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Organization of the Variant Domains of α Satellite DNA on Human Chromosome 21

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Abstract. The de novo creation of long, homogeneous, satellite DNA domains was postulated previously to occur by saltatory amplification. In this paper, pulsed field gel electrophoresis analysis of the α satellite DNA block organization of the human chromosome 21 supports this hypothesis.

Double-dimension electrophoresis indicated that the variant copies of the basic α satellite repeat of chromosome 21 are organized in a single 3,150 Kb-long domain. It was also established that the other satellite DNAs found in man (β, II, and III) are organized independently of the α satellite DNA block of the same chromosome.

Introduction

In spite of a large number of studies devoted to satellite DNAs, their structural organization is not yet totally elucidated; their mode of amplification is still subject to discussion; and their role, if any, is still unknown. The human satellite DNAs do not escape these problems. Six have been partially described: I–IV, α (Singer 1982), and β (Meneveri et al. 1985; Agresti et al. 1987; Waye and Willard 1989). Of these, the most thoroughly studied is the α satellite, which is present at the centromere of all human chromosomes (Manuelidis 1978). Almost all chromosomes have developed specific subsets of the α satellite 171-basepair (bp) basic repeat (Willard and Waye 1987), so sufficiently stringent conditions can be found to discriminate, via Southern blotting, the centromeric α satellite DNA sequences of each chromosome. Chromosomes 13 and 21 share an α satellite DNA subset with almost 100% homology, however, and cannot therefore be discriminated from each other with the corresponding probe (Jorgensen et al. 1987).

We have described the organization of the α satellite component on both chromosomes 13 and 21 (Marçais et al. 1991a–c) and proposed a mode of evolution in human populations (Marçais et al. 1991b). It implies saltatory amplification steps of α satellite DNA arrays which are presumed to occur in single individuals.

In this paper, we describe new structural characteristics of the α component of these chromosomes and discuss their possible implications in connection with the above model.

Materials and Methods

DNA Preparation. The DNA samples were prepared in agarose plugs as follows: cells obtained from blood samples (lymphocytes), from CEPH tissue culture lines, or from the WA 17 cell hybrid (Vitek et al. 1985) were directly suspended in 0.5% (final concentration) agarose blocks (Gel Pulse Agarose, Appligene, Illkirch, France) in PBS (0.145 M NaCl; 0.76 mM NaH_2PO_4, H_2O; 2.24 mM Na_2HPO_4, 7 H_2O; pH 7.2) and treated in 0.5 M EDTA, 1% lauryl sarcosyl, with 0.5 mg/ml of proteinase K (Appligene) for 48 h at 50°C. Each block contains 12 μg DNA. The DNA sample in solution was prepared at a concentration of 200 μg/ml and was distributed by CEPH.

Digestion of DNA in Agarose Inserts and Pulsed Field Gel Electrophoresis (PFGE). Prior to digestion with restriction enzymes, the DNA samples were thoroughly washed with TE

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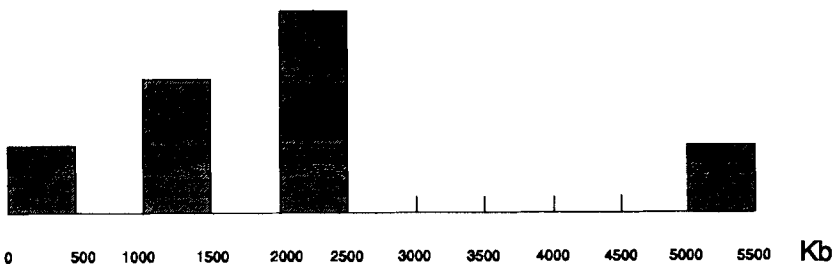
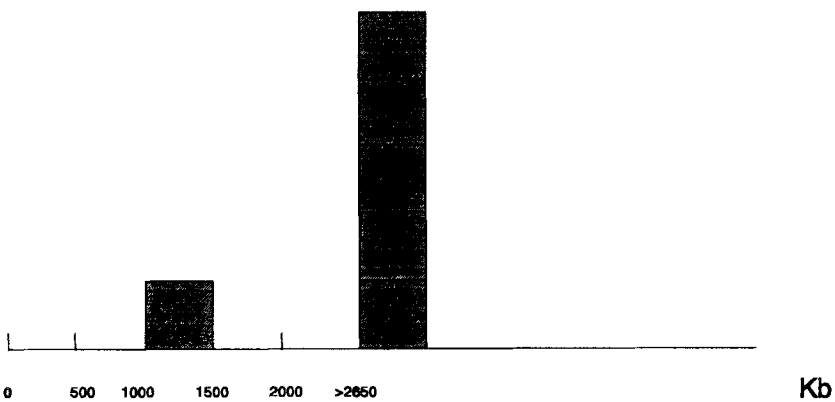
Table 1. Distribution of the different sizes of α satellite DNA blocks from chromosome 21 (upper) and 13 (lower)[a]



