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Genetics

Characterization of a cloned repetitive DNA sequence concentrated on the human X chromosome

(alphoid DNA/centromere)

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ABSTRACT **A tandemly repeated DNA sequence organized predominantly, if not entirely, in a specific manner on the human X chromosome has been cloned in pBR322 and characterized. The sequence was detected as a 2-kilobase band in ethidium bromide-stained agarose gels of *Bam*HI-digested total human nuclear DNA. Although *in situ* hybridization of the cloned sequence to human metaphase chromosomes showed a single major site of hybridization at the centromere region of the X chromosome and minor sites of hybridization at several autosomal centromeres, Southern blot analysis of restricted total human DNA indicated that the cloned probe is related to other repeated DNAs, particularly the human alphoid DNAs. Restriction enzyme analysis of the cloned fragment revealed an internal repeat structure based upon multiples of 170 base pairs, confirming this relatedness. All available data, however, suggest that the 2-kilobase spacing of *Bam*HI sites within the repeat may be specific to the X chromosome.**

The human X chromosome is a particularly interesting system for the molecular investigation of mammalian gene structure expression. A large number of medically significant genetic loci have been mapped to it; and one X chromosome in female eutherian mammals exhibits the phenomenon of inactivation, apparently to accomplish dosage compensation of X-linked gene functions (1). Thus, with appropriate DNA probes, this system provides the opportunity to analyze molecular properties of the same gene in the active as well as the inactive state in the same cell.

Recent advances in DNA technology in conjunction with modern genetic methods have permitted the identification or isolation of X-chromosome specific human DNA sequences. Beauchamp *et al.* (2) used Southern blot analysis to identify a 2-kilobase-pair (kb) fragment generated by *Bam*HI digestion of rodent–human somatic cell hybrid DNA present in hybrids containing the human X chromosome and which bears homology to human satellite III DNA. Recently, several single-copy sequences from mammalian X chromosomes have been cloned. These include cDNA of the human glucose-6-phosphate dehydrogenase gene (3), a partial cDNA clone of the mouse hypoxanthine phosphoribosyltransferase gene (4), an unidentified human single-copy DNA sequence (5), and two λ libraries constructed from flow-sorted chromosomes enriched in human X (6, 7). To date, however, no cloned repetitive sequences that are located predominantly or entirely on the human X chromosome have been reported.

We report the cloning and characterization of a 2-kb fragment of human DNA which exists in tandem arrays and is concentrated at or near the centromere region of the human X chromosome. The spacing of *Bam*HI sites argues that it is homologous to the sequence noted by Beauchamp *et al.* (2). Restriction enzyme analysis of the DNA provides data to support the hypothesis that the organization of this sequence is predominantly, if not entirely, specific to the X chromosome. In addition, the DNA displays homology to the human *Eco*RI dimer and similarities to other members of the alphoid family of highly repeated primate DNAs (8, 9).

MATERIALS AND METHODS

Human Cells and Culture. All tissue culture cells were grown and maintained, in Dulbecco's modified Eagle's minimal medium (MEM-E medium; GIBCO) containing 10% fetal bovine serum (Irvine Scientific), as monolayers in plastic tissue culture flasks (Lux Scientific; Corning) or glass roller bottles (Bellco Glass). A human fibroblast line containing five X chromosomes but diploid for autosomes and a normal human male fibroblast line both were generously provided by Uta Francke. Other male cells were purchased from the Human Genetic Mutant Cell Repository (Camden, NJ). Human placentas were obtained from the University of California at Irvine Medical Center.

Preparation of DNA. Human DNA was prepared from either cells or placental nuclei. Tissue culture cells were harvested by trypsinization, washed successively in MEM-E medium with 10% fetal calf serum, MEM-E medium without serum, and Puck's saline with 0.02% EDTA, and homogenized in cold homogenization buffer (0.25 M sucrose/3 mM $CaCl_2$/10 mM Tris, pH 7.9/1% Triton X-100) with a motor-driven Potter–Elvejehm homogenizer; nuclei were sedimented by centrifugation. Nuclei were isolated from placentas by blending the minced, saline-washed, tissue in a Waring Blender with cold homogenization buffer. Lysates were filtered through three or four layers of cheesecloth and nuclei were sedimented.

