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Multicolor FISH Mapping with *Alu*-PCR-Amplified YAC Clone DNA Determines the Order of Markers in the *BRCA1* Region on Chromosome 17q12–q21

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A gene designated *BRCA1*, implicated in the susceptibility to early-onset familial breast cancer, has recently been localized to chromosome 17q12–q21. To date, the order of DNA markers mapped within this region has been based on genetic linkage analysis. We report the use of multicolor fluorescence *in situ* hybridization to establish a physically based map of five polymorphic DNA markers and 10 cloned genes spanning this region. Three cosmid clones and *Alu*-PCR-generated products derived from 12 yeast artificial chromosome clones representing each of these markers were used in two-color mapping experiments to determine an initial proximity of markers relative to each other on metaphase chromosomes. Interphase mapping was then employed to determine the order and orientation of closely spaced loci by direct visualization of fluorescent signals following hybridization of three probes, each detected in a different color. Statistical analysis of the combined data suggests that the order of markers in the *BRCA1* region is cen–THRA1–TOP2–GAS–OF2–17HSD–248yg9–RNU2–OF3–PPY/p131–EPB3–Mfd188–WNT3–HOX2–GP3A–tel. This map is consistent with that determined by radiation-reduced hybrid mapping and will facilitate positional cloning strategies in efforts to isolate and characterize the *BRCA1* gene.

INTRODUCTION

Breast cancer is a leading cause of cancer death among women, afflicting over 150,000 individuals per year in the United States alone (Silverberg and Boring, 1990). Although this disease occurs most often in middle-aged and elderly women, the onset of breast cancer before age 40 often occurs in a familial pattern suggesting an underlying inherited susceptibility (Claus *et al.*, 1990). Genetic linkage analysis has demonstrated that a gene (*BRCA1*) associated with early-onset familial breast cancer is linked to the CMM86 (D17S74) locus on chromosome 17q12–q21 (Hall *et al.*, 1990; Narod *et al.*, 1991). Linkage studies using additional polymorphic markers then defined an interval of approximately 6–8 cM flanked by markers Mfd15 (D17S250) on the proximal side and GIP on the distal side, which is likely to include the *BRCA1* gene (Hall *et al.*, 1992; Fain, 1992). Recently, this interval has been further narrowed to the region between THRA1 and SCG43 (D17S183) (Bowcock *et al.*, 1993). Additional studies place the gene above Mfd188 (D17S579) (Chamberlain *et al.*, 1993).

Efforts to construct a high-resolution physical map of the *BRCA1* region utilize yeast artificial chromosome (YAC) and cosmid clones corresponding to genetic markers that map to 17q12–q21. These resources are being used in positional cloning strategies to link markers by hybridization to overlapping YAC clones, conventional YAC and cosmid walking, and pulsed-field gel electrophoresis (PFGE). However, these methods do not provide initial localization of DNA sequences to specific chromosomal regions or, until contigs are obtained, the ability to order DNA sequences more than a few hundred kilobases apart. Recently described somatic cell and cytogenetic mapping techniques using radiation-reduced hybrids (RRH) (Cox *et al.*, 1990) and fluorescence *in situ* hybridization (FISH) can complement the results of genetic and physical mapping analyses by providing rapid localization and physical ordering of many closely spaced loci.

Previous studies have shown that cosmids can be mapped by FISH with a resolution of ≥3 Mb to metaphase chromosome bands (Lawrence *et al.*, 1990; Lichter *et al.*, 1990; Trask *et al.*, 1991). Lichter *et al.* (1990) have demonstrated the feasibility of this approach, generat-

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ing a high-resolution physically based map of human chromosome 11. Further studies have shown that two sequences separated by >1 Mb can be ordered relative to each other when labeled with different fluorochromes and hybridized to metaphase chromosomes (Trask *et al.*, 1991). Multicolor FISH to early-metaphase chromosome preparations has recently been used to construct a fine ordered map of the Charcot–Marie–Tooth type 1A (CMT1A) gene region (Lebo *et al.*, 1992).

When applied to interphase nuclei, FISH can be used to determine the relative order of sequences separated by 50–1000 kb (Trask *et al.*, 1989, 1991; Lawrence *et al.*, 1990). Interphase mapping has been used to derive probe order by measuring the physical distance between pairs of cosmid probes hybridized simultaneously to interphase nuclei. Trask *et al.* (1989, 1991) have shown that the order derived from interphase distances of cosmids surrounding 250 kb of the CHO DHFR region, and of cosmids within 810 kb in human Xq28, was consistent with the genomic distance observed from restriction mapping results. Alternatively, interphase mapping can be achieved more easily by scoring the order of fluorescent signals after detection of three or more probes with two fluorochromes (two-color mapping) (Trask *et al.*, 1991). Although this scheme can provide information regarding the order of one probe hybridization site labeled in one color (green) relative to sites of two other probes labeled in a different color (red), additional hybridization experiments are required to determine the order of the two similarly labeled probes. Marker order can be determined more directly and rapidly by labeling each probe in a different color (three-probe, three-color mapping).