Chromosome 21



Chromosome 13

[a]Sizes were determined by the study of the chromosome 21 present in the somatic cell hybrid WA 17 (Fig. 5) and by segregation analysis using the 1418 and 1347 CEPH families (Marçais et al. 1991a, b)

buffer (10 mM Tris, 1 mM EDTA, pH 8.0) followed by restriction buffer. Each block was finally suspended in 150 μl of restriction buffer and 100 units of enzyme was added for every 12 μg of DNA. Digestion was performed overnight at the appropriate temperature. A PFGE system derived from that described by Chu et al. (1986) was used (Bellis et al. 1987). Agarose gel concentration was 1%. Runs were performed in 0.5 × TBE (89 mM Tris, 89 mM boric acid, and 2.5 mM EDTA). Pulse times were 120 s, then 90 s, and finally 60 s for successive 14-h periods.

Double-Dimension Electrophoresis. Double-dimension electrophoresis was performed according to Warburton and Willard (1990). In the first dimension, the DNA fragments, released after digestion by *Bam*HI, were resolved as described above by PFGE in gel pulse agarose. A longitudinal slice (1 mm wide) of the gel containing the different DNA bands was cut and incubated overnight at 4°C in 100 ml of the appropriate restriction enzyme buffer. Restriction hydrolysis (*Taq*I or *Eco*RI) was then performed in 10 ml of restriction buffer with 200 U/ml of enzyme. BSA (0.1 mg/ml) was also added. The hydrolysis tube was gently agitated during the time of hydrolysis (12 h). The slice was then placed at the top of an agarose gel in which conventional electrophoresis (second dimension) was performed.

DNA Hybridization. After electrophoresis, DNA samples were transferred to Hybond-N+ membranes (Amersham, UK) which were hybridized with different probes. The probe α RI (680) 21–368, specific for chromosomes 13 and 21 (Jorgensen et al. 1987), was a generous gift from A. Jorgensen (Aarhus). The probes of DNA satellite II and III were a gift from Burgoyne (Drinkwater et al. 1986; Fowler et al. 1988). The probe of β satellite DNA was obtained by polymerase chain reaction (PCR) with two primers (5′-A G T G C A G A G A T A T G T C A C A A T G C C C C-3′), (5′-T C C A A A G C C C A T G T A G G C C G A G C C A A G A C A A G A G T-3′) whose sequences were defined based on the consensus sequence of β satellite established by Waye and Willard (1989). These probes were labeled according to Feinberg and Vogelstein (1983) and hybridizations were performed in 30 ml buffer (5 × SSPE; 1% SDS; 0.1 mg/ml of nonfat powdered milk and 100 μg/ml salmon sperm

