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Chromosome-Specific Organization of Human Alpha Satellite DNA

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SUMMARY

Restriction endonuclease analysis of human genomic DNA has previously revealed several prominent repeated DNA families defined by regularly spaced enzyme recognition sites. One of these families, termed α satellite DNA, was originally identified as tandemly repeated 340- or 680-base pair (bp) *Eco*RI fragments that hybridize to the centromeric regions of human chromosomes. We have investigated the molecular organization of α satellite DNA on individual human chromosomes by filter hybridization and in situ hybridization analysis of human DNA and DNA from rodent/human somatic cell hybrids, each containing only a single human chromosome. We used as probes a cloned 340-bp *Eco*RI α satellite fragment and a cloned α satellite-containing 2.0-kilobase pair (kbp) *Bam*HI fragment from the pericentromeric region of the human X chromosome. In each somatic cell hybrid DNA, the two probes hybridized to a distinct subset of DNA fragments detected in total human genomic DNA. Thus, α satellite DNA on each of the human chromosomes examined—the X and Y chromosomes and autosomes 3, 4, and 21—is organized in a specific and limited number of molecular domains. The data indicate that subsets of α satellite DNA on individual chromosomes differ from one another, both with respect to restriction enzyme periodicities and with respect to their degree of sequence relatedness. The results suggest that some, and perhaps many, human chromosomes are characterized by a specific organization of α satellite DNA at their centromeres and that, under appropriate experimental conditions, cloned representatives of α satellite subfamilies may serve as a new class of chromosome-specific DNA markers.

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INTRODUCTION

Restriction endonuclease analysis of human genomic DNA has demonstrated the existence of a number of families of highly repeated DNA, as revealed by the presence of repeated DNA sites spaced at regular intervals [1, 2]. One of the more prominent of these families was first visualized as 340- and 680-bp DNA fragments revealed by cleavage of genomic DNA with *Eco*RI. These human DNA repeats have been studied extensively by Manuelidis et al. [2–5] and consist of adjacent, but diverged, copies of a basic 171-bp or 169-bp unit. These fundamental repeats are closely related in sequence to the prototypical α satellite repeat, a tandemly repeated 172-bp monomer first described in the African green monkey genome [6–7], but since found in a wide variety of primate genomes (reviewed in [8]). Human α satellite DNA has been described as being organized principally in long tandem arrays of the 340-bp dimer (α-dimer) or the 680-bp tetramer [4, 9]. Using purified but uncloned α-dimer fragments, Manuelidis showed by in situ hybridization that sequences homologous to the α-dimer are clustered at the centromeres of human chromosomes [10]. To examine the molecular organization of α satellite DNA on individual chromosomes, we have now used a cloned human α-dimer fragment and an α satellite-containing 2.0-kbp *Bam*HI fragment from the human X chromosome [11, 12] as probes to investigate genomic DNA from rodent/human somatic cell hybrids, each containing only a single human chromosome. We report here that individual human chromosomes are each characterized by specific and different subsets of α satellite DNA, defined both by restriction enzyme periodicity and by primary DNA sequence, and, further, that cloned α satellite-containing DNA fragments from different chromosomes can serve as chromosome-specific repeated DNA probes.

MATERIALS AND METHODS

DNA was isolated from peripheral blood leukocytes, from cultured lymphoblast cells, or from somatic cell hybrid lines as described [11]. DNA was digested to completion with restriction endonucleases using conditions provided by the supplier, electrophoresed through agarose gels, and blotted onto nitrocellulose filters by a modification of the method of Southern [13]. Hybridization with ^{32}P-labeled, nick-translated probes (see below) was carried out as described [11] at 37°C (reduced-stringency conditions) or 42°C (high-stringency conditions). Filters were washed as described [11] at reduced stringency (final wash in 0.5 M NaCl at 65°C) or at high stringency (final wash in 0.1 × SSC at 65°C). Estimated melting temperature [14] was calculated by $T_m = 0.41$ (%GC) + 81.5 + 16.6 log M (where M is concentration of monovalent cations and %GC of α satellite DNA is 36% (our unpublished data, 1984, and [4]). Estimated percent mismatch was calculated assuming that every 1°C reduction in T_m corresponds to 1%–1.5% mispairing [14, 15].

