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Four distinct alpha satellite subfamilies shared by human chromosomes 13, 14 and 21

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ABSTRACT

We describe the characterisation of four alpha satellite sequences which are found on a subset of the human acrocentric chromosomes. Direct sequence study, and analysis of somatic cell hybrids carrying specific human chromosomes indicate a unique 'higher-order structure' for each of the four sequences, suggesting that they belong to different subfamilies of alpha DNA. Under very high stringency of Southern hybridisation conditions, all four subfamilies were detected on chromosomes 13, 14 and 21, with 13 and 21 showing a slightly greater sequence homology in comparison to chromosome 14. None of these subfamilies were detected on chromosomes 15 and 22. In addition, we report preliminary evidence for a new alphoid subfamily that is specific for human chromosome 14. These results, together with those of earlier published work, indicate that the centromeres of the five acrocentric chromosomes are characterised by a number of clearly defined alphoid subfamilies or microdomains (with at least 5, 7, 3, 5 and 2 different ones on chromosomes 13, 14, 15, 21 and 22, respectively). These microdomains must impose a relatively stringent subregional pairing of the centromeres of two homologous chromosomes. The different alphoid subfamilies reported should serve as useful markers to allow further 'dissection' of the structure of the human centromere as well as the investigation of how the different nonhomologous chromosomes may interact in the aetiology of aberrations involving these chromosomes.

INTRODUCTION

The centromeres of eukaryotes are generally characterised by the presence of tandemly repeated satellite DNA sequences. Apart from their repeated nature, these sequences are, in most cases, unrelated between different species (1,2). A number of functions have been proposed for the repeats (2,3,4,5) and there is now evidence that the centromeric repeats are of biological importance in at least some species (6,7).

In the human, the centromeric region is, as a rule, associated with a large amount of tandemly repeated alpha satellite DNA. This is the only known DNA within the centromere and its detailed analysis is important for the understanding of the structure of the human centromere. The alphoid DNA has a fundamental repeat unit of 171 bp (8,9) that is in turn organised into different higher-order repeating structures (10). These higher-order structures, which define different subfamilies of alpha satellite DNA, are usually present in multiple, conserved copies within the centromere of a single chromosome (10), or a small group of chromosomes (11–14).

Alphoid subfamilies specific for a single chromosome are believed to have evolved as a result of homogenisation of new mutations by regular exchange between homologous chromosomes. The origin of alphoid subfamilies common to multiple chromosomes, on the other hand, necessarily entails a more complex process of interaction involving nonhomologous chromosomes. The identification of common alphoid subfamilies on subsets of the human acrocentric chromosomes has led us to suggest that these chromosomes may interact and exchange centromeric sequences in a way which predisposes the chromosomes to nondisjunction and translocation (15). In the present study, we have further characterised the organisation of alpha DNA on these chromosomes. We report the identification of three new and one previously studied subfamilies that are shared by chromosomes 13, 14 and 21. Preliminary evidence for the existence of a new subfamily specific for chromosome 14 is presented. These results are discussed in the light of the structure of the human centromere and the process by which the three acrocentric chromosomes may interact.

MATERIALS AND METHODS

Alpha satellite clones

The isolation of human alpha satellite clones pTRA-1, pTRA-2, pTRA-4 and pTRA-7, have been reported earlier (11,12). The sizes of these clones are approximately 1.2, 3.9, 5.1, and 1.7 kb, respectively. In the present study, these clones were further examined by restriction mapping and Southern analysis using a panel of somatic cell hybrids carrying specific human

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chromosomes. The entire sequence of each clone was determined from a series of restriction fragments subcloned in M13MP18 or MP19 by the dideoxy chain-termination method (16), utilising the sequenase kit (USB) and ^{35}S-dATP.