Although previously described studies have utilized cosmid clones for mapping, the availability of YAC clones provides a means to construct long-range physical maps at a rate much faster than can be achieved with other cloning vectors. The utility of this approach has been limited by poor recovery of YAC DNA from agarose gels and by low copy number of the human DNA insert, often resulting in decreased hybridization efficiency and increased background. Recently described techniques of *Alu*-PCR have eliminated the need for time consuming gel purifications and have allowed amplification of human DNA sequences from YAC clones with complexities sufficient for FISH experiments (Lengauer *et al.*, 1992; Breen *et al.*, 1992). Here, we report the use of *Alu*-PCR-generated products from YAC clones as probes in multicolor FISH experiments to generate a high-resolution ordered map of the *BRCA1* region on chromosome 17q12–q21. The preliminary order and orientation of markers in this region relative to the centromere was established by two-color hybridization to metaphase chromosomes. In cases in which order could not be determined by metaphase analysis, order was derived using a three-probe, three-color mapping scheme to interphase nuclei. These results provide a set of well-ordered cytogenetic landmarks, which will facilitate high-resolution mapping and positional cloning of the *BRCA1* gene.

TABLE 1

YAC Clones Used in FISH Analysis of the BRCA1 Region

Probe name	Probe symbol	Library	YAC clone
EPB3	EPB3	Wash U	A206C3
GAS	GAS	Wash U	B167B1
GP3A	GP3A	Wash U	A144B1
HOX2	HOX2	Wash U	B160B1
Mfd188[a]	D17S579	CEPH	259D2
PPY[b]	PPY	CEPH	26D6
p131[b]	D17S78	Wash U	B260E11
RNU2	U2RNA	Wash U	A167E6
TOP2	TOP2	Wash U	B51F1
THRA1	THRA1	Wash U	A138A7
WNT3[b]	WNT3	Wash U	A236C12
17HSD	EDH17B3	CEPH	26F3

[a] Detection of chimerism with whole yeast DNA.
[b] Detection of chimerism using *Alu*-PCR products.

MATERIALS AND METHODS

Cell lines and cell preparation. Mitotic chromosome preparations were obtained from PHA-stimulated peripheral blood lymphocytes from a healthy normal female by conventional cytogenetic procedures (Verma and Babu, 1989). Cells were synchronized by an FUdR (10^{-5} *M*) block for 17 h and a thymidine (10^{-7} *M*) release for 4 h, followed by exposure to colcemid (0.07 μg/ml) for 20 min. Metaphase spreads were generated by treatment in hypotonic solution (0.075 *M* KCl) and multiple exposures to fixative (3:1 methanol: acetic acid). A human fibroblast cell line (TC-4728) established from a male with fragile X syndrome was processed to obtain G_1 interphase nuclei (Trask *et al.*, 1991). Cells were held at confluency for 4 days, collected by trypsinization and then swollen and fixed as described above.

Probes. DNA from three cosmid clones and *Alu*-PCR generated products from 12 YAC clones corresponding to genetic markers previously localized to 17q12–q21 were used in this study. Cosmids OF2, OF3, and 248yg9 (Anderson *et al.*, accompanying paper) were isolated from a chromosome 17 cosmid library prepared at the Los Alamos National Laboratory. The YAC clones listed in Table 1 were isolated from total human genomic YAC libraries obtained from the Center for Genetics in Medicine, Washington University at St. Louis (Burke *et al.*, 1987; Brownstein *et al.*, 1989) and the Human Polymorphism Study Center (Centre d'Etude du Polymorphisme Humain (CEPH)) in Paris, France (Albertson *et al.*, 1990). Clones were identified using PCR-based methods (Green and Olson, 1990; Kwiatkowski *et al.*, 1990). Detailed procedures for clone identification, isolation, and characterization are described elsewhere (Chandrasekharappa *et al.*, in preparation). The YAC clone carrying marker PPY was isolated in the laboratory of Kenneth Kidd (Yale University, New Haven, CT).

