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Chromosomal location by in situ hybridization of the human Sau3A family of DNA repeats

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Summary. The Sau3A family is a human, clustered, highly repetitive, GC-rich DNA family. In situ hybridization studies with a plasmid carrying a Sau3A monomer as a probe have shown that Sau3A sequences are preferentially concentrated in the heterochromatic regions of human acrocentric chromosomes (D and G groups, both in pericentromeric regions and in cytological satellites) and in pericentromeric heterochromatin of chromosome 1. The same chromosomal locations were observed by using as probes two recombinant phages which carry Sau3A-positive genomic sectors. The two sectors differ for the relative proportions of monomer and multiples of Sau3A repeats, which show different extents of homology to the cloned monomer, and for the presence, in one of the two, of a small amount of an unrelated repeat (alphoid DNA). The similarity of the results obtained with the three probes suggests that heterogeneous Sau3A repeats share the same chromosomal localizations and that the two analyzed genomic sectors may not contain significant amounts of repetitive DNAs other than the Sau3A family. A comparison between the chromosomal locations of Sau3A and EcoRI families of repeats has confirmed that each family is characterized by specific chromosomal locations and that single heterochromatic regions may contain both.

Introduction

The human genome contains highly repetitive DNA sequences organized in tandem arrays in long DNA molecules (Corneo et al. 1967, 1970, 1971). Such DNA molecules (called satellite DNAs because of the bands, distinct from the main DNA band, which they form in dense salt gradients) contain different DNA families of repeats and show a considerable organizational complexity (Cooke and Hindley 1979; Mitchell et al. 1979).

A general feature of human satellite DNAs is their preferential concentration at the heterochromatic regions of a few specific chromosomes (Jones and Corneo 1971; Jones et al. 1973, 1974). Specific subsets, such as restriction fragments, of satellite DNA also show chromosomal specificity with respect to both sequence and organization (Beauchamp et al. 1979).

Since satellite DNAs are characterized by both sequence heterogeneity and partial homology (Cooke and Hindley 1979; Singer 1982), their use in the analysis of human heterochromatin has been limited. However, the availability of well characterized repetitive DNA cloned probes should allow the analysis of heterochromatin organization in individual chromosomes and possibly provide clues to speculate about the evolution and function of highly repetitive sequences.

By analyzing the restriction enzyme digests of total human DNA (from tissues) or of the DNA of somatic cell hybrids containing only a few human chromosomes and by screening genomic libraries enriched for specific human chromosomes, several families of highly repetitive DNA sequences have been identified and characterized. Among these, alphoid DNA (the *EcoRI 340 bp family*) shows a specific pericentromeric location in autosomes (Manuelidis 1978a, b; Wu and Manuelidis 1980; Willard 1984), while a related sequence, the *2 kb BamHI family,* is predominantly located on the X chromosome (Yang et al. 1982; Willard et al. 1983; Waye and Willard 1985). Conversely, the non-alphoid *HaeIII 3.4 kb family* of repeats has been shown to be present on the Y chromosome, although subsets of the repeat show homologous sequences on some autosomes (Cooke 1976; Kunkel et al. 1976, 1979; Jeanpierre et al. 1985; Burk et al. 1985).

The identification and mapping of new human highly repetitive DNA families might be important in order to obtain more information about the content and organization of repetitive DNA sequences in specific human chromosomes and to investigate the possible role of these chromosomal regions.

In previous work, by comparing Sau3A digestion patterns of human DNA components with different base compositions, we have identified and characterized a new human DNA family of repeats, not related to alphoid DNA or to previously described human non-alphoid families (Meneveri et al. 1984, 1985). This family of repeated DNA sequences, *Sau3A family,* has a monomeric length of 68 bp and a GC content of 53%. It is also characterized by a relatively high repetition frequency (4×10^4 copies/haploid genome) and by a clustered organization. Here we report the chromosomal location of this GC-rich clustered family of repeats by in situ hybridization studies and a partial characterization of two Sau3A-positive genomic sectors.