Somatic cell hybrids

The panel of somatic cell hybrids used in the present study was: CY4A (human chromosome 13)(D. Callen, personal communication); CF34-2-3 (human chromosomes 13, 12 and 6p)(17); CP43 (human chromosome 14)(18); F4SC13C19 (human chromosomes 14, 1 and X)(19); HORL-Ia (human chromosomes 15 and X)(19); WAVR-4d-F9-4A (human chromosome 21)(20); WEGROTH (human chromosome 22)(Geurts van Kessel et al, personal communication); PgMe25Nu (human chromosomes 22 and X)(21); 5HL-94 (human chromosome 19 only)(22) and CF-31-24 (human chromosomes 20p/Xq and 3)(23). The latter hybrid contains no alpha satellite from the X-chromosome (24).

Southern blot hybridisation

$5-10\mu g$ of high-molecular-weight genomic DNA from the somatic cell hybrids were digested with restriction enzymes and loaded onto each gel track. Following electrophoretic separation, DNA was transferred onto Hybond membrane filters and hybridised in a phosphate buffer (0.5M Phosphate buffer, pH 7.0; 1mM EDTA; 1% EDTA; 1% BSA and 7% SDS) at 75.5°C. After hybridisation, filters were washed at a final stringency of $1\times$SSC, 0.1% SDS at 81°C (25).

RESULTS

Determination of higher-order structures

For the initial determination of the higher-order structure for each of the four alphoid probes (pTRA-1, -2, -4 and -7), a hybrid cell line (WAVR-4d-F9-4A) containing a chromosome 21 as its only human complement was used. As shown in Fig.1-A, 1-B and 1-C, with pTRA-1, -4 and -7, respectively, use of 15 different restriction enzymes (also SspI and StuI; data not shown) did not reveal a consistent common band that was indicative of a higher-order repeat unit for these sequences. With pTRA-2 (Fig.1-D), HindIII and MspI both gave a predominant band at 3.9 kb which is in agreement with the higher-order structure previously described (11). These results indicated that each of the four alphoid sequences belonged to a different subfamily as evident from their distinctive restriction patterns. Based on these results, four enzymes were chosen to allow further characterization of these sequences using somatic cell hybrids carrying the other human acrocentric chromosomes. In particular, EcoRI and HindIII were included in all the analyses to allow direct comparison between the probes (see below).

Chromosomal distribution of four different alphoid sequences

pTRA-1, -2, -4 and -7 were hybridised to a panel of somatic hybrid cell lines. The cell lines used included two hybrids each carrying a human chromosome 13, two hybrids each carrying a human chromosome 14, and five different hybrids each carrying a separate human chromosome 15, 19, 20, 21 or 22 (see Materials and Methods). These hybrids were selected because previous *in situ* hybridisation experiments carried out at moderate stringency have demonstrated preferential hybridisation of the probes to these chromosomes (12).

Fig.2A shows results obtained with pTRA-7. As can be seen, the two hybrids carrying human chromosome 13, those carrying human chromosome 14, as well as the hybrid carrying human chromosome 21, all gave a similar restriction enzyme pattern with EcoRI, HindIII, PstI and HindII. Some polymorphic differences were apparent between the different hybrids. For example, in CY4a (chromosome 13) and WAVR-4d-F9-4A (chromosome 21)(Figs.2A-2 and 2A-5), only one of the two HindII bands was detected. A parallel analysis of all the other hybrids did not reveal any hybridisation of the probe (picture not shown). These results indicated the existence of a common alphoid subfamily corresponding to the pTRA-7 sequence on human chromosomes 13, 14 and 21, but not 15, 19, 20 and 22.