Nuclear pellets were resuspended in 50 mM Tris, pH 8.5/50 mM EDTA/150 mM NaCl and incubated at 37°C overnight in proteinase K (100 μg/ml; Boehringer Mannheim) containing 0.5% $NaDodSO_4$. After addition of sodium perchlorate to 0.4 M, nuclear lysates were extracted once with phenol and twice with chloroform/isoamyl alcohol, 24:1 (vol/vol). They were incubated at 37°C for 2 hr with RNase A (50 μg/ml) and then reextracted as above. The final aqueous phase was dialyzed extensively against TE buffer (10 mM Tris, pH 7.5/1 mM EDTA) at 4°C. The final dialysate was either concentrated by ethanol precipitation and resuspension in TE buffer or digested with

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Abbreviations: kb, kilobase pair(s); MEM-E medium, Dulbecco's modified Eagle's medium; bp, base pair(s).

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restriction endonucleases directly by adjusting the dialysate to incubation conditions as specified by the enzyme suppliers.

Analysis of DNA and Preparation of Probe. DNA was digested with restriction endonucleases (obtained from either New England BioLabs or Bethesda Research Laboratories) according to the supplier's instructions; a 3- to 10-fold excess of enzyme units was used per μg of DNA. Restriction fragments were fractionated as stated in the legend to Fig. 1*A*, and DNA was recovered from agarose gels by electrophoresis into DE 81 paper as described in the legend to Fig. 1*C*. Labeled probes were prepared by nick-translation using [^{32}P]dCTP (2,000–3,000 Ci/mmol in aqueous solution; 1 Ci = 3.7×10^{10} becquerels; Amersham) and the Bethesda Research Laboratories nick-translation kit.

For Southern blot analyses, DNA in agarose gels was transferred to nitrocellulose (Schleicher & Schuell) according to Meeker *et al.* (12). Hybridization was carried out for 18–36 hr at 70°C. After hybridization, filters were washed at 50°C three or four times for 2–4 hr with 15 mM NaCl/1.5 mM sodium citrate, pH 7.0, containing 0.05% $NaDodSO_4$ and then three times for 0.5 hr with 15 mM NaCl/1.5 mM sodium citrate. In some experiments, filters were washed at 50°C, autoradiographed, and then successively rewashed at 5°C increments in both wash solutions; autoradiography was performed after each complete wash. Autoradiography was performed with Kodak X-Omat film and a Dupont Cronex intensifying screen at −70°C for 2–14 hr.

Cloning and Preparation of Recombinant Plasmids. Female placental DNA was used as a source of DNA for cloning. After digestion with *Bam*HI and fractionation on preparative agarose gels, the appropriate-sized fraction was recovered from the gel, ligated to *Bam*HI-digested pBR322 DNA, and transfected into *Escherichia coli* HMS 174 as described by Meeker *et al.* (12). Ampicillin-resistant, tetracycline-positive recombinants were identified by the method of Maloy and Nunn (13). Filter hybridization of selected recombinants was performed as described by Hanahan and Meselson (14) with a ^{32}P-labeled crude probe prepared as described in *Results*. Plasmids from positive recombinants were prepared as described by Meeker *et al.* (12).

Preparation of Radioactive cRNA and *In Situ* Hybridization. *In situ* hybridizations were performed and analyzed according to Ryder and Hansen with cRNA against the 2-kb cloned DNA synthesized with four [^{3}H]XTPs (11); hybridizations were carried out at 58°C in 0.45 M NaCl/0.045 M sodium citrate, pH 7.0, for 4 hr.