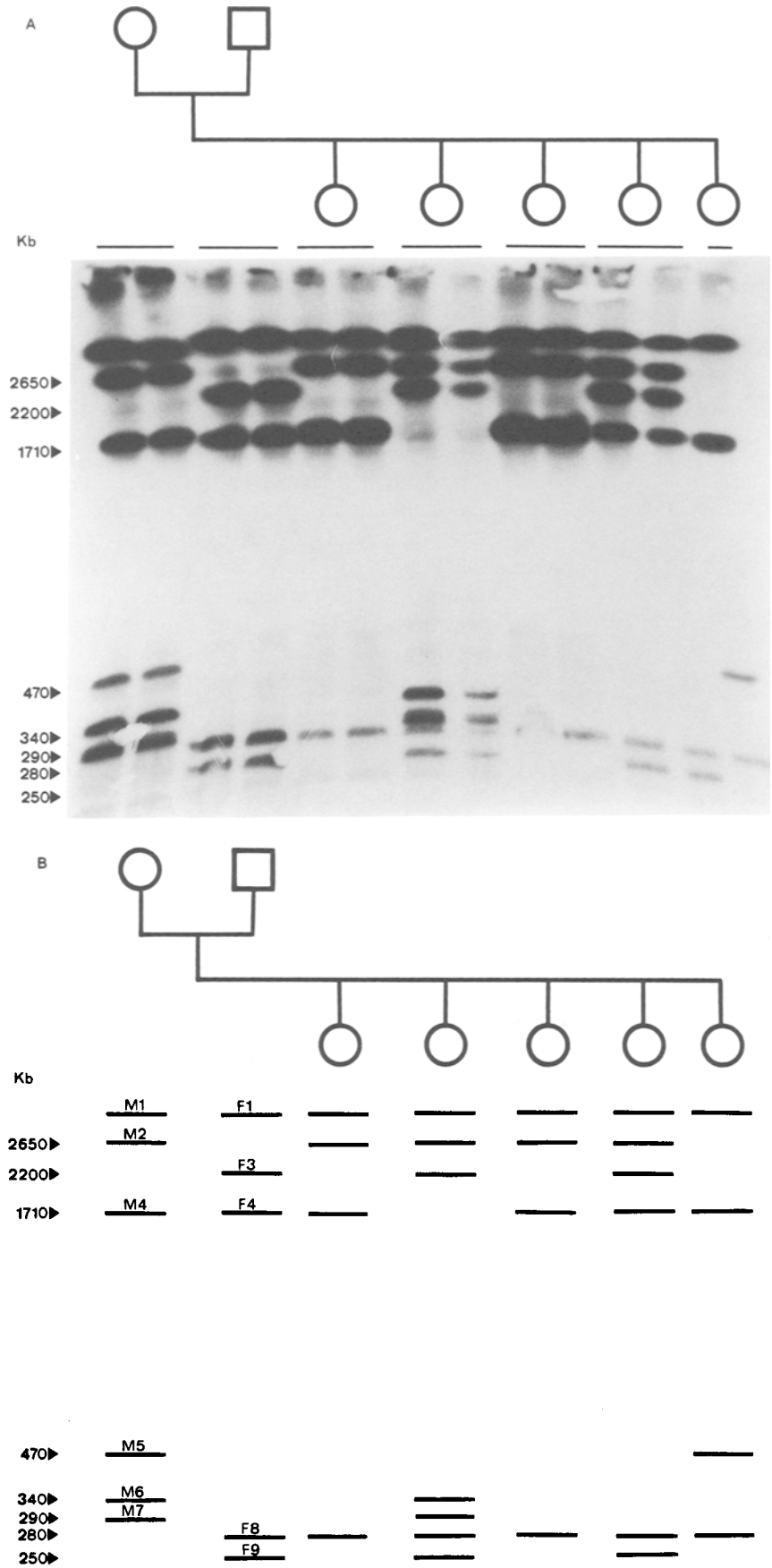


Fig. 1. **A** Segregation of the α satellite DNA bands of chromosomes 13 and 21 generated by *Bgl*II in the 1418 CEPH family. **B** Segregation analysis of different *Bgl*II fragments of the α satellite DNA of the chromosomes 13 and 21 in the 1418 CEPH family. The repartition of these fragments on the parental 13 and 21 chromosomes is as following: Maternal chromosomes 13, A1: (M4), A2: (M1 + M6 + M7), maternal chromosomes 21, B1: (M5), B2: (M2); paternal chromosomes 13, D1: (F1 + F8), D2: (F1 + F8), paternal chromosomes 21, C1: (F3 + F9), C2: (F4).

