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APPLICATION OF SYNTHETIC DNA PROBES OF HUMAN ALPHA SATELLITE CONSENSUS MONOMER FOR DETECTION OF CENTROMERE-INVOLVED CHROMOSOME ABNORMALITIES

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Summary We have synthesized the alphoid monomer of 171 bp based on the consensus sequence of human alpha satellite DNA and constructed a clone of dimeric or tetrameric sequence unit. Southern blot analysis using the clone as a probe showed restriction site periodicities in human DNA digested by *Eco*RI or *Bam*HI. The synthetic consensus unit could detect the alpha repeated centromeric regions of all human chromosomes by fluorescence *in situ* hybridization. Using the cells having a dicentric X chromosome, we showed that the two centromeric regions were stained with fluorescent alpha satellite DNA probes. Thus the probe would be useful to detect chromosomal abnormalities such as dicentrics.

Key Words alphoid, alpha satellite, centromere, dicentrics, fluorescence *in situ* hybridization

INTRODUCTION

In primate genome, the centromeric region of chromosome is dominated by a diverse class of highly repeated DNA, alphoid or alpha satellite sequences. The alpha satellite repeat units are composed of diverse, tandemly reiterated monomer units of ~171 bp and characterized by particular restriction enzyme periodicities involving multiple monomers referred to as higher-order repeat units (Waye and Willard, 1987; Alexandrov *et al.*, 1988). Analysis of these monomer units of alpha satellite DNA has revealed a certain degree of sequence heterogeneity, which is a basis for chromosome specificity of the alpha satellite DNA family. Despite such heterogeneity within the human alpha satellite repeat units, several attempts have been made to derive a human satellite consensus sequence and to identify evolutionarily conserved sequences within the monomer units (Waye and Willard, 1987; Vissel and Choo, 1987; Willard and Waye, 1987; Alexandrov *et al.*, 1988). The

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consensus nucleotide sequences were deduced from the sequence data of 153 (Waye and Willard, 1987) and 145 (Vissel and Choo, 1987) monomer units isolated from more than 12 human chromosomes.

Chromosome-specific repetitive DNA markers would be useful to identify individual chromosomes or chromosome regions (Moyzis *et al.*, 1987). Actually the alpha satellite DNA clones have been used for the identification of a certain chromosome sorted by flow cytometry and for cytogenetical analysis of human chromosomes (van Dekken and Bauman, 1988; Dale *et al.*, 1989). The chromosomal distribution in interphase nuclei was also analyzed using a certain chromosome-specific alpha satellite DNA (van Dekken and Bauman, 1988; Meyne and Moyzis, 1989). However, a useful clone which could recognize the centromeric regions of all types of human chromosomes in methaphase and interphase nuclei has to be developed in order to obtain a sufficient signals on chromosomes under a normal hybridization condition.

Here we attempted to synthesize chemically the consensus sequence of human alpha satellite DNA and obtained the results that the synthetic monomer DNA could detect the alphoid repeated centromeric regions of all human chromosomes by fluorescence *in situ* hybridization.

MATERIALS AND METHODS

The approach for generating a sequence synthetically for human alpha satellite consensus sequence is illustrated in Fig. 1. Four fragments, F1S, F1A, F2S, F2A ranging in size from 49 to 52 bases were chemically synthesized by automated DNA synthesizer. The DNA was designed to be excised out by *Eco*RI from the vector. After phosphorylation, 60 ng of each F1S and F2S were annealed with equal amount of F1A and F2A, respectively, to form pairs with complementary regions and subsequently each hybridized fragment was extended with Klenow fragment of *E. coli* DNA polymerase I. The two synthesized, blunt ended fragments were mixed and ligated at *Sma*I site of pUC118 (purchased from Takara Co., Kyoto, Japan), followed by transformation of *E. coli* MV1304. Transformants were screened by colony hybridization using a 20-bp oligomer which hybridizes the junction region between F1A and F2S. Five positive clones were obtained. *Eco*RI digestion of these plasmid DNAs generated 171-bp fragment and the sequence analysis of selected two clones named pAP-1 indicated the expected sequence. With the pAP-1 monomer clone, we subsequently constructed clones containing dimeric and tetrameric consensus units by joining *Eco*RI and *Kpn*I fragments for dimer designated as pAP-2, and inserting the *Eco*RI digested pAP-2 into *Sph*I site of pAP-2 for tetramer designated as pAP-4.