Materials and methods

DNA probes

Plasmid pUh1-39 was obtained as previously described (Meneveri et al. 1985). The recombinant plasmid contains a

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human DNA insert of 68 bp, in the BamH1 site of the pUC8 cloning vector. Plasmid pUhE-7, containing alphoid DNA (340 bp EcoRI repeat) and prepared as previously described (Meneveri et al. 1984), was also used as a probe in filter and in situ hybridization experiments. Sau3A-positive recombinant phages (A47 and A17) were obtained by screening a human genomic library with pUh1-39 plasmid DNA (Meneveri et al. 1985). Subcloning of DNA fragments from human Sau3A-positive recombinant phages has been carried out in the EcoRI site (A17*a*, A17*b*, and A47*h*) or between the EcoRI and HindIII sites of pUC8 (Maniatis et al. 1982).

Restriction enzyme mapping and DNA hybridization analyses

DNA samples were digested with restriction enzymes according to the procedures specified by the manufacturers, and fractionated by agarose gel electrophoresis in TBE buffer (0.089 *M* Tris-borate, 0.089 *M* boric acid, 0.002 *M* EDTA, pH 8). DNA was visualized by ethidium bromide staining and UV-transillumination. Restriction enzyme mapping of Sau3A-positive recombinant phages was performed by the synthetic oligonucleotides method described by Rackwith et al. (1985). DNA was transferred onto nitrocellulose filters (Southern 1975) and hybridized at 42°C in 50% formamide 750 m*M* NaCl for 16–20 h in the presence of heat-denaturated, labeled pUh1-39 and pUhE-7 purified DNA inserts (specific activity: 1×10^8 dpm/μg, $2–3 \times 10^6$ dpm/ml). Final filter washes were in 1 × SSC at 65°C (SSC = 0.15 *M* NaCl, 0.015 *M* sodium citrate) (Meneveri et al. 1985).

DNA labeling

Phosphorus-32-labeling of purified DNA inserts of pUh1-39 and of pUhE-7 plasmids was performed by Klenow or nick-translation reactions, respectively (Maniatis et al. 1982). Tritium-labeling of plasmid and phage DNAs was performed by nick-translation using ^{3}H-dATP and ^{3}H-TTP as labeled precursors (Rigby et al. 1977). Specific activities were $2–4 \times 10^7$ dpm/μg DNA.

In situ hybridization

Human metaphase chromosome spreads were obtained by standard procedures from phytohemagglutinin-stimulated cultures of whole blood from normal male and female donors. In situ hybridization was carried out essentially as described by Harper and Saunders (1982). Slides were treated with RNase (50 μg/ml) for 30 min at 37°C, rinsed twice in 2 × SSC, and then dehydrated through a graded series of ethanol solutions. Slides were heat-denatured by immersion in 2 × SSC/70% formamide at 72°C for 2–3 min followed by cooling in 70% ethanol and dehydration in a graded series of ethanol solutions.

Slides were hybridized in 50% formamide – 10% dextran sulfate in 2 × SSCP (0.12 *M* NaCl, 0.015 *M* sodium citrate, 0.02 *M* phosphate buffer pH 6) in the presence of 50–100 ng/ml of heat-denatured radioactive probe at 39°C for 16–18 h. The slides were then rinsed in three changes of 50% formamide – 2 × SSC at 39°C for 3 min each, followed by five changes of 2 × SSC at 39°C, 2 min each. The slides were then dehydrated in a graded series of ethanol solutions. Autoradiography was carried out by dipping the slides into NTB-3 photographic emulsion (Kodak). Covered slides were exposed for different periods of time (from 3 days to one month) and then developed. Human chromosomes were identified by Q banding.

Results

In situ hybridizations on human metaphase chromosome spreads (from a 46,XX normal individual) using ^{3}H-labeled DNA of the Sau3A monomer-containing plasmid pUh1-39 as a probe were carried out to localize the Sau3A family of repeats (Fig. 1B). Chromosome identification was obtained by Q banding (Fig. 1A). Hybridization signals were preferentially concentrated on six autosomal regions corresponding to the pericentromeric heterochromatin of chromosome 1 and to the heterochromatic regions of acrocentric chromosomes (D and G groups). The analysis of the distribution of 1094 grains over 83 metaphases has shown that approximately 40% of grains are on chromosomes of D group, 28% are on chromosomes of G group, and 5% are on chromosome 1; the remaining grains were randomly distributed over the rest of the chromosomes. This result indicates that Sau3A repeats are preferentially clustered at heterochromatic regions of specific chromosomes. Analogous experiments using chromosome spreads from a normal 46,XY individual gave a grain distribution similar to that reported in Fig. 1B. No hybridization was seen at the level of the heterochromatic region of chromosome Y.