Table 2. Comparison of the size blocks of α satellite DNA sequences on chromosomes 13 and 21 belonging to the 1418 CEPH family parents, as revealed by *Bam*HI (Marçais et al. 1991a) and *Bgl*II

	Bam HI	Bgl II
Chr 13		
A1	>2650 Kb	1710 Kb
A2	>2650 Kb	>2650 Kb
D1	>2650 Kb	>2650 Kb
D2	>2650 Kb	>2650 Kb
Chr 21		
B1	420 Kb	470 Kb
B2	2350 Kb	2650 Kb
C1	2330 Kb	2450 Kb
C2	1140 Kb	1710 Kb

DNA) for 48 h at 65°C. Membranes hybridized with satellite β, II, and III were washed using nonstringent conditions (2 × SSPE, 0.1% SDS, 65°C); those hybridized with α satellite DNA used stringent conditions (0.1 × SSPE, 0.1% SDS, 68°C).

Results

Polymorphism of α Satellite DNA Block Sizes in Human Populations

Pulsed-field gel electrophoresis (PFGE) following restriction analysis using the α satellite rare cutting enzyme *Bam*HI has previously shown that the α satellite DNA block sizes are highly variable (Marçais et al. 1991a,b). This is confirmed with two supplementary studies included in this paper.

The first (Table 1) summarizes the data obtained in analysis in which the DNA bands detected in PFGE could be assigned unambiguously to chromosome 13 or 21. In the numerous other unpublished results where this distinction was not possible, the same tendency toward dispersion on the α satellite DNA block sizes was also obvious.

In a second analysis, comparison was made between the PFGE restriction patterns obtained with two rare cutting enzymes, *Bgl*II (Fig. 1) and *Bam*HI (Marçais 1991a) on the CEPH family 1418. The results are presented in Table 2.

Good agreement is found between the two estimates, which indicates that the length measurements correspond properly to that of the α satellite DNA blocks present on both chromosomes. This finding was also observed on chromosome 17 by Warburton and Willard (1990) and on chromosome Y by Oackey and Tyler-Smith (1990). The discrepancy found for two chromosomes can be explained if some of the bands in the *Bam*HI or *Bgl*II digests are doublets, a situation which is observed quite frequently since PFGE is not usually able to resolve all DNA bands in a single gel.

Structural Analysis of a Variant Domain of Chromosome 21 α Satellite DNA by Double-Dimension Electrophoresis

Polymorphism is detectable in α satellite DNA not only by PFGE, as shown above, but also by conventional electrophoresis following hydrolysis with restriction enzymes that cut frequently within the α satellite DNA of chromosomes 13 and 21. *Taq*I (Marçais et al. 1991c) produces a series of bands, the main one containing 11 times the 171-bp basic repeat (11 mer). In one family (1347) of the CEPH panel, a supplementary band was detected which had an equivalent intensity to that of the 11 mer. These 9.5-mer units appear to be organized as a single domain several megabases in length (Marçais et al. 1991b,c).

Another approach has been used here to confirm this suggestion. PFGE is done on DNA cut by *Bam*HI while embedded in agarose blocks. The DNA resolved in agarose is then excised from the gel, hydrolyzed by *Taq*I, and electrophoresed in the second dimension in a conventional system.

In Fig. 2, the father of CEPH family 1347 was examined by such an analysis. It was previously established that *Bam*HI generated DNA bands numbered 3, 5, 7, and 11, which belong to chromosome 21 bearing the 9.5-mer variant domain (Marçais et al. 1991b).

DNA bands 3 and 11 are composed purely of 9.5-mer units, while DNA bands 5 and 7 appear to be composed of a mixture of both 9.5 and 11 mer. This suggests a possible organization of this chromosome 21 DNA block as shown in Fig. 3, although the exact arrangement of the different fragments is not yet established. According to this model, the 9.5-mer domain, covering an estimated length of more than 3,000 kb, is surrounded by two smaller, pure 11-mer domains.

