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Characterization of Robertsonian Translocations by Using Fluorescence In Situ Hybridization

Daynna J. Wolff and Stuart Schwartz

Division of Human Genetics, Departments of Obstetrics and Gynecology, and Pediatrics, University of Maryland School of Medicine, Baltimore

Summary

Fluorescence in situ hybridization with five biotin-labeled probes (three alphoid probes, a probe specific for beta-satellite sequences in all acrocentric chromosomes, and an rDNA probe) was used to characterize 30 different Robertsonian translocations, including three t(13;13); one t(15;15), four t(21;21), three t(13;14), two t(13;15), two (13;21), two t(13;22), one t(14;15), eight t(14;21), two t(14;22), and two t(21;22). Of 8 de novo homologous translocations, only one t(13;13) chromosome was interpreted as dicentric, while 19 of 22 nonhomologous Robertsonian translocations were dicentric. The three monocentric nonhomologous translocations included both of the t(13;21) and one t(21;22). Two of 26 translocations studied using the beta-satellite probe showed a positive signal, while rDNA was undetectable in 10 cases studied. These results indicate that most homologous Robertsonian translocations appear monocentric, while the bulk of nonhomologous translocations show two alphoid signals. A majority of the breakpoints localized using this analysis seem to be distal to the centromere and just proximal to the beta-satellite and nuclear-organizing regions.

Introduction

Robertsonian translocations represent the most common human structural chromosomal abnormality, occurring with an incidence of 1/1,000 in the general population (Hamerton et al. 1975). Chromosomal exchanges resulting in Robertsonian translocations may occur at the centromere, in the proximal short-arm satellite DNA, or within the nucleolar-organizing region (NOR), resulting in monocentric or dicentric chromosomes, depending on the site of the recombination event (Niebuhr 1972). Five previous studies have examined the centromeric composition of Robertsonian translocations by using C-banding, kinetochore staining, orcein-staining techniques, or fluorescence in-situ hybridization (Niebuhr 1972; Daniel and Lam-Po-Tang 1976; Mattei et al. 1979; Gosden et al. 1981; Cheung et al. 1990). These studies revealed that a majority (57/64) of the Robertsonian translocations could be interpreted as dicentric chromosomes. Four studies (Brasch and Smyth 1979; Mattei et al. 1979; Mikkelsen et al. 1980; Cheung et al. 1990) examined a total of 70 Robertsonian translocations by using silver staining. Only four translocations showed silver-positive material indicative of an active NOR.

Cytogenetic banding studies are useful for studying the centromeric and pericentromeric regions of the acrocentric chromosomes; however, their application is limited. C-banding selectively stains the constitutive heterochromatin located in and around the centromere. Although this technique has been used to define the number of centromeres in Robertsonian translocations, the staining is not specific for centromeric DNA, and interpretation of the results is subjective. Silver NOR-banding stains protein associated with the active NOR of the acrocentrics. Few NORs have been visualized in Robertsonian translocations; however, inactive NORs may actually be present. Thus, although cytogenetic banding analyses offer significant insights into the study of Robertsonian translocations, additional studies are warranted in order to fully define the centromeric and pericentromeric areas.

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Address for correspondence and reprints: Stuart Schwartz, Ph.D., Division of Human Genetics, University of Maryland School of Medicine, 655 West Baltimore Street, Baltimore, MD 21201.

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The availability of molecular probes specific for repetitive DNA sequences in the centromeric and short-arm regions of the acrocentric chromosomes allows for the direct analysis of these regions in Robertsonian translocations by utilizing fluorescence in situ hybridization. In addition to providing information on the number of centromeres present, this technique more precisely defines breakpoints and enables identification of the centromeric origin. On the basis of this information, mechanisms involved in the generation of Robertsonian translocation may be formulated. Recently Cheung et al. (1990) used this technique, combined with conventional cytogenetic banding, to localize breakpoints in four different Robertsonian translocations (13;14, 14;21, 14;15, and 13;21). In the present study, fluorescence in situ hybridization was used to analyze the centromeric and short-arm regions of 30 distinct Robertsonian translocations.