With pTRA-4, EcoRI, HindIII and NcoI produced band patterns that are similar for the five hybrids containing chromosome 13, 14 and 21 (Fig.2B). PstI gave a single band at 5.1 kb for the two chromosome-13 hybrids and the chromosome-21 hybrid (Fig.2B−1, −2 and −5, respectively), but two bands of similar intensity at 1.9 and 3.2 kb for the two chromosome-14 hybrids (Fig.2B−3 and −4). This result indicated that the pTRA-4 sequence on chromosome 14 has an extra PstI site within the 5.1 kb PstI fragment in comparison to those on chromosomes 13 and 21. As with pTRA-7, under the same condition of analysis, no hybridisation signal was detected in the hybrids containing human chromosomes 15, 19, 20 or 22, suggesting that pTRA-4 is specific for chromosomes 13, 14 and 21 only.

The results shown in Fig.2C confirmed those previously reported for pTRA-2 that this is a common alphoid subfamily shared by chromosomes 13, 14 and 21 (12). These results were included to allow comparison with pTRA-1, -4, and -7, and we have analysed additional hybrids in the present study. The results in Fig.2C indicated that although the pTRA-2 subfamily is present on chromosomes 13, 14 and 21, it has diverged considerably more on chromosome 14 compared to the other two chromosomes. This was most evident with HindIII and RsaI where additional sites were detected within the higher-order structure of chromosome 14. The significance of this will be discussed later.

In the previous study using pTRA-2 (12), we have detected cross-hybridising bands (not corresponding to the 3.9 kb higher-order unit for this alphoid subfamily) in a chromosome-15 and a chromosome-22 hybrid. However, use of a much higher stringency of hybridisation in the present study has completely removed all signals in these hybrids. In addition, no hybridisation signals were detected in the hybrids containing human chromosome 19 or 20.

Finally, with pTRA-1, the major bands detected were once again common between the two chromosome-13 hybrids, the chromosome-21 hybrid, and one of the two chromosome-14 hybrids (Fig.2D−1, −2, −5 and −3). The second chromosome-14 hybrid (CP43) repeatedly failed to show the major bands observed in the other hybrids, although some other weaker bands were observed. Since under the high stringency of hybridisation used, no discernible bands were detected in hybrids containing human chromosomes 15, 19, 20 or 22, it is possible that the weaker bands seen in CP43 also belonged to the pTRA-1 subfamily but representing diverged polymorphic members of this subfamily.

Because of the nature of the slightly unexpected results described above, where all four probes consistently hybridised to the same set of hybrids but not others, a number of precautions and control experiments were performed to ensure the accuracy