Human chromosome spreads were also hybridized with ^{3}H-labeled pUhE-7 plasmid containing alphoid DNA (EcoRI 340 bp). As shown in the representative metaphase plate of Fig. 1C, the main locations of alphoid sequences are at the level of the pericentromeric regions of chromosomes 1, 5, 16, and 19. Minor locations on chromosomes 4, 7, 9, and 10 are also observed.

The Sau3A repeats are characterized by a clustered organization and are present in a 0.5% fraction of a human genomic library (Meneveri et al. 1985). Sau3A-positive recombinant phages show a wide variation of signal intensity. Two of such phages, A17 and A47, were chosen for further analysis on the basis of their high (A17) and low (A47) hybridization signals with the pUh1-39 probe. Figure 2 shows the EcoRI/HindIII restriction maps of the two recombinant phages. Sau3A-positive fragments were detected by Southern blot hybridization with pUh1-39 DNA. Five fragments of phage A17 show hybridization, although to different extents, while only three out of nine fragments of phage A47 are Sau3A-positive.

Figure 3A shows a Southern blot of Sau3A-digested DNAs of the same two recombinant phages probed with pUh1-39 plasmid DNA. The two Sau3A-positive genomic sectors clearly differ in the relative proportions of monomer and multiples of Sau3A repeats, given that the same amounts of phage DNA were loaded on the gel and that the lengths of hybridizing fragments were calculated to be comparable in the two phages (Fig. 2). Southern blot hybridization of the same filter with the alphoid DNA probe pUhE-7 is reported in Fig. 3B. A single fragment of phage A17, of approximately 1300 bp in length, shows a positive hybridization signal.

The observed differences in Sau3A hybridization signals (Fig. 3A) might result (I) from different concentrations of Sau3A monomers within the genomic fragments, or (II) from sequence divergence. In order to choose between these two hypotheses, we subcloned, from each recombinant phage, two Sau3A-positive fragments showing a different degree of hybridization which is not correlated to fragment length (frag-

