Isolation and characterization of an alphoid DNA sequence recently amplified on human chromosome 3

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With the aim of isolating probes homologous to chromosome specific repetitive sequences we have cloned a charon 21A chromosome specific library of chromosome 18 in the small plasmid vector pTZ18R. Clones containing highly repetitive human DNA were identified. Pools of inserts of one hundred clones (potentially containing single or low copy number human sequences) were used as probes on Southern blots made with a panel of human hamster somatic hybrids. For one of the pools, over a background smear, discrete bands were observed which seemed chromosome specific. Using a four steps recurrent procedure we identified clone VIIB4 as being responsible for the discrete bands.

The VIIB4 insert was isolated and used for in situ hybridization on human metaphasic chromosomes. Under stringent conditions, 80% of the silver grains were located on chromosome 3 centromeric regions. Secondary hybridization could be analysed with less stringent conditions or by using a biotinylated probe and revealed that centromeric regions of chromosome 10 and 12 were the second highest hybridization sites. We believe that the VIIB4 insert derives from a fragment of chromosome 3 which contaminated the chromosome 18 preparation used to raise the library. The 635 bp insert of VIIB4 was sequenced (submitted to GenBank/EMBL databanks). It is composed of 3.7 alphoid 170-171 bp monomers with a highly conserved 24 bp sequence identical to the conserved sequence of human X chromosome specific alpha DNA (bases 107-130 fig. 4 in ref). Southern blots with human DNA digests revealed a higher order of organizational level in the chromosome 3 centromere with a large 2750 bp repeat unit consisting of 16 alphoid monomers. The presence of sequences related to VIIB4 was searched for in primates. Under moderate stringent conditions, representatives of the pongidae group were the only animals to exhibit an hybridization signal. Under the high stringency used to reveal the chromosome 3 specific hybridization the signal disappeared in all species except man.

The availability of the VIIB4 clone should contribute to the analysis of the structure and relationship of chromosome centromeres. It also enables through a simple non radioactive in situ hybridization the identification of chromosome 3 centromere on marker chromosomes in the highly rearranged karyotype of solid tumors.

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Reference : Waye, J.S. and Willard H.F. (1986) Molec. and Cell Biol. 6, 3156-3165.