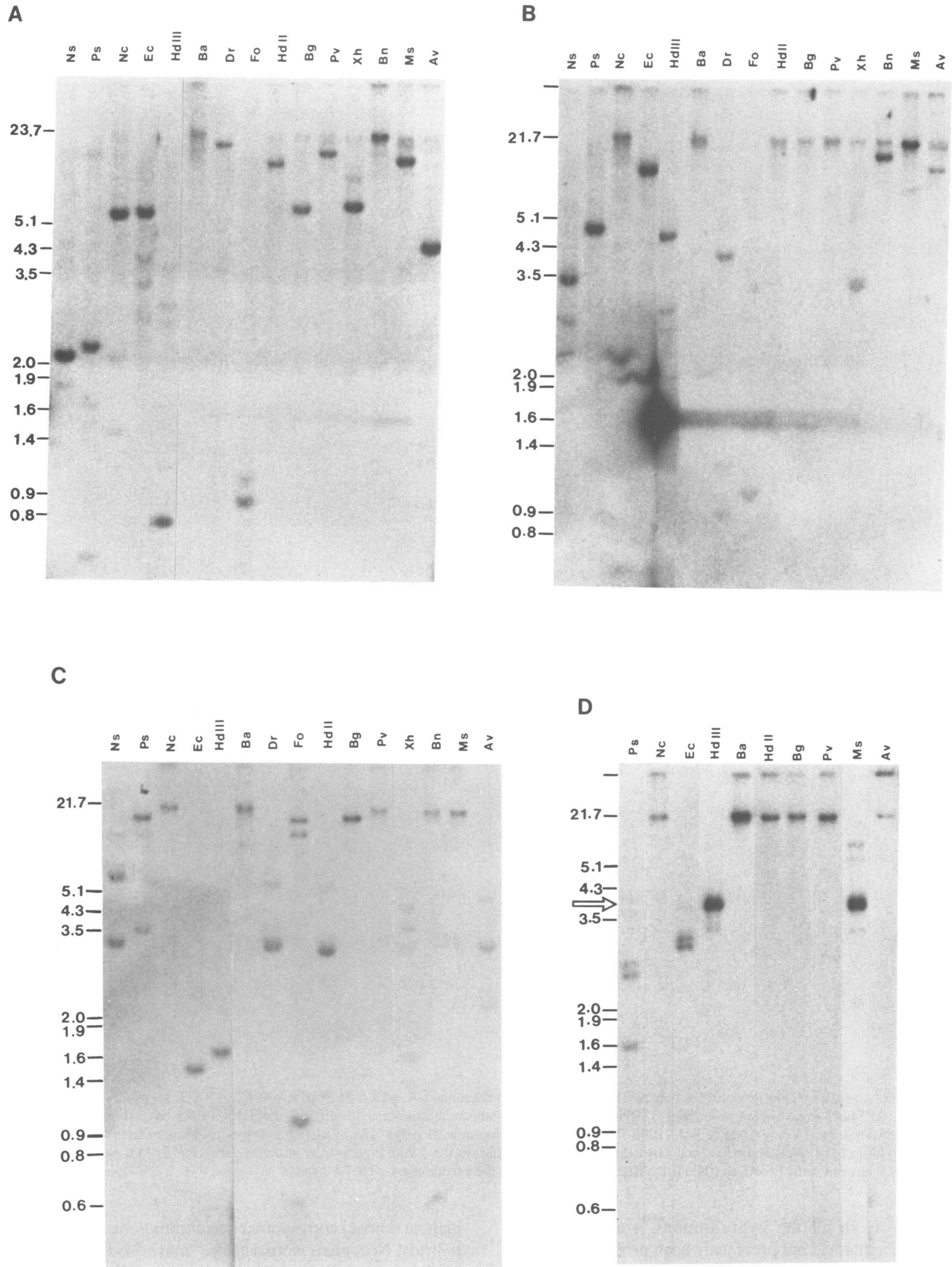

Fig. 1. Southern blot analysis of a hybrid cell line WAVR-4d-F9-4A (carrying human chromosome 21) using as probes: A, pTRA-1; B, pTRA-4; C, pTRA-7; and D, pTRA-2. The enzymes used were: NsiI (Ns), PstI (Ps), NcoI (Nc), EcoRI (Ec), HindIII (HdIII), BamHI (Ba), DraI (Dr), FokI (Fo), HindII (HdII), BglII (Bg), PvuII (Pv), XhoII (Xh), BanI (Bn), MspI (Ms), and AvaII (Av). Open arrow points to the 3.9 kb higher-order repeat unit for pTRA-2. Mol. wt. markers were lambda phage DNA cleaved with HindIII and EcoRI. [The strong signal around 1.6 kb in Fig.1B (EcoRI and HindII lanes) was due to contamination from an adjacent molecular weight marker track on the original autoradiogram. This contamination has not obliterated any band in the EcoRI and HindII tracks (see Fig.2B)]