Preferential amplification of insert DNA from purified genomic yeast clones was achieved using *Alu* primers CL1 and CL2 (Lengauer *et al.*, 1992). PCR was carried out in a total volume of 100 μl with 200 ng total yeast DNA, primers at 0.25 μ*M* in 50 m*M* KCl/10 m*M* Tris–HCl (pH 8.3)/2.5 m*M* $MgCl_2$/0.01% gelatin, 200 μ*M* each dNTP and 1 unit *Taq* DNA polymerase. After initial denaturation at 96°C for 4 min, amplification proceeded for 30 cycles of 96°C denaturation (1 min), 37°C annealing (30 s) and 72°C extension (6 min) in an automated thermal cycler (Perkin–Elmer/Cetus). Ten microliters of amplified DNA was electrophoresed through 0.8% agarose gels in 1× TAE. All probes were ethanol precipitated, resuspended in sterile distilled water and labeled by nick translation with either biotin–14-

dATP or digoxigenin–11-dUTP (BM) using a commercially available kit (BRL). Labeled DNA was separated from unincorporated nucleotides using G-50 sephadex spin columns. Hybridizations were carried out with 250 ng of each probe DNA precipitated with 40 μg Cot_1 fraction human placental DNA (BRL) and resuspended in 10 μl of a hybridization mixture containing 50% formamide, 2× SSC, and 10% dextran sulfate. Following denaturation at 70°C for 5 min, all probes were preannealed for 5 h at 37°C before being applied to denatured slides.

Hybridization and detection. Prior to hybridization, slides were digested with RNase and proteinase K, fixed in 4% paraformaldehyde and denatured in 70% formamide, followed by dehydration in an ethanol series, according to the method of Pinkel *et al.* (1986). Labeled denatured probes were applied to slides and hybridization proceeded overnight at 37°C followed by three 5-min washes in 50% formamide/2× SSC at 42°C, three 5-min washes in 0.1× SSC at 60°C, and one 5-min wash in 4× SCC at room temperature. After posthybridization washes, slides were processed via modification of previously described techniques (Lichter *et al.*, 1990; Trask *et al.*, 1991) as follows: (1) one 30-min preblock in 4× SSC/3% BSA at 37°C, (2) one 30-min incubation in 5 μg/ml avidin–Texas red in 4× SSC/1% BSA/0.1% Tween 20 at 37°C, (3) three 5-min washes in 4× SSC/0.1% Tween at 42°C, and (4) one 3-min wash in PN Buffer (0.1 *M* NaH_2PO_4 and 0.1 *M* Na_2HPO_4) containing 0.1% NP-40. In the remaining steps, detection incubations were at room temperature for 45 min with each reagent diluted in PNM (PN + 5% nonfat dry milk) as follows: (5) 15 μg sheep anti-digoxigenin Fab fragments (BM) and 10 μg biotinylated goat anti-avidin/ml (6) 1:50 dilution of FITC-conjugated rabbit anti-sheep IgG and 5 μg avidin–Texas red/ml, and (7) 1:50 dilution of FITC-conjugated goat anti-rabbit IgG. Slides were washed between incubations three times for 7 min each in PN + 0.1% NP-40, followed by a 5-min preblock in PNM, all performed at room temperature. After a final wash, an antifade solution containing 1 μg DAPI/ml was applied to each slide prior to viewing. Slides were viewed with a Zeiss Axioskop epifluorescence microscope coupled to a cooled CCD camera (Photometrics). Camera control and digital image acquisition were as described by Ried *et al.* (1992) using an Apple Macintosh Quadra computer. A triple-band pass filter (Omega Optical) was used to view FITC, Texas-red, and DAPI fluorescence simultaneously, whereas individual fluorochromes were selectively imaged and captured as gray-scale images with filter sets obtained from Zeiss. Gray scale images were pseudocolored and simultaneously merged using the software package Gene Join (Yale University). Photographs were taken directly from the computer monitor using Kodak 100 Ektachrome color print film.

Metaphase and interphase analysis. To ensure that *Alu*-PCR products derived from YAC clones would provide sufficient hybridization signals for mapping, and to test for chimerism, DNA products from each clone were individually hybridized *in situ* to metaphase chromosomes. For chimerism analysis, a minimum of 15 cells were analyzed. Positive hybridization was determined by the presence of fluorescent signals on both chromatids. These results were compared with those obtained using whole yeast DNA as FISH probes.