The probes used were pBamX7, a cloned copy of the 2.0-kb *Bam*HI repeat [11], and pαRI-12, a cloned 340 bp *Eco*RI α-dimer. The pαRI-12 clone was obtained by complete digestion of human genomic DNA with *Eco*RI, gel purification of the 340-bp band after visualization by ethidium bromide staining, cloning into *Eco*RI-digested pUC9, and randomly screening plasmids with inserts for the presence of α satellite DNA. Approximately 30% of plasmids so obtained contained α-dimer DNA. The nucleotide sequence of pαRI-12 has been determined (our unpublished data, 1984), by the M13-chain termi-

nation method [16] and is 96% identical to the consensus α-dimer sequence published by Wu and Manuelidis [4].

Three of the somatic cell hybrids used in this study have been described. AHA-11a is a mouse/human hybrid, provided by F. H. Ruddle, which contains only a human X chromosome [17]. Y15/1 is a mouse/human hybrid, provided by M. Markus, which contains only a human Y chromosome [18]. FA9-C2T10B2-c12B is a mouse/human hybrid containing only human chromosome 3 [19]. SCC16 is a chromosome 21-only mouse/human hybrid provided by D. Cox. W4-3AAZc15 is a subclone of the Chinese hamster/human hybrid W4-3AAZ [19] that has lost the X/14 translocation chromosome and contains only a human chromosome 4 in all cells examined.

In situ hybridization was performed essentially as described by Harper and Saunders [20]. Hybridization was carried out in 3 × SSC, 50% formamide, 10% dextran sulfate at 37°C for 16 hrs. Slides were washed in 2 × SSC, 50% formamide at 37°C, or at 50°C–51°C and processed for autoradiography.

RESULTS

The 2.0-kb *Bam*HI fragment used in this study is a member of the DXZ1 family of reiterated DNA that consists of approximately 5,000 copies of a cognate 2.0-kb repeat, organized in tandem arrays and clustered in the pericentromeric region of the human X chromosome [11, 12]. Restriction mapping of the cloned repeat had previously indicated the existence of multiple restriction sites at 170- and 340-bp intervals [12], and recent DNA sequence analysis has established that this X-linked repeat is comprised of 12 imperfectly duplicated units of approximately 170 bp in length, each 65%–75% identical in sequence to the reported [4] consensus sequence of uncloned human α-dimer (J. S. Waye and H. F. W., manuscript in preparation). To examine relationships among the X-linked DXZ1 α satellite family, the previously described α-dimer, and α satellite DNA elsewhere in the human genome, we performed Southern blot hybridization analysis of digested human genomic DNA using the cloned 2.0-kb X repeat (pBamX7) as probe. Under conditions of reduced stringency that permit an estimated 25% sequence mismatch (see MATERIALS AND METHODS), the probe hybridized to a large number of *Eco*RI or *Hin*dIII restriction fragments (including the 340-bp and 680-bp *Eco*RI fragments visible after ethidium bromide staining), thus revealing a ladder of bands corresponding to multiples of approximately 170 bp (fig. 1*A*). This pattern is quite similar to that reported by Yang et al. with a different cloned member of the same DXZ1 family [12]. In this type of analysis, no differences were noted among DNAs from different individuals. Most of the material in the genome hybridizing to the X-linked probe under these conditions, however, is *not* X-linked, since a much simpler pattern of hybridization was observed when DNA from a mouse/human somatic cell hybrid containing a single human X chromosome as its only human component was examined in the same experiment under the same conditions (fig. 1*C*). Under these conditions, no hybridization is detected with rodent DNA. Further, when an identical experiment was performed under conditions of high stringency (allowing no more than 1%–3% sequence mismatch), most of the non-X-linked fragment lengths were not detected, even after much longer exposures (fig. 1*B* and *D*). This indicates that the majority of the autosomal (and/or Y-linked) α satellite sequences in the genome are somewhat distantly related to the X-chromosome α satellite repeat.