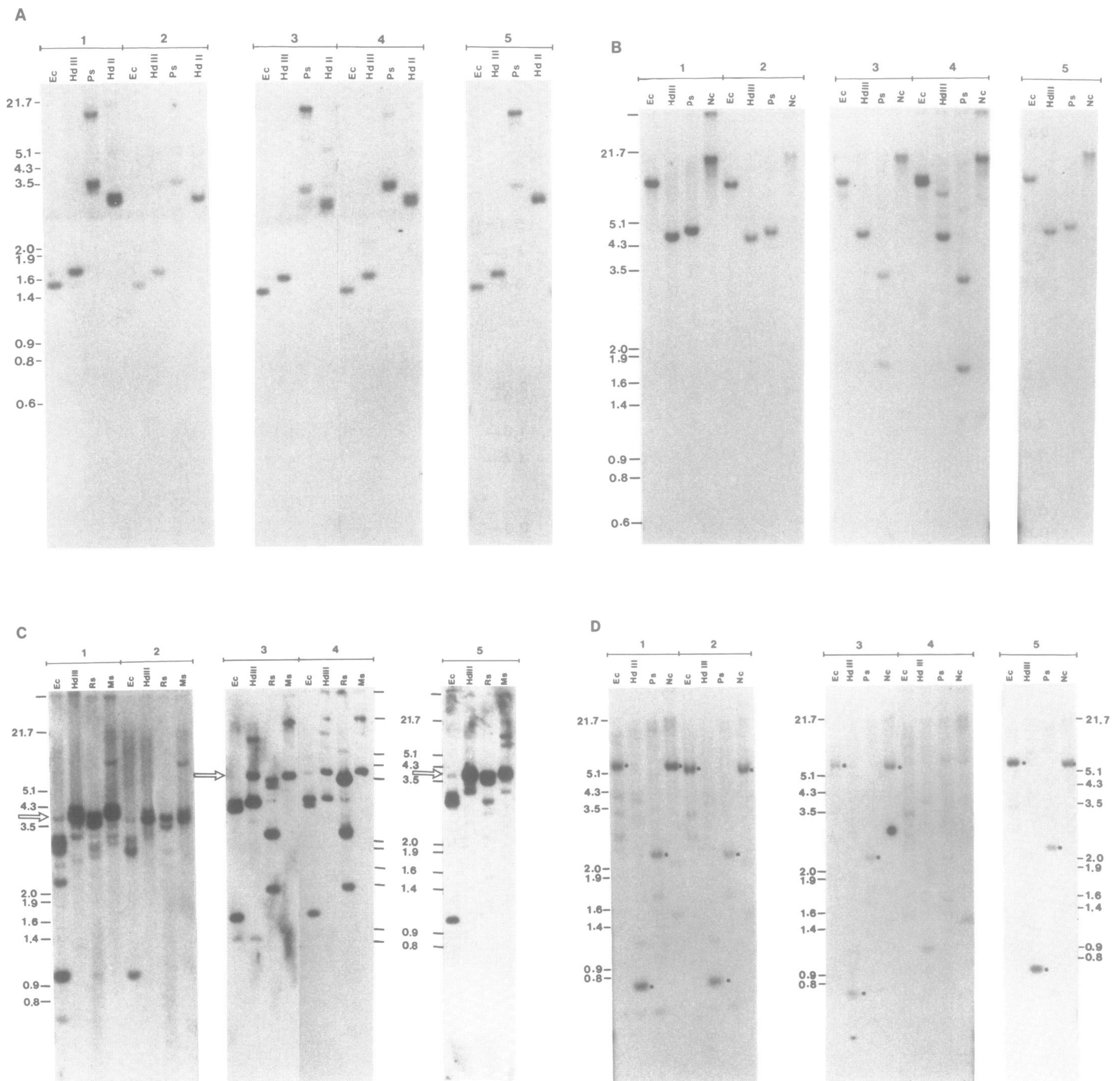

Fig.2. Southern blot analysis and chromosomal distribution of different alphoid sequences: A, pTRA-7; B, pTRA-4; C, pTRA-2; D, pTRA-1. The somatic hybrid cell lines were: 1, CF34-2-3 (human chromosomes 13, 12 and 6p); 2, CY4A (human chromosome 13 only); 3, F4SC13C19 (human chromosomes 14, 1 and X); 4, CP43 (human chromosome 14 only); and 5, WAVR-4d-F9-4A (human chromosome 21 only). The restriction enzymes used were: EcoRI (Ec), HindIII (HdIII), PstI (Ps), HindII (HdII), NcoI (Nc), RsaI (Rs), and MspI (Ms). Arrow in C indicates the 3.9 kb higher-order structure for pTRA-2. Mol. wt. markers were either lambda phage DNA cleaved with EcoRI and HindIII. Asterisk in D indicates the predominant pTRA-1 bands.