RESULTS

Identification of Sequence. *Bam*HI digestion of XY, XX, and XXXXX DNAs followed by agarose gel electrophoresis and staining revealed a 2-kb DNA band that exhibited increasing intensity with increasing X chromosome dosage (Fig. 1*A*). This result suggests that a repetitive sequence with *Bam*HI sites spaced 2 kb apart is located predominantly on the human X chromosome. This sequence is probably the same as that found by Beauchamp *et al.* (2) in rodent–human cell hybrid DNA. Based on densitometer scans of gel negatives (Fig. 1*B*) we estimate that the 2-kb sequence comprises approximately 0.8% of the 5X genome.

In order to isolate and characterize this sequence, a crude probe from the fragment was prepared from *Bam*HI-digested female placental nuclear DNA (Fig. 1*C*). Fragments approximately 2 kb in size were eluted from the gel and redigested with *Bst*NI because there are no sites for this enzyme in the X chromosome dose-related 2-kb *Bam*HI band (data not shown). The doubly digested sample displayed a significant enrichment of the 2-kb *Bam*HI band when fractionated on an agarose gel (Fig. 1*C*). This band was eluted from the gel and nick-translated for use as a probe.

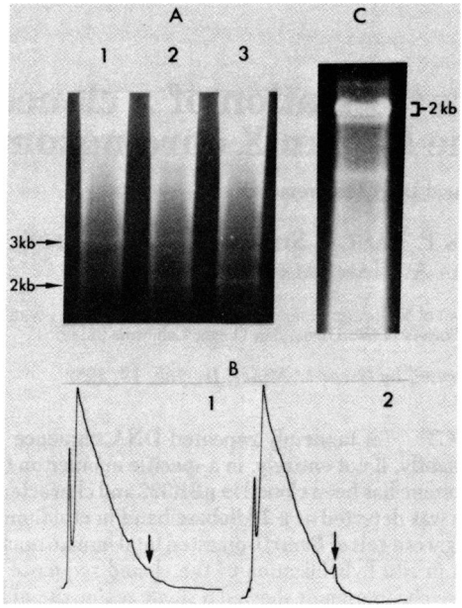

FIG. 1. Identification of 2-kb band and preparation of crude probe. (*A*) Ethidium bromide-stained 1% agarose gel of *Bam*HI-digested human DNA from male (lane 1), normal female (lane 2), and 5X female (lane 3). Restriction fragments were fractionated on horizontal agarose gels in 40 mM Tris/10 mM sodium acetate/2 mM EDTA, pH 7.8. (*B*) Densitometer tracing of photographic negative from lanes 1 and 3 in *A* (shown as panels 1 and 2, respectively). Arrows indicate position of 2-kb band. Direction of migration is toward the right. Tracing was performed on a Joyce–Loebl densitometer. (*C*) Ethidium bromide-stained 1% agarose gel of enriched 2-kb *Bam*HI band. Female DNA was digested with *Bam*HI and fractionated on a 1% agarose gel. The 2-kb DNA was recovered from the gel and enriched by redigestion with *Bst*NI and fractionation on a 1% agarose gel. Recovery was performed using DE 81 paper as follows (R. Reeder, personal communication). The DNA fragment of interest was located by ethidium bromide staining and a slot was cut in the gel just below the desired band. DE 81 paper (Whatman) was inserted into the slot and the DNA was electrophoresed into the paper. The paper was removed, placed in a 1 ml syringe, and washed with 0.1 M NaCl/0.1 mM EDTA/10 mM Tris, pH 8, by centrifugation in a 15-ml Corex (Corning) tube. The DNA was eluted from the paper with 1 M NaCl, ethanol precipitated, and redissolved in TE buffer.