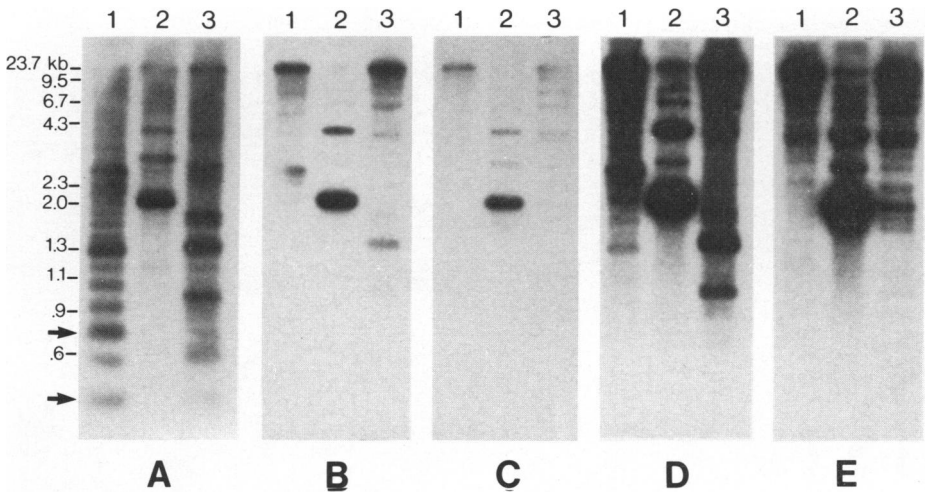

FIG. 1.—Southern blot analyses of human genomic DNA and DNA from AHA-11a, a mouse/human somatic cell hybrid containing only a single human chromosome, the X. DNA prepared from peripheral blood leukocytes from a normal male or from cultured hybrid cells was digested with the restriction enzymes *Eco*RI (*lanes 1*), *Bam*HI (*lanes 2*), or *Hin*dIII (*lanes 3*), electrophoresed through 1.5% agarose gels, blotted onto nitrocellulose filters, and hybridized with ^{32}P-labeled, nick-translated pBamX7. *A*, Human DNA at reduced stringency (see MATERIALS AND METHODS). *B*, Human DNA at high stringency. *C*, AHA-11a hybrid DNA at low stringency. *D*, Same as *B*, except longer exposure time; *E*, same as *C*, except longer exposure time. *Nos. on left* are size markers in kb. *Arrows* indicate migration of 0.34- and 0.68-kb *Eco*RI bands visible after ethidium-bromide staining.

To gain insight into the location of these non-X-linked sequences in the genome, we performed in situ hybridization to human metaphase chromosomes under conditions of high and low stringency, using ^{3}H-labeled pBamX7 as probe. Under stringent conditions, the probe hybridized almost exclusively to the X chromosome, specifically to the pericentromeric region (fig. 2*A* and inset). In this experiment, greater than 60% of the grains were found over the X centromere, and the X had greater than 10 times as many grains as any other individual chromosome. Under conditions of reduced stringency, however, a strikingly different pattern was observed: the probe bound to each human chromosome, specifically labeling the pericentromeric region of all 22 autosomes and the X and the Y chromosomes (fig. 2*B*). No consistent sites of hybridization other than the centromeres were observed.

