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The CA Repeat Marker D17S791 Is Located within 40 kb of the WNT3 Gene on Chromosome 17q

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Recent developments in genetic linkage mapping of the human genome have generated a large number of short tandem repeat polymorphic markers (13). Eventual integration of these markers into a physical map is a natural and a necessary step. A large number of genomic clones have been generated for the early-onset breast and ovarian cancer (BRCA1) region at 17q21 (1–3, 5, 7, 9, 10) and are being used to construct a physical map of this region. An attempt was made to link CA repeat markers from this chromosomal region to yeast artificial chromosome (YAC) and cosmid clones. We report here the localization of the CA repeat marker D17S791 (155xd12) to within 40 kb of WNT3, a human homolog of the gene activated by proviral insertion in mouse mammary tumors (12).

Three YAC clones were isolated by screening the total human genomic YAC library constructed at the Center for Genetics in Medicine at Washington University, St. Louis, MO (4), with a set of PCR primers generated from the mouse wnt3 cDNA sequence (12). The primers (5′-CCA TCC TGG ACC ACA TGC AC-3′ and 5′-GGT GTG CAC ATC GTA GAT GC-3′), chosen from the fourth exon of wnt3, amplify the same-sized (470 bp) PCR product from total human and total mouse genomic DNA templates, allowing for the screening of human WNT3 by PCR-based methods (8, 11). Each of the three YAC clones, A236C12, B19E12, and B82F6, contained single YAC inserts of 235, 140, and 125 kb, respectively. The methods for the characterization of the YAC clones were as described earlier (6). DNA from the three YAC clones was tested by PCR with the primers for the marker D17S791. As shown in Fig. 1 (lanes **1–3**), the clones A236C12 and B19E12, but not B82F6,

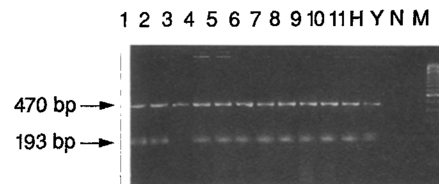

FIG. 1. PCR with DNA from YAC or cosmid clones as templates and primers for WNT3 and D17S791. DNA (2 ng/μl) from YAC clones or cells from cosmid clones were subjected to PCR reaction, separately, using primers for WNT3 and D17S791. The sequences of the PCR primers for D17S791 were 5′-GTTTTCTCC AGTTATTCCCC-3′ and 5′-GCTCGTCCTTTGGAAGAGTT-3′ (13). The primers for WNT3 are given in the text. The PCR reactions were carried out for 35 cycles, with each cycle consisting of 94°C for 1 min, 62°C for 1 min, and 72°C for 1 min for WNT3 primers, whereas the annealing temperature was 58°C for 1 min for the D17S791 primers. Ten microliters from both PCR reactions for each DNA was loaded on the same lane, since the WNT3 PCR product (470 bp) resolves very well with that of D17S791 (193 bp). Lanes **1** to **3** represent YAC clones A236C12, B19E12, and B82F6; lanes **4** to **11** represent the cosmid clones 12F2, 69C8, 75A9, 89A12, 106D12, 142E3, 156A2, and 158H2; and lanes **H**, **Y**, and **N** were control PCR reactions with human DNA, yeast DNA, and no DNA, respectively. Lane **M** contains the 100-bp ladder (BRL/GIBCO) as size markers. None of three unrelated cosmids tested showed a positive PCR signal with either set of primers (data not shown).

generated a PCR product (193 bp) of the expected size. Since the smaller YAC that shared both the sequence-tagged sites (STSs) was only 140 kb, D17S791 and WNT3 must be no more than 140 kb apart.

An arrayed chromosome 17 cosmid library, prepared and supplied by Larry Deaven of the Los Alamos National Laboratory, was screened for clones containing WNT3. The 470-bp WNT3 PCR product, amplified from human DNA, was labeled and used as probe. Eight cosmids (12F2, 69C8, 75A9, 89A12, 106D12, 142E3, 156A2, and 158H2) were identified by screening five genomic equivalents of the library. Individual colonies from these clones were tested by PCR for WNT3 as well as for the marker D17S791, and all eight were positive for both (lanes **4–11**, Fig. 1). The colocalization of the marker D17S791 to the same cosmids containing an STS for WNT3 shows that the marker is no farther than 40 kb, the average size of an insert in a cosmid, from WNT3. Given that eight of eight cosmids are positive for both, the two markers are likely to be much closer than 40 kb.

The presence of both the marker D17S791 and WNT3 on two YAC and eight cosmid clones is in general agreement with two other studies: genetic linkage analysis places, among other markers, D17S579 (Mfd188) and HOX2B as the two flanking markers for D17S791 (2), and physical mapping by fluorescence *in situ* hybridization mapped WNT3 between the same two flanking markers (7). Both WNT3 and D17S791 have been excluded from the BRCA1 locus (2–3, 5). However, the marker D17S791, with 11 alleles, can serve as a tightly linked marker for the WNT3 locus, which has no known polymorphism associated with it.

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An Oligonucleotide Probe Specific to the Centromeric Region of Human Chromosome 5

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We have designed a 28-mer alpha satellite oligonucleotide probe that, at high stringency, hybridizes specifically to the centromeric region of human chromosome 5. Using fluorescence *in situ* hybridization (FISH) we also find that, under low-stringency conditions, this oligomer hybridizes to chromosomes 1, 5, and 16. The hybridization to chromosome 16 was somewhat unexpected since clonally derived probes from this alphoid DNA subset hybridize to chromosomes 1, 5, and 19 at high stringency. We also compare and contrast the sequences of previously cloned alphoid DNAs from chromosomes 5, 16, and 19 in the region corresponding to the oligomer. This oligonucleotide should facilitate the genomic and cytogenetic analysis of human chromosome 5.

Alphoid DNA is a family of tandemly repeated 171-bp elements located at the centromeric regions of primate chromosomes. In the human genome, this family is composed of three suprachromosomal subfamilies: subfamily I, composed of chromosomes 1, 3, 5–7, 10, 12, 16, and 19; subfamily II, consisting of chromosomes 2, 4, 8, 9, 13–15, 18, and 20–22; and subfamily III, which includes chromosomes 1, 11, 17, and X (1, 10). In addition, the alphoid DNA on each chromosome is organized in numerous tandemly repeated higher-order structures, composed of *n* diverged monomers, the subject of which has been extensively reviewed (3, 9, 11).

Baldini *et al.* (1989) showed that a 17-kb cosmid insert (pC1.8) containing chromosome 5-derived alphoid DNA hybridized (in order of decreasing intensity) to chromosomes 5, 1, and 19. No conditions that eliminated hybridization to any of these three chromosomes were found. A complex higher-order repeat unit consisting of at least six 340-bp dimers was also demonstrated for this alphoid DNA subset (2). We decided to utilize the partial sequence of this clone to construct an oligonucleotide specific to human chromosome 5. This oligomer, termed α-CHR-05 (5′-GGT*TTT*TTT*CCT*GT*AAGGCT*AGACAGAA-3′), spans positions 141–170 of the alpha satellite DNA consensus (3). The

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