Southern Blot Analysis. The crude probe described above was hybridized to Southern blots of DNAs containing different numbers of X chromosomes. Fig. 2 shows the expected increase in hybridization of probe to DNA from XY, XX, and XXXXX cells, documenting the X-specificity of the sequence. Autoradiograms consistently showed hybridization to DNAs 2 and 4 kb long whose intensity increased with X chromosome dosage. Upon longer exposure, a minor band at 3 kb also was observed

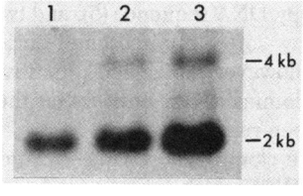

FIG. 2. Autoradiograms of Southern blot hybridization of ^{32}P-labeled crude 2-kb probe to *Bam*HI-digested human DNA from male (lane 1), normal female (lane 2), and 5X female (lane 3). Equal amounts of DNA were loaded into each lane. Specific activity of the ^{32}P-labeled probe was $>2 \times 10^7$ cpm/μg.

which did not reproducibly increase significantly in intensity as the X chromosome dosage increased (data not shown). This hybridization probably reflects homology with another repeat which may exist on the X in low copy number but whose predominant location is autosomal. A minor 6-kb band that sometimes resolved represents either a trimer of the 2-kb monomer *Bam*HI repeat unit or a dimer of the 3-kb band; the available data do not permit distinction between these two alternatives. Some gels also showed hybridization to an 8-kb fragment, probably representing a multimer of the X-linked 2-kb monomer unit. These data suggest that the X chromosome contains a tandemly repeated DNA sequence with *Bam*HI sites spaced 2 kb apart. Alteration of *Bam*HI sites within the tandem array generates multimers of the monomer unit, resulting in the characteristic ladder generated by restriction digestion of tandemly repeated DNAs.

Cloning and Restriction Analysis of Cloned 2-kb Repeat. The 2-kb repeat was cloned into the *Bam*HI site of pBR322. Of 800 ampicillin-resistant, tetracycline-sensitive recombinants, 9 displayed strong positive signals after screening using the crude probe. One recombinant containing a monomer insert (pXBR-1) was subjected to restriction enzyme analysis. *Bam*HI digestion of the plasmid (Fig. 3*A*) yielded two fragments, 2 and 4.3 kb (pBR322). In addition, digestion with *Eco*RI, *Hin*dIII, or *Bst*NI demonstrated the absence of sites for these enzymes within the cloned insert, in agreement with results from genomic double digests (data not shown).

The 2-kb insert was removed from the plasmid, purified, and radioactively labeled with ^{32}P by nick-translation. This probe was hybridized to Southern blots of restriction digests of human DNA. Comparison of the *Bam*HI digest shown as lane 3 in Fig. 4*A* with Fig. 2 shows identical patterns of hybridization with the cloned probe and the crude probe. The restriction enzyme and Southern blot analyses of the cloned sequence confirm that it is a monomer of the X-linked 2-kb *Bam*HI repeat.

Southern blot analysis of human DNA digested with *Eco*RI, *Hae* III, *Hin*fI, or *Hin*dIII demonstrated numerous bands of hybridization, some of which formed ladders based on multiples of 170 base pairs (bp) (Fig. 4). The *Eco*RI-generated ladder (Fig. 4*B*, lane 1) has been identified previously as characteristic of