of the results: 1) In all the hybridisations, freshly prepared membrane filters that had not previously been probed were used; 2) The karyotypes of all the hybrids were independently determined in our own laboratory just prior to their use in this work; 3) Southern blot hybridisation with an alpha RI probe (specific for human chromosomes 13 and 21; ref.13) has demonstrated the expected results with hybrids CF34-2-3, CY4a and WAVR-4d-F9-4A, thus confirming the presence of an alphoid domain corresponding to chromosome-13 or −21 in these hybrids. No signals were detected in hybrids CP43, F4SC13C19, HORL-Ia and PgMe25Nu with this probe; 4) Southern blot hybridisation with an alpha XT probe (specific for human chromosomes 14 and 22; ref.14) has, on the other hand, demonstrated strong hybridisation to hybrids CP43, F4SC13C19 and PgMe25Nu but not CF34-2-3, CY4a and WAVR-4d-F9-4A, thus establishing the presence of an alphoid domain corresponding

Table 1. Alphoid subfamilies on human acrocentric chromosomes.

Subfamily	Clone Designation	Chromosome 13	14	15	21	22	References
I	pTRA-1	+	+		+		Present study
II	pTRA-2	+	+		+		Present study; (11,12)
III	pTRA-4	+	+		+		Present study
IV	pTRA-7	+	+		+		Present study
V	αRI-680, L1.26	+			+		(13,29)
VI	αX, αT, αXT		+			+	(14)
VII	pTRA-54		+				Present study
VIII	p82H		+				(27)
IX	p22/1:2.1					+	(26)
X	pTRA-20			+			(25)
XI	pTRA-25			+			(25)
XII	Undesignated			+			(25)
No. of Domains		**5**	**7**	**3**	**5**	**2**	

The presence of a subfamily (or domain) is indicated by +. The number of alphoid domains so far identified on each chromosome is shown at the bottom. XII represents up to two alphoid subfamilies that are specific for chromosome 15 but appear to be different from pTRA-20 (X) and pTRA-25 (XI)(25).

to chromosome-14 or −22 in the first three hybrids; 5) Southern blot hybridisation with two alphoid probes (pTRA-20 and -25; specific for human chromosome 15; ref.25) has demonstrated the presence of human chromosome 15 in hybrid HORL-Ia but not in the other hybrids; 6) Hybridisation of hybrids 5HL-94 and CF-31-24 with pTRA-2 probe under a low stringency of 3×SSC at 55°C has revealed strong signals (with a characteristic 171 bp ladder with some enzymes), indicating the presence of alphoid domains that are expected to be present on chromosome 19 and 20 in these hybrids; 7) Analysis of the different hybrids with a chromosome 22-specific alphoid probe has also provided compatible results (see below).

Sequence analysis

The complete nucleotide sequences for the four alphoid subfamilies (pTRA-2, -4, -7 and -1) were determined. These sequences have been submitted to the EMBL Data Library (accession nos. X55367, X55368, X55369 and X55370, respectively). All four clones were shown to be alpha DNA with their 171 bp monomers showing an average of 91%, 95%, 95% and 96% homology, respectively, to the published human alphoid concensus (8). We have compared sequences of the individual 171 bp monomers that constitute these four subfamilies both within and between the different clones. The results indicated that, at the level of their primary sequence, the four alpha subfamilies are characterised by structures that are as unrelated to each other as the different alpha subfamilies from other chromosomes (B.V. and K.H.C., unpublished). These results corroborate those obtained by Southern analysis of somatic cell hybrids that pTRA-2, -4, -7 and -1 are different alphoid subfamilies.