For mapping experiments amplified products from two YAC clones, one labeled with biotin and the other with digoxigenin, were hybridized simultaneously to early metaphase preparations. The position of one probe relative to the other along the length of individual chromatids was scored as proximal, distal, or in some cases even with each other. To determine probe orientation, red and green hybridization sites were scored relative to the centromere of chromosome 17. In experiments in which marker order could not be determined (i.e., signals were on top of each other or side by side), three closely spaced probes were hybridized simultaneously to interphase nuclei and detected in three different colors (three-color, three-probe mapping). One probe was labeled with biotin, the second with digoxigenin and the third probe was a mixture of biotinylated and digoxigenin-labeled DNA which produced a yellow-orange signal. Probe order in interphase nuclei was scored by direct visualization of the three-color array. The orientation of markers was determined by comparing the order of three-probe sets which included overlapping markers.

TABLE 2

Two-Color Metaphase Mapping

Position of	Relative to	Number of chromatids: Distal	Proximal	Even	Probability of correct order
TOP2	RNU2	2	22	4	0.999990
p131		18	2	8	0.999889
GAS	PPY	0	34	4	1.000000
WNT3		14	0	2	0.999969
p131	EPB3	4	6	4	0.725586
17HSD		6	20	28	0.997038
17HSD[a]	p131	1	20	5	0.999995
17HSD	Mfd188	0	20	0	1.000000
PPY		0	32	4	1.000000
EPB3		2	18	12	0.999889
WNT3		20	2	6	0.999967
GP3A		36	3	24	1.000000

[a] This hybridization was performed using a 17HSD plasmid clone obtained from Fernand Labrie (Laval Medical Center, Quebec, Canada) and a p131 cosmid clone obtained from the American Type Culture Collection (Rockville, MD).

Statistical analysis. For both two-color metaphase and three-color interphase mapping data, we calculated the Bayesian posterior probability that the most frequently observed locus order is the true locus order using a newly described analysis (S.-W. Guo, M. Boehnke, and W. L. Flejter, in preparation). In brief, this analysis is as follows. Let $k = 2$ (metaphase) or 3 (interphase) be the number of possible locus orders. Assume that for each scorable cell, the correct order is inferred with probability $1 - \epsilon$, whereas each incorrect order is inferred with probability $\epsilon/(k-1)$. Assume further that ϵ is uniformly distributed on the interval 0, corresponding to perfect scoring, to $(k-1)/k$, corresponding to random scoring. Under these assumptions, the posterior probability of correct ordering $P(\mathrm{CO})$ for a metaphase experiment given the data is

$$P(\mathrm{CO}) = \int_0^{1/2} \epsilon^{n-n^*}(1-\epsilon)^{n^*}d\epsilon \Big/ \int_0^1 \epsilon^{n-n^*}(1-\epsilon)^{n^*}d\epsilon$$

where n^* is the number of observations for the most frequently observed position (proximal or distal) and n is the total number of observations in which a proximal or distal position could be inferred.

For the interphase data, under the same assumptions, the posterior probability of correct ordering given the data is

$$P(\mathrm{CO}) = \int_0^{2/3} (\epsilon/2)^{n-n^*}(1-\epsilon)^{n^*}d\epsilon \Big/ \sum_{i=1}^{3} \int_0^{2/3} (\epsilon/2)^{n-n_i}(1-\epsilon)^{n_i}d\epsilon$$

where n_1, n_2, and n_3 are the numbers of observations for each of the three possible locus orders, n^* is the maximum of n_1, n_2, and n_3, and $n = n_1 + n_2 + n_3$.

RESULTS

Metaphase Ordering

Two-color FISH mapping to metaphase chromosomes was used to cytogenetically order a number of markers within the *BRCA1* region (Table 2). Pairwise combina-