A similar result was obtained with the same DNA sample when *Eco*RI was used instead of *Taq*I in the second dimension (Fig. 4). *Eco*RI reveals the polymorphism as 12 mer instead of 9.5 mer. Again, DNA bands 3, 5, 7, and 11 bore this 12-mer variant domain.

These results do not exclude, however, that several other repeat types might be present in such domains; but, in this case, they should represent either subdomains or a minor portion of the repeats.

We did not go further in this analysis, but it provides support once more for the independent organization and generation of large α satellite DNA arrays in the centromeric regions of human chromosomes.

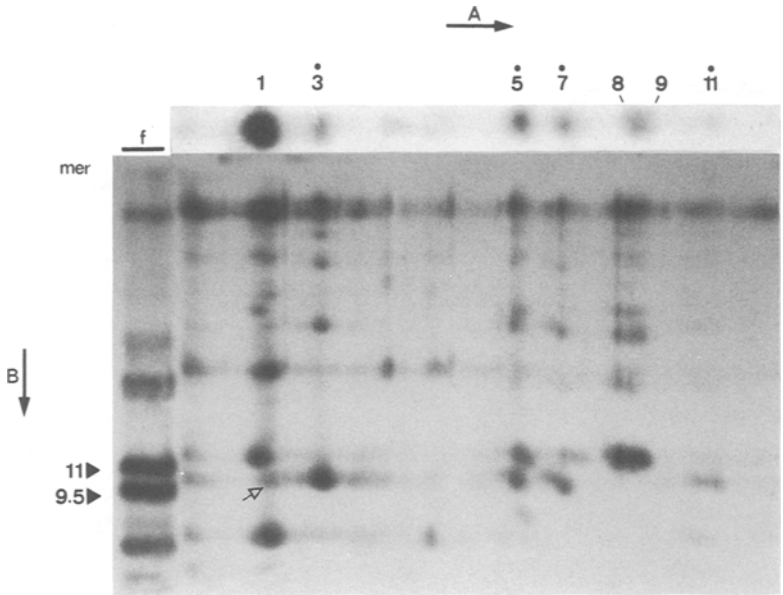


Fig. 2. Analysis by two-dimensional electrophoresis of the *Taq*I 9.5 and 11 mer present in the different *Bam*HI fragments separated by PFGE. The *Bam*HI fragments from chromosomes 13 and 21, separated in the first dimension, are digested by *Taq*I and separated in a second dimension by conventional electrophoresis. **A** In the first dimension, *Bam*HI fragments belonging to chromosomes 13 and 21 present in the father of the 1347 CEPH family are separated by PFGE. In this restriction profile, the fragments of chromosome 21 bearing the *Taq*I 9.5 mer polymorphism are 3, 5, 7, and 11 and are marked by a *solid circle* (Marçais et al. 1991b). **B** In the second dimension, are separated, by conventional electrophoresis, the *Taq*I restriction fragments of chromosomes 13 and 21 obtained after digestion by *Taq*I of the DNA present in the different *Bam*HI fragments separated in the first dimension. *Lane f: Taq*I restriction profile of the α satellite DNA of chromosomes 13 and 21 of the father, electrophoresed in the same gel. A faint 9.5-mer band is detectable in the *Taq*I digest of band 1 (*arrow*). This probably originates from a partial *Bam*HI digestion of chromosome 21, which carries the 9.5-mer polymorphism. Band 1 does not contain α satellite DNA from this chromosome 21 (Marçais et al. 1991b).

The Human α Satellite DNA is Organized Independently From the Other Satellite DNAs Detectable in the Human Genome

Several different satellite DNAs frequently coexist within the centromeric regions of the chromosomes from higher organisms, but their relative structural organization is not yeat clearly established. The situation is similar in man, and it is not certain that the DNA bands detected in the PFGE analyses described above are in fact composed of a pure α component, to the exclusion of DNA sequences belonging to other human satellite DNA categories.

Human satellite β has been shown by different authors (Agresti et al. 1987; Waye and Willard 1989; Meneveri et al. 1993) to be present on the short arm of all acrocentric chromosomes as well as on chromosomes 1, 3, 9, and Y. Satellite DNAs II and III have been shown previously (Gosden et al. 1975) to be detectable also in the pericentromeric regions of a number of human chromosomes. We therefore tested for the presence of such satellite DNA sequences on PFGE blots which had been probed with α satellite DNA specific for chromosomes 13 and 21.