Identification of a new alphoid subfamily specific for human chromosome 14

We have studied the chromosomal distribution of a previously described alphoid probe, p22/1:2.1 (26) using our panel of hybrids. As shown in Figs.3−3 and 3−4, the probe showed the expected strong hybridisation to the two hybrids containing human chromosome 22. In addition, the probe revealed a set of weaker bands that was common to the two chromosome-14 hybrids (Figs.3−1 and 3−2) but absent in both the chromosome-22

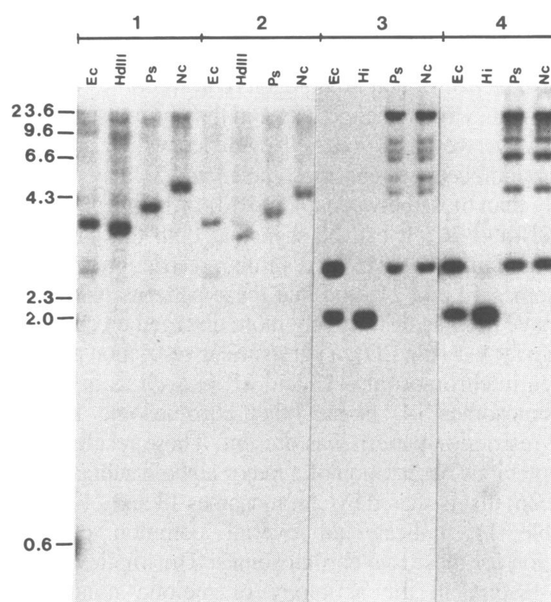

Fig.3 Southern blot analysis using p22/1:1.2 probe. The somatic hybrid cell lines used were: 1, CP43 (human chromosome 14 only); 2, F4SC13C19 (human chromosomes 14, 1 and X); 3, PgMe25Nu (human chromosomes 22 and X); and 4, WEGROTH (human chromosome 22 only). The restriction enzymes used were: EcoRI (Ec), HindIII (HdIII), PstI (Ps) and NcoI (Nc). Mol. wt. markers were lambda phage DNA cleaved with HindIII.

hybrids (Figs.3−3 and 3−4). The detection of no hybridisation signals in the other hybrids (CF34-2-3, CY4a, HORL-Ia and WAVR-4d-F9-4A) further indicated the absence of this sequence on chromosomes 13, 15 and 21. Since the pattern of bands seen in the chromosome-14 hybrids was distinctively different from any of the previously published alphoid subfamilies (see Discussion), it presumably represented a new subfamily of alpha DNA that is specific for human chromosome 14. We have tentatively designated this alphoid subfamily as pTRA-54. The detection of this subfamily using the p22/1:2.1 probe under high stringency condition suggested a high degree of sequence homology between p22/1:2.1 and pTRA-54.

DISCUSSION

Four common alphoid subfamilies on human chromosomes 13, 14 and 21: evidence for a greater degree of homology between 13 and 21

We describe the identification of four different alphoid subfamilies that are shared by human chromosomes 13, 14 and 21. In a previous study, using *in situ* hybridisation technique, three of these subfamilies (pTRA-1, -4 and -7) were shown to cross-hybridise extensively to chromosomes 13, 14, 15, 19, 20, 21 and 22, with pTRA-7 showing an increased hybridisation to chromosome 14 (12). The discrepancy in the two studies can be attributed to the stringency of hybridisation used. As was previously discussed (12), the condition of hybridisation used in the *in situ* experiments allowed the detection of closely related sequences. The use of a significantly higher hybridisation and washing stringency in the present Southern blot analysis has eliminated the problem of cross-hybridisation and has demonstrated the specificity of all four alphoid subfamilies for chromosomes 13, 14 and 21 only.