To examine the molecular organization of α satellite DNA sequences on individual human chromosomes, we performed Southern blot analysis of DNAs from a number of mouse/human or hamster/human hybrids, each containing only a single human chromosome. Human DNA and hybrid DNAs were each digested with *Eco*RI, *Bam*HI, and *Hin*dIII and a Southern blot prepared, which was sequentially hybridized under conditions of reduced stringency with the X-derived pBamX7 probe and a cloned 340-bp α-dimer probe (pαRI-12). The results of sequential hybridization to digested human DNA (fig. 3*A*) established that the two probes hybridize to an essentially identical series of fragments, with the major differences between the patterns being quantitative

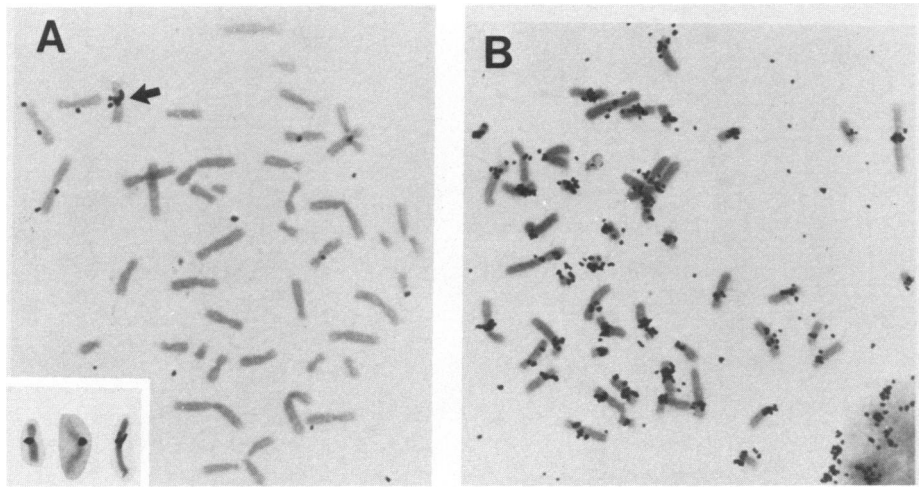

FIG. 2.—In situ hybridization analysis of α satellite DNA in the human genome. Metaphase chromosomes were prepared from 46,XY lymphoblasts (*A*) or 49,XXXXY lymphoblasts (*B*) and hybridized to ^{3}H-labeled, nick-translated pBamX7. Slides were washed at 50°C–51°C (high stringency) (*panel A*) or 37°C (reduced stringency) (*panel B*). Exposure times were 2 days (*A*) or 3 days (*B*). In *A*, *the arrow* indicates the X chromosome. *Inset:* X chromosomes from three other cells from the same experiment.

rather than qualitative (particularly when a number of different exposures are compared). In a similar fashion, we examined DNA from hybrids containing only the human X chromosome, the Y chromosome, chromosome 3, chromosome 4, or chromosome 21 (fig. 3*B*). In each hybrid DNA, the probes hybridized to a specific, but distinct, subset of the fragments detected in total genomic human DNA.

These experiments allow definition of a particular enzyme periodicity characteristic of α satellite DNA on a single chromosome. Thus, α satellite DNA is found on the human Y chromosome primarily in two *Hin*dIII fragment lengths, of 6.4 kb and > 24 kb; on chromosome 3 in a number of *Hin*dIII fragment lengths including prominent bands at 3.0 and 2.7 kb; on chromosome 4 in large *Eco*RI domains > 25 kb with a relatively minor component in *Eco*RI fragments of approximately 1.4 kb; and on chromosome 21 in a number of *Eco*RI fragments (fig. 3*B*).

DISCUSSION

The data presented above establish that the DXZ1 repeated sequences found at the centromere of the X are members of a larger, diverse DNA family that includes the centromeric regions of all human chromosomes (figs. 1 and 2). Our in situ hybridization data, indicating that essentially all copies of the DXZ1 family are confined to the Xq11→Xp11.1 region, are consistent with similar data of Yang et al. [12] and with our previous assignment of these sequences to the region Xq11→Xp11 by Southern blot analysis of somatic cell hybrids containing only portions of the human X due to X-autosome translocations [21].