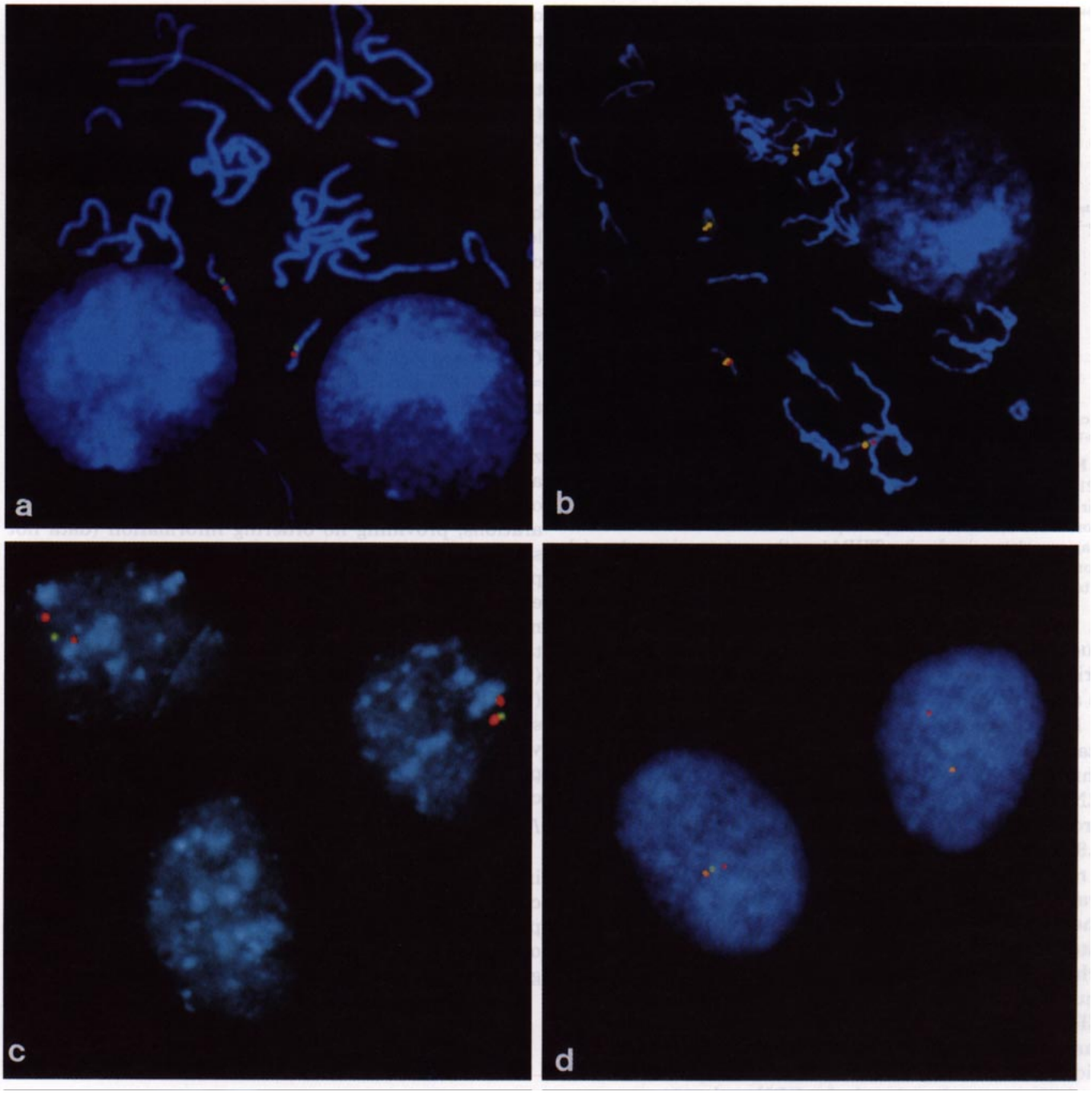

FIG. 1. Multicolor FISH with *Alu*-PCR-generated probes derived from YAC clones. Probes labeled with digoxigenin were detected with FITC-conjugated antibody (green), and biotin-labeled DNA probes were detected with avidin–Texas red (red). For interphase ordering, the third probe was labeled with both digoxigenin and biotin (orange). Metaphase chromosomes and interphase nuclei were counterstained with DAPI. (**a**) The hybridization site of TOP2 (green) was proximal to that of RNU2 (red) in 91% of chromatids scored. (**b**) Cohybridization of WNT3 (red) and PPY (yellow) placed PPY proximal to WNT3. *Alu* products from the PPY YAC also hybridized to the distal long arm of a D group chromosome. (**c**) The order of probes in two interphase nuclei maps GAS (green) between TOP2 (red) and 17HSD (orange) in 86% of cells examined. (**d**) 17HSD (green) was located between GAS (red) and RNU2 (orange) in 78% of nuclei scored. In a second cell, only the hybridization sites of GAS and RNU2 were observed.

tions of probes, one labeled with Texas-red (red) and the other with fluorescein (green), were visualized simultaneously to orient each hybridization site on blue DAPI-stained chromosomes (Fig. 1). All probes produced strong fluorescent signals on chromosome 17 and *Alu*-PCR products generated from YACs for PPY, p131 (D17S78), and WNT3 each produced additional fluorescent signals on a second chromosome pair, indicating that these YAC clones are chimeric. Although *Alu*-PCR products derived from clone Mfd188 (D17S579) pro-