For Southern blotting, high molecular weight DNA was isolated from human placenta. The DNA was digested with restriction enzymes, separated by agarose gel electrophoresis, and transferred to nitrocellulose filter. The filter was incubated 3 hr at 42°C in hybridization solution (50% formamide, 5×SSC (0.15 M NaCl, 0.015

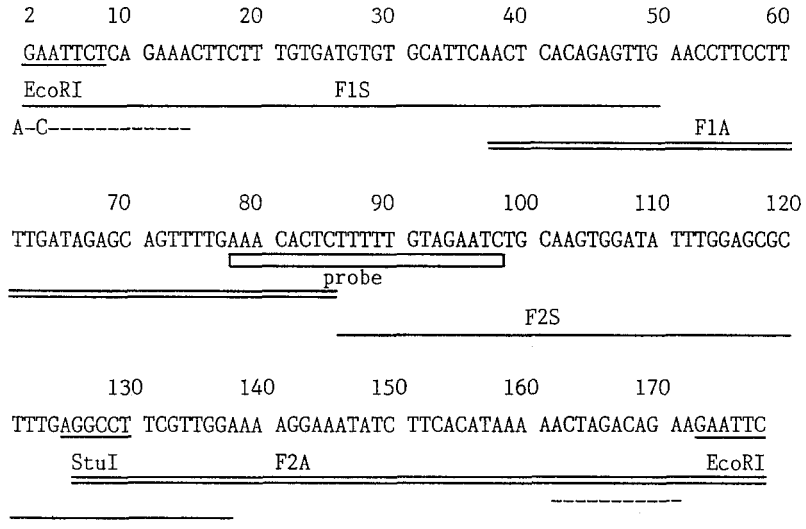

Fig. 1. DNA sequence and synthetic oligonucleotides of the human alpha satellite monomer unit. The monomer unit was assembled from 4 chemically synthesized oligonucleotides which are indicated under the sequence; — 'sense', = 'antisense' strand. The sequence was numbered according to Waye and Willard (1987). These oligonucleotides formed pairs consisting of a 'sense' and 'antisense' strands. Enzymatic extention of fragment pairs by Klenow fragment was used to produce blunt-ended duplexs. Synthetic DNA was designed so as to be excised out by *Eco*RI for our use later on. The sequence of probe used for screening of the proper ligation was indicated by underline. The base at position 1 was added and the base at position 3 was corrected by site-directed mutagenesis using 5′ACTAGACAGAAAGCATTCTCAGAAA3′ (that is indicated as broken line) when the construction of dimeric and tetrameric alpha consensus sequences.

M sodium citrate), 0.1% SDS (sodium dodecyl sulfate), 10 × Denhardt's solution and 100 μg denatured salmon sperm DNA per ml), and then hybridized for 16 hr at 42°C with hybridization solution containing 8×10^6 dpm per ml of radioactive *Eco*RI fragment from pAP-4 (684 bp) under the above condition. After hybridization, the filter was washed at 60°C with three 30 min-washes of 0.1 × SSC–0.1% SDS solution. Autoradiography was then performed at room temperature for 3 hr.

In situ hybridization was carried out essentially according to Pinkel *et al.* (1986). Metaphase cells were obtained from EB virus-transformed human lymphocytes. After colcemid arrest in metaphase, the cells were incubated in 75 mM KCl for 10 min at 37°C and fixed 3 times in 3:1 methanol: acetic acid. The cells were spread on microscope slide with dropping. The plasmid pAP-4 DNA was digested with *Hae*III and labeled with Photoprobe™ biotin (Vector Laboratories Inc., USA) according to the manufacturer's directions. The metaphase chromosomes were denatured in 70% formamide, 2 × SSC at 70°C for 2 min followed by ethanol series of increasing concentrations (50, 75, 95, and 100%) at 4°C. The hybridization mix-

ture contained 100 ng/ml biotinylated alphoid monomer, 200 μg/ml sonicated salmon sperm DNA, 10% dextran sulfate, and 2×SSCP (SSC+15 mM Na phosphate) in 30% (or 50%) formamide at pH 7. After overnight hybridization at 37°C, the slides were washed 3 times for 3 min each in 30% (or 50%) formamide, 2×SSC at 42°C, followed by 3 washes for 3 min each in 2×SSC at 42°C. The slides were then incubated with anti-biotin rabbit IgG, followed by fluorescinated anti-rabbit IgG goat serum (Cappel, Pa. USA). Finally, the metaphase chromosomes were counterstained with propidium iodide (PI, 0.8 μg/ml) and diamidinophenylindol (DAPI, 0.4 μg/ml) to allow simultaneous observation of chromosomes and hybridized probe. The slides were mounted with antifade solution and screened with Nikon fluorescence microscope. The lymphoblastoid cell line No. 2444 is a kind gift from Dr. Nakagome (National Children's Medical Research Center, Japan).