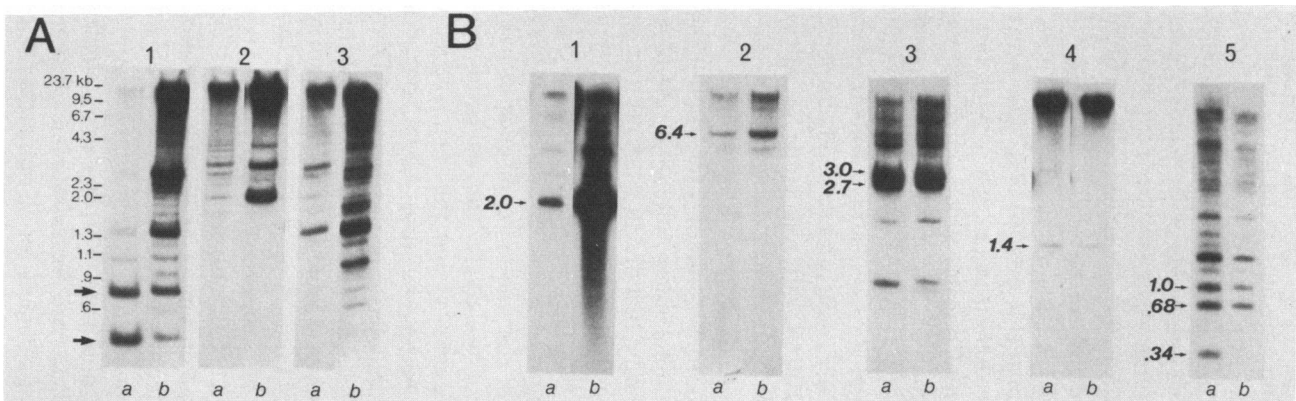

FIG. 3.—Hybridization analysis of α satellite DNA in human genomic DNA and in DNAs from rodent/human somatic cell hybrids, each containing only a single human chromosome. *A*, Human male DNA (46,XY) was digested with *Eco*RI (*lane 1*), *Bam*HI (*lane 2*), or *Hin*dIII (*lane 3*) and a Southern blot experiment performed at reduced stringency. Filter was sequentially hybridized with pαRI-12, (*a lanes*) and then with pBamX7 (after removing the first probe) (*b lanes*). Exposure times were chosen to equalize intensity of 340-bp and 680-bp *Eco*RI bands hybridizing to the two probes (*arrows*). *B*, DNA from five somatic cell hybrids was digested and analyzed as in *A*. *Nos. to left* indicate sizes of selected bands, in kb. *Panel 1*, X-chromosome hybrid DNA digested with *Bam*HI. *Panel 2*, Y chromosome hybrid DNA digested with *Hin*dIII. *Panel 3*, chromosome 3 hybrid DNA digested with *Hin*dIII. *Panel 4*, chromosome 4 hybrid DNA digested with *Eco*RI. *Panel 5*, chromosome 21 hybrid DNA digested with *Eco*RI. Exposure times in *panels 1, 2*, and *3* are equivalent and were selected to normalize hybridization of the two probes to the DNA in *panel 3*. In *panels 4* and *5*, exposure time in *a lanes* is five times that in *b lanes*.

Data obtained using hybridization and/or washing conditions of different stringencies suggest that the X-linked and non-X-linked members of the α satellite family differ from one another with respect to degree of sequence relatedness (figs. 1 and 2). Further, the data in figure 3 establish that at least some individual human chromosomes, including the X and Y chromosomes, are characterized by a distinct subset of α satellite DNA, as defined by restriction site organization. The relative intensities of the bands observed in figure 3 must be interpreted with caution and presumably reflect possible differences in copy number as well as differences in the rate of formation and melting temperature of heteroduplexes whose degree of mismatch with the two probes used varies. The role of the latter effect is most striking in comparing hybridization of the two probes to DNA from the X-only hybrid. The less intense signal obtained with the α-dimer probe demonstrates that α satellite DNA on the X and the 340-bp *Eco*RI α-dimer show considerable sequence divergence. The α satellite family on chromosome 21 provides another example of this heterogeneity. Although the two probes used in this study (neither of which is derived from chromosome 21) detect a series of α satellite-containing *Eco*RI fragments on chromosome 21, only three intense bands (of lengths 0.34, 0.68, and 1.0 kb) are observed when other, different cloned α satellite probes are used in this type of analysis (our unpublished data, 1984).