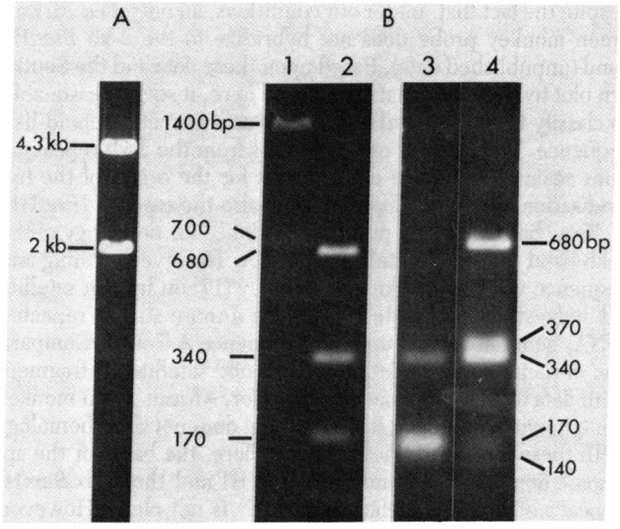

FIG. 3. Restriction enzyme analysis of pXBR-1 and cloned insert: ethidium bromide-stained gels. (*A*) pXBR-1 digested with *Bam*HI. Electrophoresis was performed on a 1% agarose gel. (*B*) Cloned 2-kb fragment (insert only) digested with *Pst* I (lane 1), *Ava* II (lane 2), *Hin*fI (lane 3), and *Hae* III (lane 4). Electrophoresis was performed on 2% agarose gels.

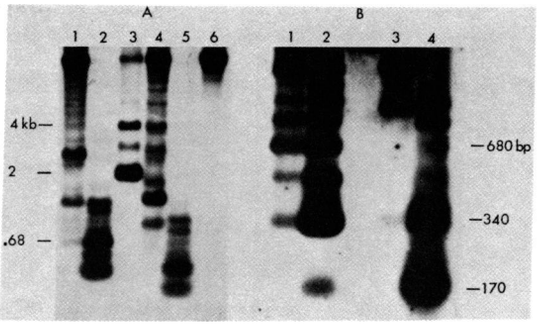

FIG. 4. Autoradiograms of Southern blot analysis with probe of ^{32}P-labeled, cloned, 2-kb *Bam*HI band hybridized to female DNA digest. (*A*) Digestion with *Eco*RI (lane 1), *Hae* III (lane 2), *Bam*HI (lane 3), *Hin*dIII (lane 4), *Hin*fI (lane 5), and *Kpn* I (lane 6). Restriction fragments were fractionated on 1% agarose gels. (*B*) Digestion with *Eco*RI (lane 1), *Hae* III (lane 2), *Hin*dIII (lane 3), and *Hin*fI (lane 4). Restriction fragments were fractionated on 2% agarose gels.

several possibly overlapping classes of repeated human DNAs including primate alphoid DNAs (9, 15) and human satellites (refs. 16 and 17, and *Discussion*). The *Hae* III and *Hin*fI ladder of 170-bp multiples (lanes 2 and 4) also have been associated with these families of repeated DNAs (9, 16, 17). However, under our conditions, although a faint band was seen at 340 bp, the 2-kb cloned *Bam*HI probe did not hybridize to a 170-bp alphoid-human-DNA-like ladder generated by *Hin*dIII (lane 3) which was noted by Maio *et al.* (9) in hybridization with an uncloned African green monkey component-α probe. The relative stabilities of the duplexes formed between the cloned insert and the different repetitive human DNA sequences detected was determined by successive washings of hybridized filters at 5°C increments from 50°C to 80°C followed by autoradiography. No losses of radioactive bands were detected up to 80°C (data not shown), suggesting that the sequences in the bands that hybridized to the cloned probe were highly homologous to it. These data support the conclusion that the X-linked *Bam*HI band is a member of one or more of these repeated human DNA families (see *Discussion*).