Material and Methods

Sample Procurement

Forty-four individuals in 30 families in which a Robertsonian translocation was segregating were studied (table 1). The families were ascertained from six genetic centers, including University of Maryland, Hershey Medical Center, Washington Children's Hospital at the National Medical Center, Eastern Virginia Medical Center, Genetics Associates of Miami, and Tulane University. In addition, six Robertsonian translocation cell lines (GM00479, GM00392, GM01296, GM01700, GM00085, and GM06943) were purchased from NIGMS Human Genetic Repository (Camden, NJ).

Table 1

Centromeric Composition of Robertsonian Translocations

Translocation	No. Monocentric	No. Dicentric	Total no. of Individuals (no. of families)
Homologous:			
t(13;13).....	2	1	3 (3)
t(15;15).....	1	0	1 (1)
t(21;21).....	4	0	4 (4)
Total	7	1	8 (8)
Nonhomologous:			
t(13;14).....	0	7	7 (4)
t(13;15).....	0	3	3 (2)
t(13;21).....	3	0	3 (2)
t(13;22).....	0	1	1 (1)
t(14;15).....	0	1	1 (1)
t(14;21).....	0	13	13 (8)
t(14;22).....	0	5	5 (2)
t(21;22).....	1	1	2 (2)
Total	4	31	35 (22)

Probe Preparation

Five biotin-labeled probes were used for the analysis: (1) αXT(680)22-94, specific for alphoid repeat sequences on chromosomes 14 and 22; (2) L1.26, which hybridizes to alphoid sequences on chromosomes 13 and 21; (3) pTRA 20, chromosome 15 alphoid sequence specific; (4) pβ4, specific for beta-satellite sequences in all acrocentrics; and (5) 5B, which hybridizes to rDNA sequences. Information identifying the DNA probes used in the present study is given in table 2.

Large-scale plasmid preparations were performed for each probe. The purified whole plasmids were biotin-labeled using the BioNick Labeling System (BRL). Labeled probes were recovered from a G-50 sephadex spin column and were stored at −20°C. In order to rapidly prepare labeled insert DNA from plasmid αXT(680)22-94, PCR amplification techniques were employed. The reaction mixture contained the appropriate buffer, unlabeled nucleotides, biotin-16-dUTP (Boehringer Mannheim), pUC 13 insertion-site primers, and *Taq* polymerase (Beckman). The PCR cycle consisted of three steps: a 2-min annealing (55°C), a 3-min extension (68°C), and a 2-min denaturation (94°C). At the end of the 25th cycle, the extension step was increased to 10 min. DNA was recovered after ethanol precipitation.

In Situ Hybridization

Cell cultures were grown and harvested, and slides were made using either standard methodology (Moorhead et al. 1960) or an ethidium bromide technique (Ikeuchi 1984). The chromosomes from each sample had previously been analyzed using standard G-banding techniques. Fresh slides were aged by heating at 60°C overnight. In situ hybridization was performed according to the technique of Pinkel et al. (1986), with minor modification. In brief, aged slides were denatured for 2 min in denaturing solution (70% formamide, 2 × SSC; 70°C) and dehydrated in a −20°C ethanol series. The denatured probe mixture (40 ng biotinylated DNA probe, 50% formamide, 1% dextran sulfate, 0.5 mg herring testes DNA/ml, 2 × SSC) was applied to prewarmed slides (37°C) which were