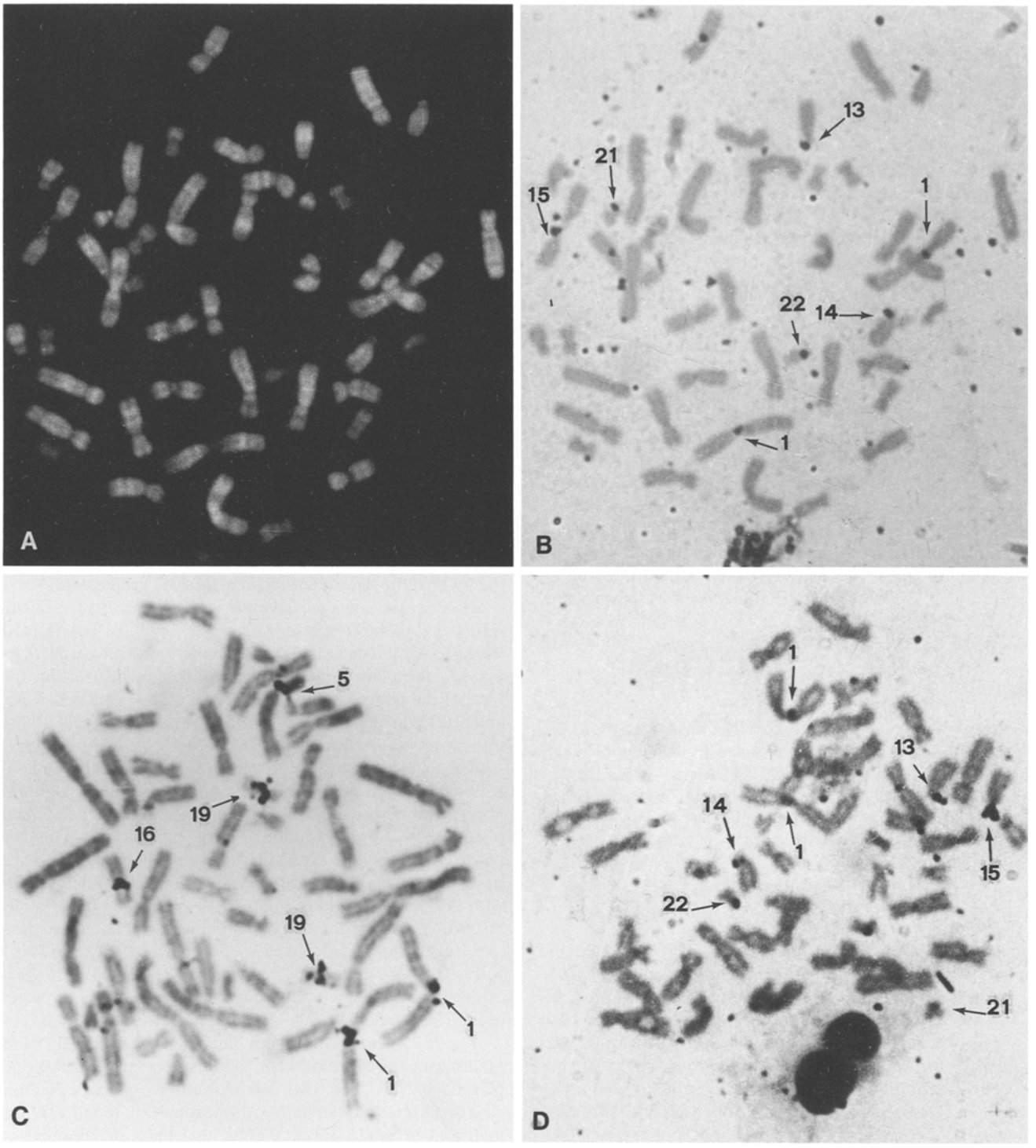

Fig. 1A–D. In situ hybridization of H-labeled DNA probes to human metaphase chromosome spreads. **A** Q banding (46,XX); **B** Giemsa-stained chromosomes (same metaphase as **A**) probed with pUh1-39 (Sau3A family); **C, D** Giemsa-stained chromosomes (46,XY and 46,XX, respectively) probed with pUh-E7 (EcoRI family) and A47 recombinant phage DNA (Sau3A-positive genomic sector), respectively. *Arrows* identify the more frequently labeled chromosomes

ments *a* and *b* of phage A17, and fragments *a* and *h* of phage A47, see Fig. 2).

The subcloned genomic fragments, digested with EcoRI/Sau3A (A17*a* and *b*; A47*h*) or with EcoRI/HindIII/Sau3A (A47*a*) are shown in Fig. 4A. Southern blotting hybridization of these digests with the EcoRI/HindIII purified insert of pUh1-39 (a 98 bp sequence which contains 30 bases of pUC8 polylinker and the 68 bp Sau3A prototype sequence) is shown in Fig. 4B. The content of Sau3A monomers in the subclones can be evaluated from the band intensities in the ethidium bromide stained gel. For example, in the subclone A47*h*, the sum of the higher molecular weight bands accounts for approximately 900 bp of the 2.4 kb of the full insert (assuming that the 140 bp band, which is relatively more intense than the

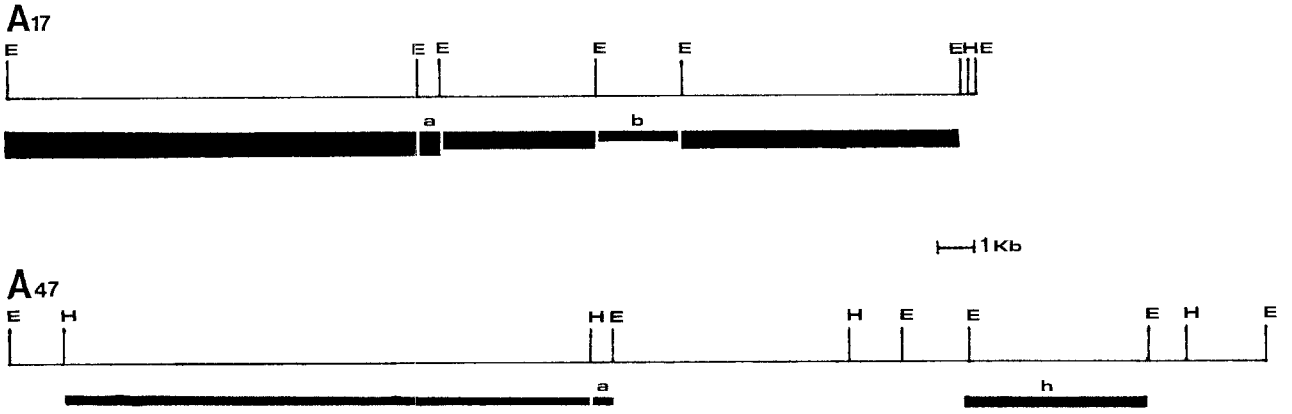

Fig. 2. EcoRI/HindIII maps of genomic inserts of Sau3A-positive phages A17 and A47. Sau3A-positive fragments are identified by *bars* the thickness of which is proportional to the intensity of the hybridization signal. The fragments *a* and *b* of phage A17, and *a* and *h* of phage A47 have been subcloned