Three unrelated individual DNAs, plus DNA from a mouse human hybrid cell line containing human chromosome 21 (WA 17), were cut by *Bam*HI, separated by PFGE, and probed with α satellite, β satellite, and satellites II and III DNAs (Fig. 5). No communal DNA bands were detected, indicating an independent organization of these different satellite DNAs although it cannot be excluded that a minor portion of the bands bearing the alphoid sequences also contain DNA sequences from other satellite DNA families since a junction between satellites III and α has been recently characterized in chromosome 21 by Vissel et al. (1992).

Discussion

The satellite DNAs of man, like those of many other organisms, are continuously changing in DNA sequence, copy number, and organization. These changes are, however, occurring in a cohesive manner (Dover 1982), both at the molecular and at the population levels. Among the numerous mechanisms which are speculated to play a role in this process, saltatory amplification could be an important one (Dod et al. 1989; Marçais et al 1991b).

In this paper, we provide further evidence that the α satellite DNA variant domains which are often detected on human chromosomes might be consid-

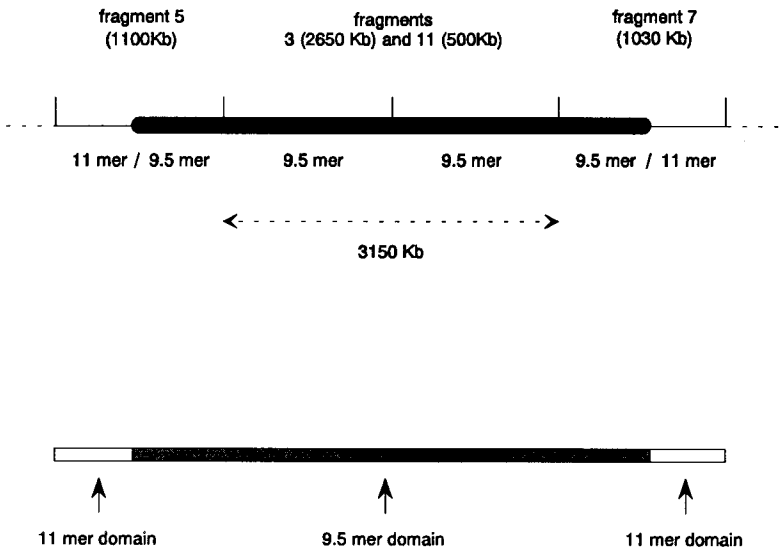


Fig. 3. Domain organization of the α satellite DNA block belonging to the chromosome 21 bearing the 9.5-mer variant repeat. Fragments 5 and 7, possessing the two types of repeats (9.5 mer and 11 mer), are assigned at the extremities of the block and are oriented in such a way that their 9.5 portions are adjacent to the two fragments 3 and 11 which consist of only the 9.5-mer variant repeats. From this one obtains an organization in three domains: one central varient domain flanked by the two normal domains. The size of fragments 3 and 11 allow determination of the minimum size of the varient domain as 3,150 kb.