RESULTS AND DISCUSSION

The consensus sequences of the alpha satellite monomer units were deduced from the sequence data on 153 and 145 cloned monomers (Waye and Willard, 1987; Vissel and Choo, 1987). Since the two reported sequences have differences only in a few bases, we have synthesized DNA fragments based on the report by Waye and Willard (1987) and constructed the alpha satellite monomer unit, subsequently cloned into a pUC vector (Fig. 1). After construction of the monomer unit, plasmids carrying dimeric and tetrameric monomer units were also constructed. The plasmid, pAP-4, containing tetrameric units in tandem was quite stable during the bacterial cell growth and storage in the cell at −80°C.

It was reported that the most abundant alphoid sequences form bands of 340 and 680 bp after *Eco*RI digestion and higher-order repeat units were seen when digested with other restriction enzymes (Willard and Waye, 1987; Alexandrov *et al.*, 1988). So we examined total human DNA by Southern analysis with the synthetic consensus sequence as a probe, Human genomic DNA was digested with *Eco*RI or *Bam*HI, and subjected to Southern blot analysis using ^{32}P-labeled 684-bp *Eco*RI fragment of pAP-4. The prominent bands were seen at around 340, 680 bp with a less intense bands of 540, 870, 1,050, and 2,200 bp in the *Eco*RI digested DNA under a stringent condition. While prominent bands were seen at around 2,200, 2,600, and 2,800 bp in the *Bam*HI digested DNA (data not shown). The prominent, distinct bands at around 340 and 680 bp may be ascribed to repeated units of alphoid monomer in various chromosome types. Similarly the prominent bands at around 2.0–2.8 kb may reflect higher-order repeat units dominant in certain chromosome types.

Next, we examined if the chemically synthesized alpha consensus monomer would recognize the alpha satellite DNA at the centromeric region of each chromosome. The DNA of 171 bp consensus monomer was excised from pAP-4 and biotinylated. The DNA was hybridized to metaphase chromosomes as described

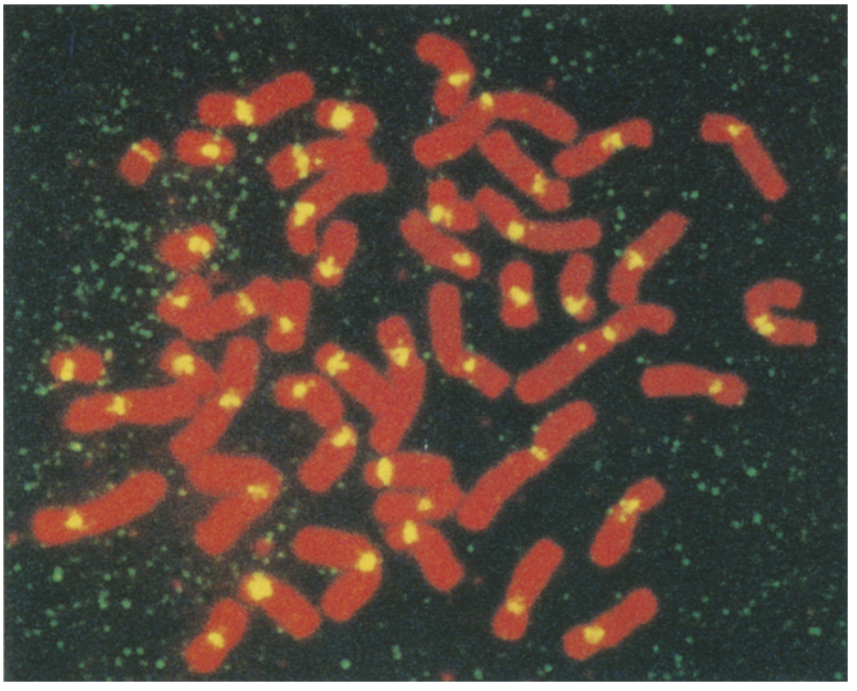

Fig. 2. Fluorescence *in situ* hybridization with synthetic alpha monomer as a probe to human lymphoblast metaphase spreads. The biotin-labeled probe DNA was hybridized to chromosomal DNA on metaphase spreads as described in MATERIALS AND METHODS. The hybridized probe was detected with FITC-antibodies. FITC (yellow-green) and PI (orange) fluorescences were observed with excitation at 480 nm.