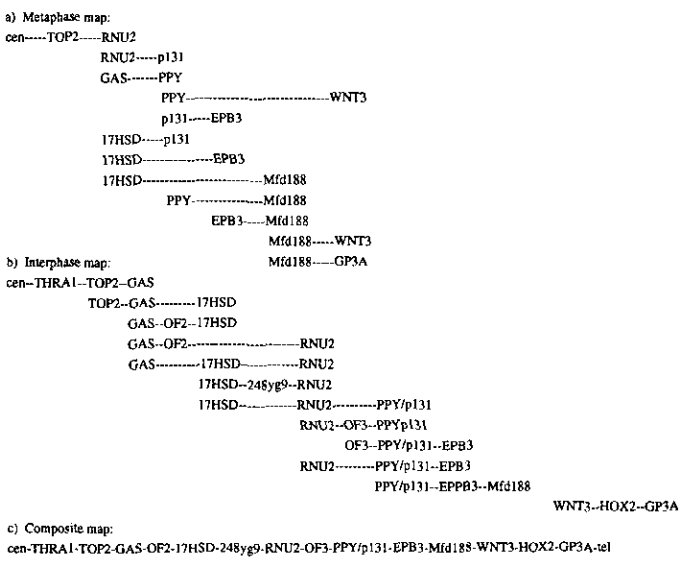

FIG. 2. FISH maps of the *BRCA1* region on chromosome 17q12–q21. (**a**) Tentative order of markers derived from two-color FISH to early-metaphase chromosome preparations. (**b**) Probe order obtained from three-color FISH to G_1 interphase nuclei. The orientation of the map was determined using THRA1 as the centromeric marker. (**c**) Composite map of the *BRCA1* region derived from all metaphase and interphase mapping data.

duced a fluorescent signal on chromosome 17 only, hybridization of whole yeast DNA indicated that this clone is also chimeric.

Initial mapping experiments revealed that the hybridization site of TOP2 was proximal to RNU2 in 22 of 24 chromatids scored (Fig. 1a), and p131 was distal to RNU2 in 18 of 20 chromatids examined, with posterior probabilities of correct ordering of 0.999990 and 0.999889, respectively (Table 2). When PPY was used as a reference clone to order four additional markers, GAS was located proximal to PPY (P(CO) = 1.000000) and the hybridization site of WNT3 was distal to the PPY site (P(CO) = 0.999969) on all of the chromatids for which order could be determined (Table 2, Fig. 1b). Simultaneous hybridization of PPY with either p131 or EPB3 resulted in fluorescent signals that were equidistant from the centromere on all chromatids scored, providing no ordering information (data not shown). The inability to resolve signals for PPY and p131 on metaphase chromosomes is consistent with STS mapping, which shows that they lie within the same 100-kb YAC clone and the same 45-kb cosmid clone (Chandrasekharappa *et al.*, in preparation). In contrast, the physical distance between PPY and EPB3 has not yet been determined. The mapping result may reflect close proximity or the effects of chromosome condensation. To test this hypothesis, EPB3 and p131 were cohybridized to extended chromosome preparations. Although the data suggest that p131 is located proximal to EPB3 (P(CO) = 0.725586), there was no clear evidence for the order of these loci (Table 2). We conclude that the relative distance between EPB3 and PPY/p131 is below the limits of resolution on metaphase chromosomes (<1 Mb). In two other experiments the hybridization site of 17HSD appeared proximal to that of EPB3 approximately three times more often than it was found distal to EPB3 (P(CO) = 0.997038) and proximal to p131 in 20 of 21 chromatids scored (P(CO) = 0.999995).

The next series of experiments was performed to determine the order of five markers relative to Mfd188 (Table 2). The hybridization sites of 17HSD and PPY were located proximal to Mfd188 on all chromatids where order could be resolved, resulting in posterior probabilities for each of these positions of 1.000000. EPB3 was also found proximal to Mfd188 in 18 of 20 chromatids scored (P(CO) = 0.999889). Alternatively, WNT3 and GP3A were located distal to Mfd188 in 20 of 22 (P(CO) = 0.999967) and in 36 of 39 (P(CO) = 1.000000) chromatids analyzed, respectively.

In each of five other pairwise comparisons, cohybridization of THRA1 and TOP2, GP3A and WNT3, 17HSD and GAS, RNU2 and GAS, RNU2 and EPB3 produced overlapping fluorescent signals on early metaphase preparations, providing no ordering information (data not shown). Again, these data presumably reflect the close proximity (<1 Mb) of markers relative to each other in each pairwise combination. The results of two-color metaphase mapping suggested that the order and orientation of markers used in these analyses is cen–TOP2–(GAS–17HSD–RNU2)–PPY/p131–EPB3–Mfd188–(WNT3–GP3A)–tel with PPY and p131 residing on the same YAC and cosmid clones (Fig. 2a). These data provide a starting point for further multicolor FISH mapping to determine the exact order and orientation of closely spaced markers.