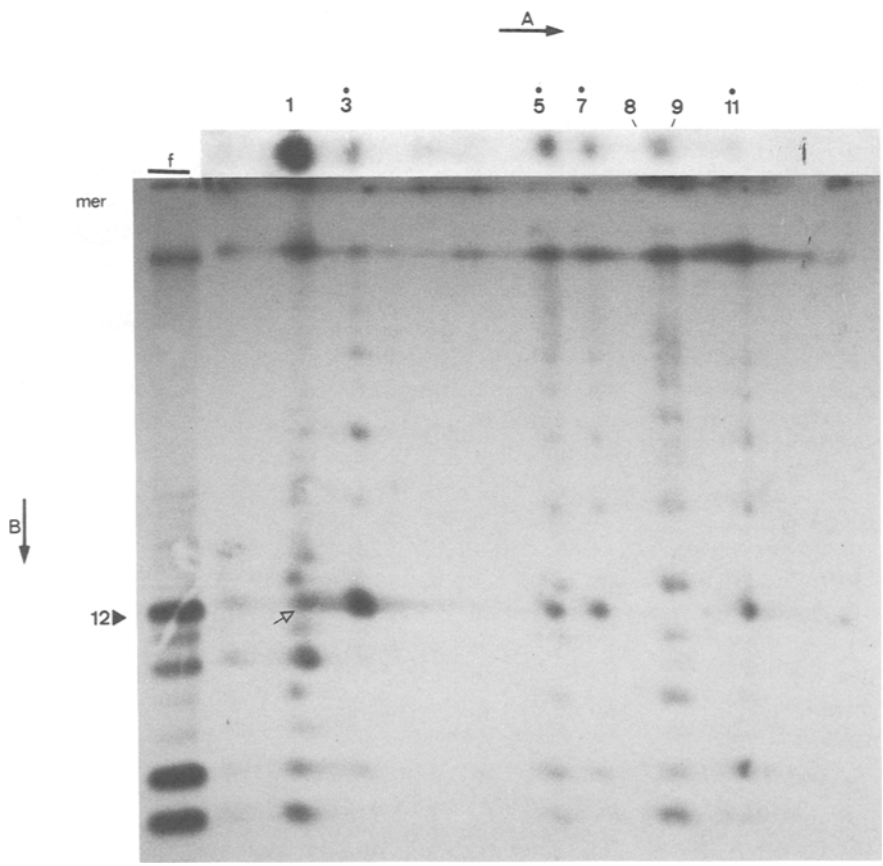


Fig. 4. Two-dimensional analysis of *Eco*RI higher-order repeat length variants in chromosome 21 from the father of the 1347 CEPH family bearing the *Eco*RI 12-mer polymorphism. **A** *Bam*HI-restricted α satellite DNA from chromosomes 13 and 21 of this parent is separated in the first dimension. **B** *Eco*RI restriction profile of the different *Bam*HI fragments separated in the first dimension. All *Bam*HI fragments from chromosome 21 bearing the 12-mer *Eco*RI polymorphism, marked by a *solid circle* (3, 5, 7, and 11), contain the 12-mer variant repeat. *Lane f:* As in the *Taq*I analysis (Fig. 2), *Eco*RI-digested α satellite DNA from chromosomes 13 and 21 of the father is electrophoresed on the same gel. Again, the *arrow* indicates a 12-mer band that probably results from partial *Bam*HI digestion of the DNA from the chromosome 21 containing the 12-mer polymorphism. The *Taw*I and *Eco*RI digests were both performed on a slice of gel containing the α satellite DNA that was separated in the same PFGE.

ered as if they are independent, unrelated satellite DNAs. This notion comes from the experiments described here, in which a parallel can be drawn between them. On one chromosome 21 we have analyzed an α satellite variant domain (repetition unit = 9.5 mer) which was shown to cover more than 3,150 kb in length, flanked by two other identical domains composed of the normal (11 mer) type of repetition unit. This indicates that the 9.5-mer domain has been formed as a long homogeneous array, which added to the normal block of satellite DNA sequences already present at the centromere, thus contributing to the large variation of α satellite DNA block sizes which occur naturally in the population. We have also shown that other satellite DNAs are organized independently from the α satellite, as large DNA fragments generated by rare restriction cutting enzymes and resolved by PFGE