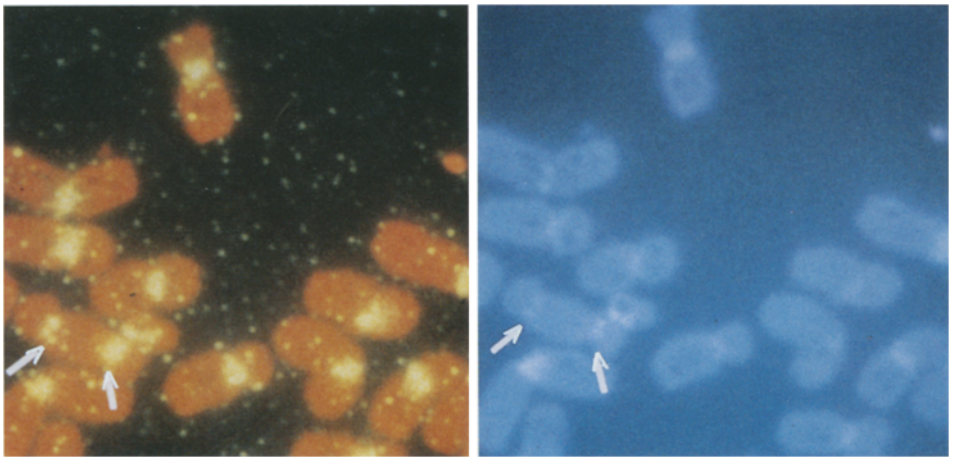

Fig. 3. Fluorescence *in situ* hybridization with synthetic alpha monomer to human lymphoblast cell line 2444. Left, FITC (yellow-green) and PI (orange) fluorescence; Right, DAPI (blue) fluorescence. Arrows indicate centromeric regions on X chromosome.

in MATERIALS AND METHODS. Under a higher stringency condition (50% formamide at 37°C), intensity of fluorescence varied with chromosomes, and the centromeric region of Y could not be labeled (data not shown). To ensure that centromeric regions of all chromosome types are stained, we reduced the hybridization stringency; 30% formamide at 37°C. Figure 2 shows that all centromeric regions of human chromosomes in a typical metaphase spread were labeled with FITC.

So far, alpha satellite repeat units specific to each human chromosomes type have isolated except for chromosome 2, and 15 (Kidd *et al.*, 1989). Our result suggests that all the centromeric regions of human chromosomes are dominated with a variety of human alphoid sequences, which are recognized by the consensus sequence derived from 153 and 145 monomer units. There has been reported one such ubiquitous, cloned sequence called p82H detecting all centromeric regions of human chromosomes under a lower hybridization condition. Recently, however, the sequence was shown to be derived from a subfamily of alpha satellite DNA on chromosome 14 (Waye *et al.*, 1988).

In most cases with a constitutional dicentric chromosome, the both centromere regions are stained with C-banding, although only active centromere is stained with Cd-banding (Nakagome *et al.*, 1986). Nakagome *et al.* reported that in a cell; 46,X, psu dic(X)(q27), case 2444, the inactive centromere of dicentric X chromosome was not stained even with C-banding. Thus we tried to detect the dicentromeric regions of the X in metaphase cell of 2444 using the synthetic alphoid monomer probe. As shown in Fig. 3, the synthetic monomer could detect both centromeric regions of the X chromosome.

The dicentric chromosome is an important parameter in the evaluation of dose, dose-rate or other characteristics of ionizing radiation. The present technique should be useful in the distinction of a dicentric chromosome from that with a twisted arm. Further the probe could be used for detection of other centromere involved chromosome abnormality or heterogeneity (Dale *et al.*, 1989).

In preparation of this paper, we found a similar approach has been tried by Meyne *et al.* (1989) using a synthetic fragment of consensus monomer unit. However, we experienced that the 171 bp monomer probe gave us a more intense signals compared with ~35 bp fragment probes.

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