Interphase Ordering

Interphase mapping experiments were performed using three-probe combinations of markers which were located in close proximity to one another based on the preliminary metaphase ordering information. Probe order was determined by direct visualization of the red, green, and orange fluorescent signals (Table 3 and Fig.

TABLE 3

Probe Order in G_1 Interphase Nuclei

Probe combinations (green/red/orange)	Observed color scheme			Probability of correct order
	RGO	GOR	GRO	
GAS/TOP2/THRA1	3	2	15	0.999719
GAS/TOP2/17HSD	19	2	1	0.999999
OF2/17HSD/GAS	14	2	0	0.999982
OF2/RNU2/GAS	20	6	8	0.995919
17HSD/GAS/RNU2	14	3	1	0.999758
248yg9/RNU2/17HSD	18	2	2	0.999993
PPY/RNU2/17HSD	0	2	23	1.000000
p131/RNU2/OF3	0	27	3	1.000000
OF3/PPY/EPB3	2	1	17	0.999996
PPY/EPB3/RNU2	9	3	1	0.984101
PPY/Mfd188/EPB3	7	12	6	0.829697
HOX2/WNT3/GP3A	16	3	2	0.999879

1). The chromosomal orientation of an initial three-probe set was determined using THRA1 as the centromeric marker. Simultaneous hybridization of GAS, TOP2, and THRA1 (Table 3) placed TOP2 between THRA1 and GAS in 15 of 20 nuclei that could be scored, yielding a posterior probability for that order of 0.999719. Once this initial three-probe ordered array was established, subsequent hybridizations were performed using overlapping probe combinations. In this way, most probes were used in more than one hybridization experiment, including one combination in which each probe mapped between two flanking markers (Fig. 2b). For example, in the combination of GAS, TOP2, and 17HSD, GAS was located between TOP2 and 17HSD in 19 of 22 nuclei scored (P(CO) = 0.999999) and in the next three-probe set, OF2 was positioned between GAS and 17HSD in 14 of 16 interphase cells examined (P(CO) = 0.999982). Combining these results suggests the order of cen–THRA1–TOP2–GAS–OF2–17HSD (Fig. 2b). In two subsequent experiments (Table 3) the hybridization site of RNU2 was located distal to OF2 in 20 of 34 interphase cells scored and distal to 17HSD on 14 of 18 interphase nuclei yielding posterior probabilities for these positions of 0.995919 and 0.999758, respectively. The hybridization site of 248yg9 was between those of 17HSD and RNU2 in 18 of 22 interphase cells examined (P(CO) = 0.999993), whereas PPY was positioned distal to 17HSD and RNU2 on 23 of 25 nuclei scored (P(CO) = 1.000000). Additional experiments revealed that OF3 was located between RNU2 and p131 in 27 of 30 interphase cells (P(CO) = 1.000000) and the hybridization site of PPY was between OF3 and EPB3 in 17 of 20 interphase nuclei scored (P(CO) = 0.999996). In two subsequent experiments the hybridization site of PPY was found between RNU2 and EPB3 more often than it was located on either distal end of the three-probe array (P(CO) = 0.984101). Cohybridization of probes representing PPY, Mfd188, and EPB3 suggest that the order PPY–EPB3–Mfd188 is more likely (P(CO) = 0.829697) than either EPB3–PPY–Mfd188 or PPY–Mfd188–EPB3 (Table 3).

Two-color metaphase mapping showed that WNT3 and GP3A are located distal to Mfd188 but could not be ordered relative to each other. These data suggest that the physical distance between Mfd188 and WNT3 or GP3A exceeds the maximum range for interphase mapping. As a result, we selected HOX2, previously localized distal to WNT3 (Fain, 1992), as the third marker to establish the order and orientation of WNT3 and GP3A in interphase nuclei. Interphase mapping placed HOX2 between GP3A and WNT3 in 16 of 21 nuclei examined (P(CO) = 0.999879) and suggests the order Mfd188–WNT3–HOX2–GP3A–tel. Combining these data with that obtained from all metaphase and interphase mapping results described above provides a consistent order for all 15 markers within the *BRCA1* region of cen–THRA1–TOP2–GAS–OF2–17HSD–248yg9–RNU2–OF3–PPY/p131–EPB3–Mfd188–WNT3–HOX2–GP3A–tel (Fig. 2c).

