, and 14 and 21 would give rise to t(13q14q) and t(14q21q), respectively. In addition, an important feature to reconcile in this hypothesis is the observed site of the Robertsonian translocations. Although these translocations are known to take place at the centromere, in the satellite-III DNA, or in the ribosomal DNA, direct cytogenetic study has revealed that most are at the centromere (27). The pTRA-2 clone represents the first sequence which seems to satisfy most of

these requirements since it is repetitive, centromerically located, and shares close sequence homology between chromosomes 13, 14 and 21. We do not have any information on the chromosomal orientation/"inversion" of the pTRA-2 sequences, or whether the inversion, if exists, involves a portion or the entirety of the pTRA-2 repetitive arrays. Despite this, the identification of this sequence provides the best evidence to date in support of the above model for frequent 13q14q and 14q21q translocations. Further, the observation of pTRA-2 sequences on all five acrocentric chromosomes is consistent with the occurrence of the baseline number of observed Robertsonian translocations involving all the other possible combinations of the acrocentric chromosomes (18). These translocations could arise because the tendency of centromeric repetitive sequences homologous to the different acrocentric chromosomes to pair and undergo recombination may sometimes create "errors" such as "U-type exchanges" (29) which could lead to whole-arm transfer.

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