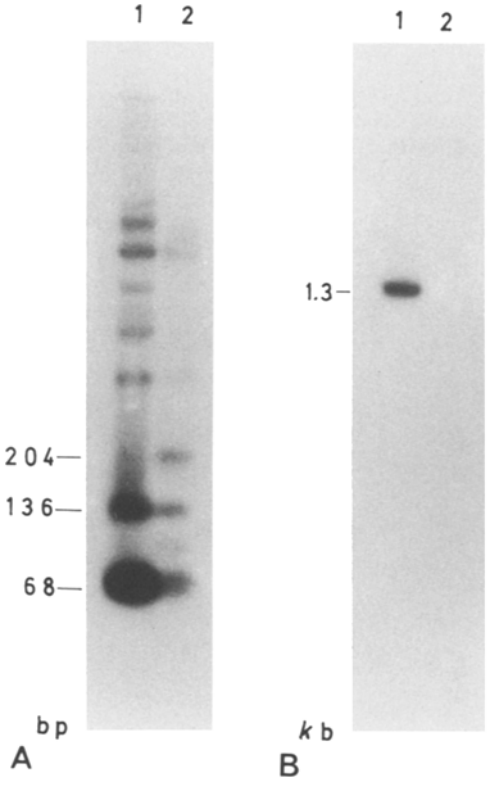

Fig. 3A, B. Sau3A digests of equal amounts (2.5 μg) of human Sau3A-positive A17 (*1*) and A47 (*2*) phages fractionated by electrophoresis in 2% agarose. **A, B** Southern blot hybridizations with radiolabeled purified 68 bp insert of pUh1-39 (Sau3A family) and 340 bp insert of pUhE-7 (EcoRI family), respectively

neighboring ones, is composed of two bands, one from the plasmid and one from the insert). The remaining 1.5 kb could be constituted by approximately 22 copies of the Sau3A monomer, as supported by the high intensity of the 68 bp band. Analogous calculations (also from other digestions, not shown here) suggest that the Sau3A monomer is represented approximately seven times in subclone A17*b* (1.15 kb, twice in subclone A17*a* (360 bp), and three times in subclone A47*a* (360 bp).

A comparison of the band patterns of the ethidium bromide stained gel and of the Southern blot clearly indicates that there is no correspondance between monomer concentration and hybridization signal intensity, i.e., that there is sequence divergence. In particular, the hybridization signal from the 68 bp band of subclone A47*h* (22 copies) is much fainter than those of A17*a* (two copies) and of A47*a* (three copies). The subclone A17*b* shows a complete lack of hybridization at the level of the 68 bp band.

Hybridization at the level of the dimer forms (136 bp) is evident in subclone A17*b* and completely lacking in subclone A47*h*, while hybridizing bands of approximately 110–120 bp are present in subclones A17*a* and A47*a*. The hybridizing band at 143 bp present in pUC8, A17*a*, A17*b*, and A47*h* is due to the BamHI-HindIII region of the polylinker, which is present in the probe and absent in A47*a* because of the different cloning strategy (EcoRI/HindIII instead of EcoRI/EcoRI).

The considerations applicable to the 68 bp band cannot be extended to higher molecular weight bands. Hence the lack of hybridization of the 360 and 290 bp bands of A47*h* and of A17*b* might be due either to sequence divergence or to the presence of unrelated sequences.

The evidence reported above indicates that the two recombinant phages differ in Sau3A monomer quality and possibly content, and for the presence of other unrelated repetitive sequences (alphoid DNA). It was then of interest to investigate the chromosomal locations of sequences homologous to those carried by the two recombinant phages and to compare them with that of the Sau3A monomer of pUh1-39.