The organization of other restriction sites in the cloned sequence was also determined. No *Msp* I, *Hpa* II, *Sal* I, or *Mbo* I sites were detected (data not shown). *Hin*fI digestion resulted in the production of two major fragments, 170 and 340 bp long (Fig. 3*B*). Because the sum of the lengths of these two fragments does not account for the entire insert, these data indicate that the 2-kb *Bam*HI fragment is made up of tandemly repeated 170-bp internal subunits defined by *Hin*fI sites. The 340-bp band is probably a dimer of the 170-bp repeat generated by an alteration in at least one of the *Hin*fI sites. Analogous results were seen with *Ava* II and *Hae* III digestion (Fig. 3*B*). In both cases, major bands were seen at 170, 340, and 680 bp. In the case of *Hae* III, the fragments visible at 360 and 140 bp probably arose from the ends of the cloned 2-kb sequence which has a *Bam*HI site on one side and an *Hae* III site on the other. The absence of bands in the *Hin*fI and *Ava* II digests at sizes other than $(170)_n$ argues that these restriction sites are close to the *Bam*HI site because small terminal fragments would not be resolvable in the gels used. These restriction digests argue that the 2-kb *Bam*HI fragment is composed of tandemly repeated 170-bp subunits defined by *Hin*fI, *Ava* II, and *Hae* III. This internal organization is characteristic of members of the alphoid DNA family (9, 15) and human satellite DNAs (16, 17).

Digestion of the cloned insert by *Pst* I (Fig. 3*B*) resulted in two fragments—700 and 1,400 bp long—indicating a single *Pst* I site between the 2-kb-spaced *Bam*HI sites. The Southern blot

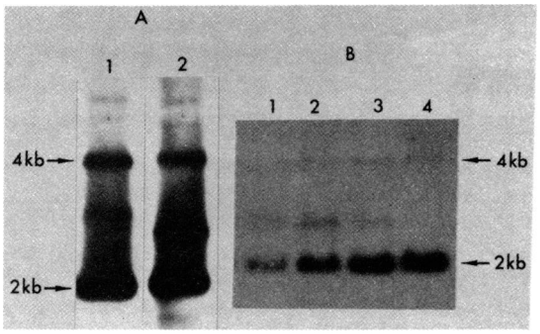

FIG. 5. Autoradiograms of Southern blotted total human DNA. (*A*) Digested with *Bam*HI (lane 1) and *Pst* I (lane 2) and hybridized with ^{32}P-labeled cloned 2-kb fragment. (*B*) Total human DNA from male (lane 1), normal female (lane 2), or 5X female (lanes 3 and 4) digested with *Pst* I (lanes 1–3) or *Bam*HI (lane 4) hybridized to ^{32}P-labeled cloned 2-kb fragment. Equal amounts of DNA were loaded into each lane.

of *Pst* I-digested human DNA hybridized with the cloned probe (Fig. 5*A*) demonstrated a pattern identical to the 2-kb ladder seen with *Bam*HI digestion, supporting the idea that *Pst* I sites are tandemly arranged and spaced at 2-kb intervals in the repeat. The intensity of the 2-kb *Pst* I site exhibits X chromosome-related dosage both in ethidium bromide stained gels (data not shown) and in Southern blots (Fig. 5*B*), arguing that the 2-kb *Pst* I pattern is also X-linked such that both *Bam*HI and *Pst* I sites are spaced at 2-kb intervals within the same tandemly repeated DNA sequence.

***In Situ* Hybridization.** The chromosomal localization of the 2-kb sequence was determined by *in situ* hybridization of a ^{3}H-labeled cRNA to metaphase chromosomes of a normal male cell line and the 5X cell line (Fig. 6). The major site of hybridization in both cell lines was the centromere region of the X chromosome. In addition, analysis of several chromosomal spreads on several slides showed consistent but significantly lower levels of hybridization to chromosomes 1, 11, and 17 and less-frequent, weak signals to chromosomes 2, 4, 15, 18, 19, 20, and 22. Consistent hybridization to chromosomes other than X is a consequence of cross-hybridization either to related sequences or to identical sequences. However, the X chromosome-related dosage of *Bam*HI sites 2 kb apart suggests that autosomal homologous sequences either possess a different spacing of *Bam*HI sites or lack tandemly spaced *Bam*HI sites.