The presence of multiple common alphoid subfamilies on three nonhomologous chromosomes would involve exchange of alphoid DNA between the different chromosomes. In this process, it is expected that the degree of homology of a particular alphoid subfamily on a pair of non-homologous chromosomes will depend on the frequency of exchange between the chromosomes. In the present study, we have obtained evidence which suggest that homogenisation between chromosome 13 and 21 may occur more efficiently than those between 14 and 13, or between 14 and 21. In Figs.2B and 2C, it can be seen that both the pTRA-4 and pTRA-2 subfamilies gave very similar restriction patterns for chromosomes 13 and 21, and that these patterns, while clearly related, have become distinctively more diverged on chromosome 14. With pTRA-1 (Fig.2D), a very similar restriction pattern was again seen in chromosomes 13 and 21, as well as in one of the two chromosomes 14; in the other chromosome 14, a very different restriction pattern was present. These results, together with the previous observation of a major alphoid subfamily (alpha RI or L1.26) that is shared by chromosomes 13 and 21 (see below and Table 1), indicate an overall common centromeric organisation for these two chromosomes. The implication of this unusual feature in the aetiology of meiotic nondisjunction involving these two chromosomes has previously been discussed (15).

With pTRA-7 (Fig.2A), the otherwise common restriction pattern seen on chromosomes 13, 14 and 21 was disrupted by the presence of two major HindII bands (approx. 2.8 and 3 kb) in both chromosome 14 and one chromosome 13 (CF-34−2-3), but only one band (2.8 kb) in the other chromosome 13 (CY4A) and the chromosome 21. An explanation for this result is that recombination has occurred between chromosomes 13 and 14 in which sequences containing the HindII doublet has been exchanged from a chromosome 14 onto the chromosome 13 in CF-34-2-3. Since in the above analyses the same set of human chromosomes 13, 14 and 21 was used, the detection of the different patterns of sequence-homology with the four alphoid subfamilies suggests that the different subfamilies can undergo at least some degree of independent recombination involving these three chromosomes.

Identification of a new chromosome-14 alphoid subfamily

During our study of the different alphoid probes isolated by other workers, we have identified a new alphoid subfamily (pTRA-54) which appears to be present on human chromosome 14 but not 13, 15, 21 and 22. Earlier, Waye et al (27) have reported an alphoid sequence (p82H) that is specific for human chromosome 14. The pTRA-54 subfamily is different from this sequence by virtue of the different restriction patterns obtained with EcoRI, HindIII and PstI. In particular, HindIII cleaved within the two chromosome-14 DNA (Fig.3) to give an approximately 3 kb pTRA-54 band, whereas the same enzyme did not cut within the p82H sequence as evident from a large hybridising band at greater than 15 kb (27).

Presence of multiple distinct alphoid subfamilies within each acrocentric centromere

Table 1 summarises all the alphoid subfamilies that have so far been identified on the human acrocentric chromosomes. There are at least 5, 7, 3−4, 5 and 2 different subfamilies on chromosomes 13, 14, 15, 21 and 22, respectively. The existence and maintenance of several discrete alphoid domains within a centromere suggest that the centromere can no longer be regarded as a single amorphous block of alpha satellite DNA in which two homologous chromosomes can pair and undergo unrestricted unequal crossing-over as is generally assumed. Instead, a more stringent subregional pairing of the different alphoid subfamilies, or microdomains, must prevail. Such a requirement has not been stated previously and may be important for the functional operation of the centromere, including the precise recognition, pairing and segregation of the homologous chromosomes. Clarke and Baum (28) have recently demonstrated the functional importance of centromere-specific, tandemly repeated sequences for the full operation of the centromere in meiosis in the fission yeast, S.pombe. The different alphoid subfamilies reported in the present study should form useful markers along the length of the centromere of the different acrocentric chromosomes to allow further dissection of these centromeres, including the search for a possible 'core sequence' similar to that identified in S.pombe (28).

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