Tritium-labeled DNAs of the recombinant phages were in situ hybridized to human chromosome spreads. The distribution of radioactive signals obtained using phage A47 DNA as a probe is shown in Fig. 1D and is essentially identical to that obtained with pUh1-39 DNA (Fig. 1B). The grain distribution for phage A47, evaluated on 27 mitoses and a total of 485 grains, shows 17%, 46%, and 28% of total grains on chromosome 1, D group, and G group chromosomes, respectively. Phage A17 DNA, although containing EcoRI sequences, gave a comparable result (data not shown), in agreement with the low relative concentration of EcoRI sequences. More details of the chromosomal location of Sau3A repeats are shown in Fig. 5 which displays a collection of D and G chromosomes selected from different metaphases in situ hybridized with plasmid or recombinant phage DNAs. The location of radioactive signals at the level of cytological satellite regions and of pericentromeric heterochromatin is evident for both probes.

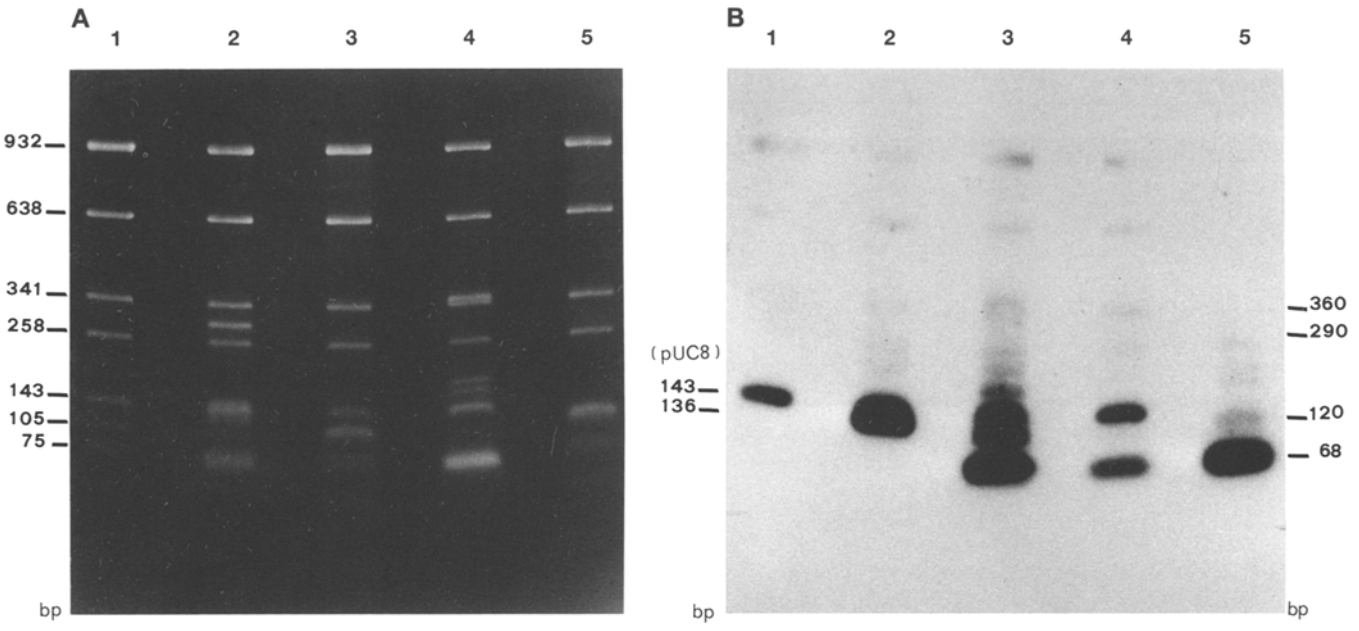

Fig. 4A, B. Restriction enzyme digests of Sau3A-positive subcloned fragments from phages A17 and A47 (Fig. 2), fractionated by electrophoresis in 2% agarose. **A** Ethidium bromide staining; **B** Southern blot hybridization with radiolabeled EcoRI/Hind III purified insert of pUh1-39 plasmid. *1,* Sau3A-pUC8 control; *2–4,* EcoRI/Sau3A digests of subclones A17*b*, A17*a*, and A47*h*; *5,* EcoRI/Hind III/Sau3A digest of subclone A47*a*