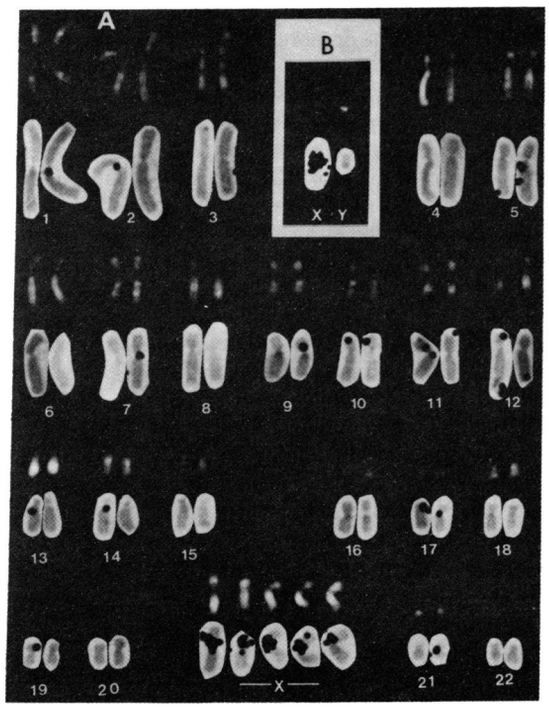

FIG. 6. *In situ* hybridization of ^{3}H-labeled cRNA synthesized from the cloned 2-kb fragment to human 5X female metaphase chromosomes (*A*) and male sex chromosomes (*B*).

DISCUSSION

A repetitive DNA sequence that is highly concentrated near or at the centromere region of the human X chromosome has been cloned and characterized. This sequence is visualized as a distinct 2-kb band in ethidium bromide-stained agarose gels of total human nuclear DNA after digestion with *Bam*HI. The sequence appears to be organized as a tandem repeat with a unit monomer length of 2 kb, defined by *Bam*HI sites. Comparative analysis of *Bam*HI-digested DNA from human cell nuclei containing different numbers of X chromosomes shows a correlation between the relative intensity of the 2-kb band and the number of X chromosomes, both in ethidium bromide-stained gels and in Southern blots. The low background smear seen in Southern blots relative to the intensity of the 2-kb ladder bands strongly indicates that this sequence is not interspersed throughout the genome and therefore must be organized as clustered tandem repeats. Thus, the organization and 2-kb spacing of the *Bam*HI sites within the tandem repeat appears to be predominantly located on the X chromosome and in fact may be specific to it.

Southern blot analysis of total human DNA digested with other restriction enzymes indicates that the 2-kb *Bam*HI sequence has homology to at least one class of repeated human DNA revealed after digestion by *Eco*RI. Sequence analysis of the 340-bp fragment (the *Eco*RI dimer) seen in the *Eco*RI-generated 170-bp-based ladder illustrates extensive regions of homology between the *Eco*RI dimer and the 170-bp repeat unit in African green monkey component-α DNA (18). Preliminary sequence data on 170-bp subunits of the 2-kb *Bam*HI fragment also show extensive regions of homology with both the human *Eco*RI dimer and African green monkey component-α DNA despite the fact that, under our conditions, an uncloned African green monkey probe does not hybridize to the 2-kb *Bam*HI band (unpublished data). Based upon these data and the Southern blot hybridization data presented here, it seems reasonable to classify the 2-kb *Bam*HI fragment as a primate alphoid-like sequence. The absence of *Eco*RI sites from the 2-kb repeat argues against alternative explanations for the origin of the hybridization seen with *Eco*RI. This is also the case for *Hin*dIII.

Beauchamp *et al.* (2) provided evidence for homology of the 2-kb band to human satellite III DNA. However, cloning and sequence work by Cooke and Hindley (17) on human satellite III suggests that satellite III may be a more simple repeated DNA, generally based upon the sequence A-T-T-C. Comparison of their sequence data from a cloned satellite III fragment with data from the human *Eco*RI dimer, African green monkey component-α, and 2-kb *Bam*HI repeat does not show homology with these alphoid sequences. Therefore, the basis for the apparent homology of human satellite III and the 2-kb *Bam*HI repeat noted by Beauchamp *et al.* (2) is not clear. However, because of some structural similarities between alphoid DNAs and human satellite DNA as revealed by restriction digests (16, 17), it is possible that alphoid DNAs comprise a minor component of satellite III.