It is interesting that of the chromosomes so far examined (chromosomes X, Y, 3, 4, and 21 [fig. 3] and chromosomes 5 and 17, our unpublished data, 1984), only chromosomes 5 and 21 contain a significant number of prototype α-dimer *Eco*RI fragments. Thus, it is apparent that much, if not most, α satellite DNA in the human genome is found in restriction fragment lengths other than the ones originally defined [4, 9, 22] and that characteristics of repeated DNA within a particular fragment length may not, therefore, be representative of the genome as a whole. Further, it is evident that the mere absence of hybridization of a fragment to an α-dimer probe under relatively stringent conditions of hybridization cannot be taken alone as evidence that the fragment is not α satellite. Indeed, such experiments are likely, because of sequence divergence, to significantly underestimate the abundance of this repeated DNA family.

Here we have demonstrated that some, and perhaps all, human chromosomes are characterized by a distinct, chromosome-specific subset of α satellite DNA as defined both by different restriction enzyme periodicity and by degree of DNA sequence relatedness. Heterogeneity within human α satellite DNA has been noted previously [9, 22, 23], but not identified as being related to chromosome-specificity, although chromosome-specific organization has been suggested for components of human satellite III DNA [24, 25] and for satellite DNA on the mouse X chromosome [26]. Extension of the somatic cell hybrid approach originally used by Lee and Singer [27] to study a single African green monkey chromosome and applied here to the human genome should allow definition of α satellite-containing domains for all human chromosomes. Based on the results presented here, we would predict that some of the "unusual" human α satellite domains identified previously [9, 22] might, in fact, represent α satellite DNA configurations characteristic of a particular human chromo-

some. If so, hybridization analysis under high-stringency conditions should permit mapping of these domains in somatic cell hybrids. One such possible domain has been described [28] since the completion of this work and has been assigned to the centromere of human chromosome 6. Using their probe, Jabs et al. [28] independently demonstrated chromosome-specific organization of centromere DNA. As yet, however, that fragment has not been shown directly to contain α satellite DNA.

The availability of cloned α satellite-containing DNA fragments from different chromosomes should provide significant information about human chromosome structure and about the evolution and chromosomal dispersion of this repeated DNA family. In addition, our experience with cloned members of the X α satellite subfamily, as well as with cloned representatives of the α satellite subsets characteristic of chromosomes 5 and 17 (our unpublished data, 1984), suggests that α satellite members on a single chromosome are much more closely related in sequence than are members on different chromosomes. This repeated DNA family might, therefore, provide a source of useful molecular probes to specifically identify individual human chromosomes under appropriate experimental conditions. A collection of repeated DNA probes specific for each human chromosome might be useful for molecular cytogenetic analysis in certain clinical situations.

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INTERNATIONAL MEETING ON HUMAN POPULATION GENETICS: Use of genetic markers, especially HLA, for studying interregional variability; Toulouse, France, October 21–23, 1985. Principal topics: analysis methods in human genetic populations; use of red cell blood groups, tissue antigens (HLA-A, -B, -C, -DR . . .), serum allotypes (Bf, C4, Gm, Gc . . .), and enzymes; results of a study in 15 areas in France (and in Quebec) and comparison with data from other populations. Information and registration: INSERM U.100, Meeting Secretary, CHU Purpan, 31052 Toulouse Cedex, France. Telephone: (61)49.36.33. Free papers planned: abstract forms provided upon request; deadline for abstract submission: June 1, 1985; all accepted abstracts will be distributed at the time of the meeting. Publications: The proceedings of the meeting will be published by INSERM. Organizers: Prof. E. Ohayon and Dr. A. Cambon-Thomsen, INSERM U.100, CHU Purpan, 31052 Toulouse Cedex, France. Telephone: (61)49.36.33. Télex: INSERM 521.042 F. Sponsored by: INSERM.