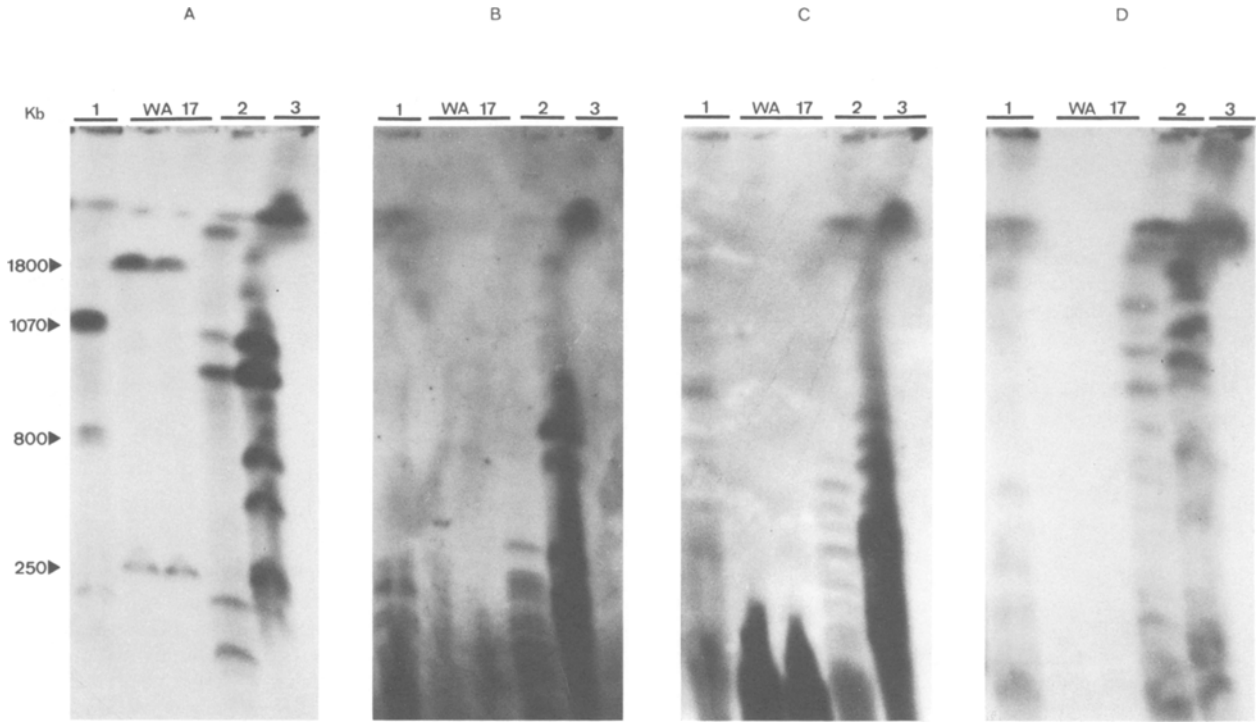


Fig. 5. *Bam*HI PFGE restriction profile of the DNA from three unrelated individuals (1, 2, 3) and the somatic cell hybrid WA 17, hybridized successively on the same membrane with (**A**) the α satellite DNA specific for chromosomes 13 and 21, (**B**) the β satellite, (**C**) the satellite II, and (**D**) the satellite III.

bore exclusively the α satellite DNA sequences. The limited literature available that deals with the organization of variant domains of a given satellite DNA or of several different satellite DNAs within the same genome is in agreement with our finding.

In the mouse, two satellite DNAs have been described which are organized as separated arrays on different chromosomes (Dod et al. 1989). In *Drosophila,* Lohe and Brutlag (1987) examined the sequence junctions between the different satellite DNAs detected and showed that they are also organized independently from each other on different DNA arrays. In man, the relative organization of different satellite DNAs is only partially known in very few cases. On the Y chromosome, an independent organization is clearly established (Tyler-Smith, personal communication). Concerning the variant domains found in the human α satellite DNA, Warburton and Willard (1990) show also the presence of localized homogeneous sequence domains within array of α satellite DNA of chromosome 17, and Choo et al. (1991) have recently reviewed the numerous cases which establish such an independent organization. Satellite DNAs composed in their basic repeat units of a mosaic of sequences have been described in bovine (Pech et al. 1979) and in the human β satellite (Meneveri et al. 1993). However, they are similar to the other types of satellite DNAs in that they too are composed of long homogeneous arrays of nearly identical basic repeat units.

Taken together, these data support our view that a molecular mechanism must exist which is able to create long, homogeneous satellite domains, either slightly variant or drastically different from adjacent sequences. Clearly, the simplest way of doing this is saltatory amplification of a particular small (in length) subset.

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