The *in situ* hybridization pattern of the 2-kb *Bam*HI fragment is significantly different from that of other human repeated

DNAs previously mapped. One notable difference is that the major site of this sequence is on the human X chromosome, a location that does not show significant hybridization with other repeated DNAs, including the 340-bp *Eco*RI dimer fragment (19) and each of the four human satellite DNAs (20).

The organization of the 2-kb tandem repeat (defined by spacing of *Bam*HI sites) superimposed upon an internal repeat structure (defined, for example, by the 170-bp spacing of *Hinf* I sites) suggests a mechanism by which this organization may have been generated during evolution. This mechanism is similar to that proposed by Southern to explain long-range periodicities in mouse satellite DNA (21). First, one must postulate the existence of a progenitor sequence on the X chromosome and on a number of autosomes organized as a 170-bp tandem repeat defined by the spacing of particular restriction sites (*Hinf* I, *Hae* III, *Ava* II). At some point, a *Bam*HI site was generated by mutation in one of the 170-bp monomer units, followed by duplication and tandem amplification of a continuous block of 170-bp units including the unit containing the new *Bam*HI site. If a block of 12 contiguous 170-bp units each containing a single *Bam*HI site were amplified as a single unit, a 2-kb-spaced *Bam*HI tandem repeat would result. In addition, the 170-bp spacing of *Hinf* I (*Hae* III, *Ava* II) repeats present in the ancestral sequences would remain intact. The presence of a single *Pst* I site within the 2-kb repeat unit that is organized similarly to the *Bam*HI sites supports the argument that a contiguous block of 170-bp units 2 kb long was amplified as a single unit. If the initial generation of the *Bam*HI site and the amplification event occurred only on the X chromosome, then the resultant 2-kb spacing of *Bam*HI sites would be specific to the X. Furthermore, sequences between the *Bam*HI sites would retain homology to autosomal repeated sequences that were derived from the original progenitor.

The possible existence of minor amounts of the 2-kb *Bam*HI repeat on chromosomes other than the X cannot be ruled out with the available data. In fact, data from Beauchamp *et al.* (2) indicate that the 2-kb-spaced *Bam*HI sites also exist on human chromosome 22. Although we believe we have cloned a monomer of the same 2-kb sequence, we see only a very weak hybridization signal *in situ* to chromosome 22. It is possible that the 2-kb *Bam*HI band apparently associated with chromosome 22 is actually a 2-kb *Bam*HI fragment homologous to a component of human satellite III that has diverged significantly from the X-linked repeat and therefore exhibits minimal cross-hybridization to the cloned X-linked sequence. Alternatively, the somatic cell hybrid used in their studies may have retained a fragment of a human X chromosome centromere that was undetectable cytologically. Southern blot analysis with the cloned probe on several rodent–human somatic cell hybrids that lack the human X chromosome will be useful in determining the precise chromosomal specificity of the cloned *Bam*HI repeat.

The cloned fragment has also been used to study the organization and localization of homologous sequences in higher primates (unpublished data). Furthermore, partial sequence analysis of the fragment could permit comparison to published sequences of several alphoid DNAs from primates including man (22). Finally, the location of the cloned sequence on the human X chromosome provides us with a probe for human heterochromatin and, in particular, for the Barr body.

Note Added in Proof. In recent experiments using an alternative method to determine the thermal stability of duplexes formed between the cloned 2-kb sequence and other human repetitive DNAs, we observed selective elution of hybrids containing *Eco*RI- and *Hin*dIII-digested DNA when filters were washed at 80°C. These data indicate that there is some divergence between the cloned sequence and other human repeats.

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