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 EcoRI or BamHI. 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
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 metaphase 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 EcoRI 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 SmaI 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. EcoRI 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 EcoRI and KpnI fragments for dimer designated as pAP-2, and inserting the EcoRI digested pAP-2 into SphI 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