DISCUSSION

The application of FISH as a method of gene mapping has proved to be a reliable and rapid technique for defining an ordered array of adjacent DNA markers within a given chromosomal region. In this study, we have used early-metaphase chromosomes and interphase nuclei as mapping targets to determine the order and orientation of 15 markers located within the *BRCA1* region on chromosome 17q12–q21. In contrast to previous studies that utilize cosmid clones for mapping, we demonstrate the efficiency of using *Alu*-PCR-generated products derived from YAC clones as FISH probes. Prior to use in mapping experiments, *Alu*-amplified DNA from each YAC clone was hybridized to metaphase chromosomes to ensure detection of hybridization signals and to test for chimerism. In our hands, hybridization efficiency is equal to that obtained with cosmid clones and fluorescent signals often appear brighter and larger than those observed with cosmids. *Alu*-PCR-generated products from three of the 12 YAC clones tested each demonstrated a second hybridization site on metaphase chromosomes, indicating that these clones are chimeric. In addition, one clone (Mfd188), which did not reveal chimerism with *Alu* products, was found to be chimeric when total yeast DNA was used. In a related study, we found that *Alu*-amplified DNA from 6 of 15 chimeric YAC clones each produced hybridization signals only on chromosome 17 (Chandrasekharappa *et al.*, in preparation). These results suggest that the use of *Alu*-PCR DNA products from YAC clones is not always useful in detecting chimeras and is dependent on the *Alu* content of the human DNA fragment.

When used in interphase mapping experiments, noncontiguous DNA sequences in the chimeric clones occasionally produced additional fluorescent signals of similar color clustered in close proximity to those of the other two colored sites. However, in the majority of nuclei scored hybridization of chimeric sites did not interfere with analysis, consistent with the observation that interphase nuclei exhibit territorial organization of chromosomal domains (Lichter *et al.*, 1988).

In addition to using *Alu*-PCR generated DNA from YAC clones in mapping experiments, we have also demonstrated the advantage of a three-probe, three-color interphase mapping strategy. This approach allows determination of probe order by a single experiment, in contrast to two-color interphase studies, which must be carried out with at least two different color-labeling schemes to confirm order for any given set of three probes. Moreover, using a three-color scheme, marker orientation can be determined by as few as one additional experiment using overlapping probe combinations if the position of a single flanking marker is known. Although error probabilities for individual experiments

essentially add when a minimal ordering strategy is used, the results of sequential hybridizations of multiple overlapping marker pairs and triplets reinforce the data.

Multicolor FISH has provided a more complete map of the *BRCA1* region than has been achieved previously by genetic linkage analysis. Two-color metaphase analysis was used to orient the map relative to the centromere and grouped markers in tentative clusters within 17q12–q21. Interphase mapping was used to further delineate the order of markers that could not be resolved on metaphase chromosomes. Although the true physical distance between markers used in this study can be estimated only from published genetic linkage analysis data, our results were in general agreement with those obtained by Lawrence *et al.* (1990) and Trask *et al.* (1989, 1991), who found that the relative order of adjacent loci located within 1 Mb cannot be determined by metaphase analysis and must be separated by ≥50 kb in somatic interphase nuclei.

For the statistical analysis of the data, we made two assumptions. First we assumed that for interphase data, the two incorrect locus orders were equally likely to be inferred for each cell. Although this may not be true, simulation results suggest that this assumption is slightly conservative in the sense that making the assumption results in a small underestimate of the probability of correct locus ordering (S.-W. Guo, M. Boehnke, and W. L. Flejter, in preparation). Second, having no strong prior information about the possible values of the overall error probability ϵ, we assumed it is uniformly distributed over its possible range values. Limited experimental data suggest that this assumption may give too much weight to larger error probabilities, again resulting in a slight underestimate of the probability of correct locus ordering.

Given the results of our statistical analysis of the FISH mapping data presented, we conclude that the order of 15 markers in the *BRCA1* region is cen–THRA1–TOP2–GAS–OF2–17HSD–248yg9–RNU2–OF3–PPY/p131–EPB3–Mfd188–WNT3–HOX2–GP3A–tel. This order is consistent with and complements data obtained by RRH and genetic mapping of this region (Abel *et al.*; Anderson *et al.*, accompanying papers). Together, the results provide a high-density physical map of the *BRCA1* gene region which will serve to order additional markers within 17q12–q21. Marker positions identify starting points from which further positional cloning techniques can be initiated to assist in the eventual isolation and identification of the *BRCA1* gene.

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