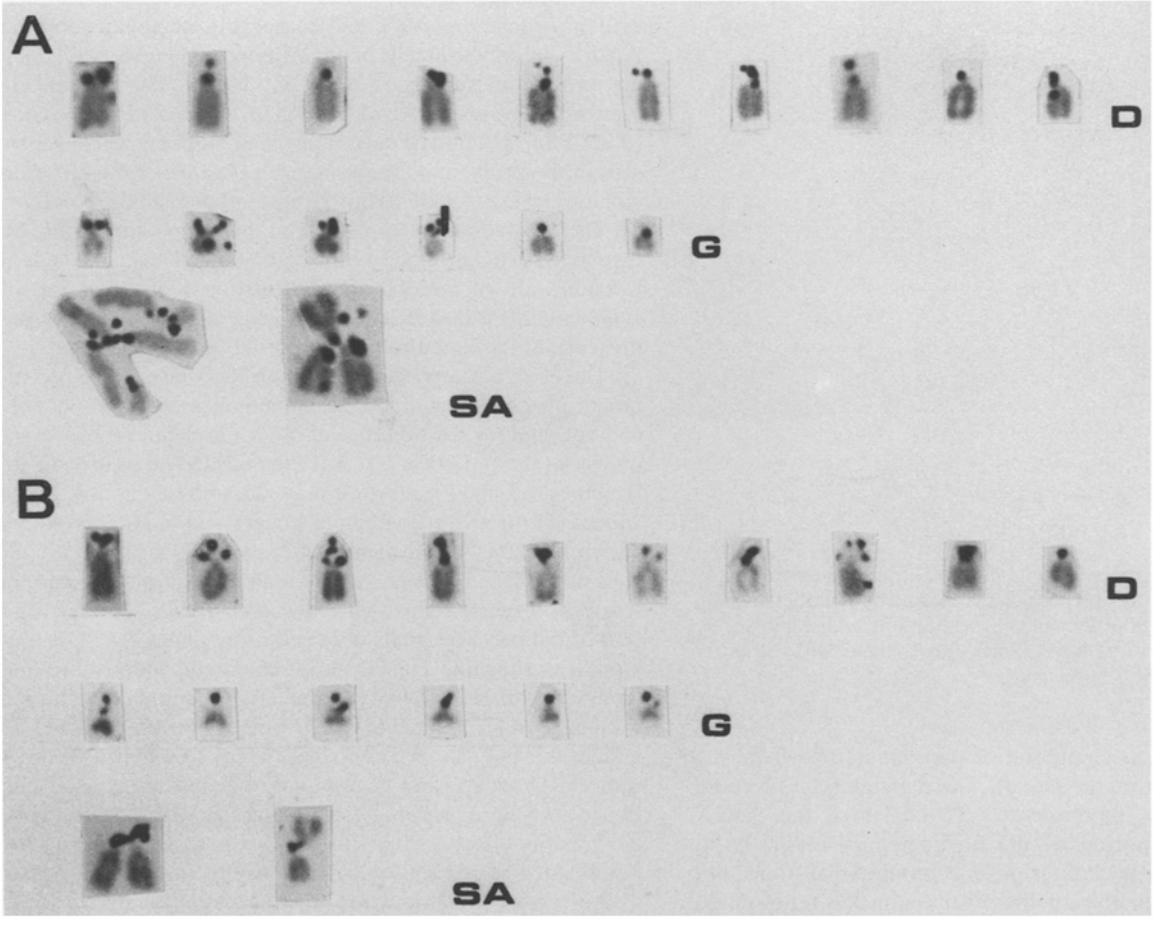

Fig. 5A, B. Representative collection of D and G chromosomes selected from different metaphases in situ hybridized with ^{3}H-pUh1-39 (**A**) and ^{3}H-A47 (**B**) DNA probes. SA, satellite associations

Examples of acrocentric chromosome associations with intense hybridization signals are also shown.

Discussion

We have recently described a new family of human DNA repeats characterized by a clustered organization and a relatively high GC content, the *Sau3A family* (Meneveri et al. 1985). In this paper we report in situ hybridization data indicating the preferential chromosomal location of Sau3A sequences on the short arms of human acrocentric chromosomes 13, 14, 15, 21, and 22, at the level of both satellite regions and centromeric heterochromatin. The centric heterochromatic region of chromosome 1 also shows positive hybridization. Although the Sau3A probes hybridize to chromosomal regions containing ribosomal DNA, previous molecular hybridization experiments have excluded that this family of repeated sequences is part of the ribosomal DNA unit (Meneveri et al. 1985).

Comparable grain distributions were observed using three different Sau3A-containing probes: the plasmid pUh1-39, which contains a Sau3A 68 bp monomer, and the recombinant phages A47 and A17, which contain Sau3A-positive genomic sectors and are characterized by (I) different concentrations of sequences homologous to pUh1-39 sequences, (II) the presence of Sau3A-divergent sequences, and (III) the presence of a low concentration of unrelated repeats (alphoid DNA). The identity of the observed chromosomal distributions suggests that Sau3A sequences homologous to the prototype pUh1-39 are the only repetitive DNAs sufficiently represented in the genomic sectors to give hybridization signals. As we know that the genomic sectors also contain divergent Sau3A repeats and, one of them, a small amount of alphoid DNA, the absence of additional chromosomal locations can be explained in two ways: (I) divergent repeats could be not homogeneous enough to constitute an efficient probe (in fact the 340 bp EcoRI sequence of phage A17 does not give its characteristic locations, probably because of its low concentration in the genomic sector) and (II) the divergent Sau3A sequences, even if some subset were homogeneous enough to constitute an efficient probe, might have the same chromosomal locations as the family identifier prototype sequence. A direct evaluation of the extent of Sau3A familial divergence as well as of the presence of unrelated sequences requires direct sequencing of genomic sectors (work in progress).

In situ hybridization patterns identical or very similar to the ones reported here for the Sau3A family have been recently described by Devine et al. (1985) who used as a probe a recombinant phage isolated from a human genomic library and containing repetitive sequences concentrated on chromosome 21. Although the repeated sequences present in this recombinant phage have not yet been characterized, it is possible that they share sequence homology with the Sau3A family of repeats.

In order to compare the chromosomal location of the Sau3A family to that of an AT-rich family of repeats, a recombinant plasmid representative of human alphoid DNA *(EcoRI 340 bp)* was in situ hybridized to human chromosome spreads. The main chromosomal location of alphoid DNA sequences was found in the pericentromeric heterochromatin of chromosomes 1, 5, 16, and 19. This result differs from that reported by Manuelidis (1978b), who used a partially purified probe instead of a cloned sequence, and who found similar locations, but also a main localization on chromosome 7 and only a minor one on chromosome 16. The discrepancy may be due to the different probes used, i.e., to the presence of divergence in the family. Alternatively, the chromosomal loci involved might show polymorphism of repetitive sequences content.

The in situ hybridization data on Sau3A and EcoRI families reinforce the notion that each family of repetitive DNA sequences is characterized by a specific chromosomal location. The simultaneous mapping on chromosome 1 of both the repetitive DNA sequences studied by us and of the non-Y-specific sequences of the *3.4 kb HaeIII* family (Burk et al. 1985) indicate that a single heterochromatic chromosomal region may contain several different families of repeats. This evidence is analogous to the finding that the same heterochromatic blocks of human acrocentric chromosomes contain, in addition to Sau3A repeats, both AT-rich satellite DNA *(sat DNA III)* and non-Y-specific sequences *(3.4 kb HaeIII)* (Godsen et al. 1981; Burk et al. 1985). In the case of *sat DNA III,* the size or the intensity of fluorescence of cytological satellite regions was found to be unrelated to the *sat III DNA* content (Godsen et al. 1981), indicating that other repeated sequences, such as *Sau3A* and *non-Y-3.4 kb HaeIII* repeats may contribute to the known polymorphism characteristic of human acrocentric heterochromatin.

The finding that the short arms of human acrocentric chromosomes and the pericentromeric heterochromatin of chromosome 1 share the same families of repeated sequences is in agreement with a statistically significant metaphase association of nucleolus organizing regions (NOR) with the centric region of chromosome 1 (Tuck-Muller et al. 1984). This nonrandom association has been proposed to result from "heterochromatin attraction" which may be directed by identical sequences (Schmid et al. 1975).

The acrocentric chromosomes are involved in Robertsonian translocations and in the only viable autosomal trisomies (trisomies 13 and 21). The availability of DNA probes which map in chromosomal regions possibly involved in these events, should be useful to study the molecular basis of the interaction of specific chromosome regions and the exchanges of genetic material between them. Finally, DNA probes such as Sau3A and EcoRI might help to clarify the role of repetitive DNA sequences in heterochromatin organization by studying the possible presence of sequence-specific DNA binding proteins.

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