Table 2

DNA Probe Information

Probe Name	Repeat Sequence	Chromosomal Location	Source	Reference
L1.26	Alphoid	13 and 21 centromeric	P. Devilee	Devilee et al. 1986
αXT(680)22-94	Alphoid	14 and 22 centromeric	A. Leth Bak	Jorgensen et al. 1988
pTRA 20	Alphoid	15 centromeric	K. H. Choo	Choo et al. 1990
pβ4	Beta satellite	Either side of NOR	H. F. Willard	Waye and Willard 1989
5B	rDNA	Stalk	D. Schlessinger	Bowman et al. 1981

subsequently placed in a humid chamber and incubated at 37°C overnight. The slides were washed in a 43°C wash series (3 × 50% formamide, 2 × SSC, [3 min]; 1 × 2 × SSC; [2 min]) followed by a room temperature wash in 2 × SSC (2 min). For the beta probe, the washing conditions were less stringent (3 × 50% formamide, 2 × SSC; 37°C [3 min each]). Signal was detected by using the avidin-fluorescein isothiocyanate amplification method (Pinkel et al. 1986).

Each homologous translocation was hybridized utilizing the appropriate probe (e.g., L1.26 for t(21;21)). For the nonhomologous translocations, two appropriate probes were placed on the same slide, so that the hybridization solutions mixed in the middle. This technique facilitated the study of each probe signal individually, as well as in combination (fig. 1). For the t(13;21) and t(14;22) translocations, only one probe specific for both chromosomes involved in the translocations was used. In each case, approximately 15–50 metaphase spreads were analyzed.

Results and Discussion

The pericentromeric composition of 30 distinct Robertsonian translocations was determined using fluorescence in-situ hybridization. Results of the analysis using the alphoid DNA probes are given in table 1. Three different types of homologous translocations were studied. Seven of the eight translocations were interpreted as monocentric (fig. 2, *upper panels*), while the remaining one, a t(13;13), was dicentric (fig. 2, *lower panel*). These data agree with the hypothesis that homologous Robertsonian translocations may represent isochromosomes (Grasso et al. 1989). Antonarakis et al. (1990) analyzed 10 dup(21q) by using DNA polymorphisms and reported that 8/10 were isochromosomes. Shaffer et al. (1991) used similar techniques to show that 8/8 of their rea(21q21q) were isochromosomes. These findings imply that most "homologous Robertsonian translocations" may indeed be isochromosomes originating via mechanisms different than those responsible for nonhomologous Robertsonian translocation formation.

Alphoid probes were also used to analyze eight different types of nonhomologous Robertsonian translocations. Eighty-six percent (19/22) were dicentric (fig. 3, *upper panels*). This finding is consistent with previous cytogenetic banding studies (Niebuhr 1972; Daniel and Lam-Po-Tang 1976; Mattei et al. 1979; Gosden et al. 1981), which found that 90% of the Robertsonian translocations studied contained two centromeres. In some cases, the fluorescent signal of an alphoid probe at one of the two centromeres did not show typical centromeric constriction; similar findings have been reported with C-banding (Vig 1984). It has been hypothesized that the nonconstricted area may represent the inactive centromere (Vig 1984).

The three t(13;21) chromosomes (two from the same family) were interpreted as monocentric (fig. 3, *lower panel*) and were NOR silver-staining negative. In addition, beta-probe signal, which is visualized normally on both sides of the rDNA repeats of the acrocentrics (Waye and Willard 1989), was undetectable in the translocation chromosomes studied in a case from each family. The ribosomal probe did not hybridize to the translocation chromosome in one case

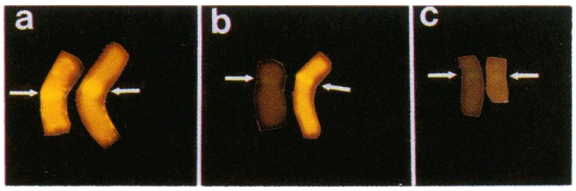

Figure 1 Hybridization of alphoid probes L1.26 (left in each pair) and XT(680)22-94 (right in each pair) to t(13;14) translocation (*a*) and two t(14;21) translocations (*b* and *c*). Signal is detected for each probe, indicating that there is centromeric material present from each of the involved acrocentrics.

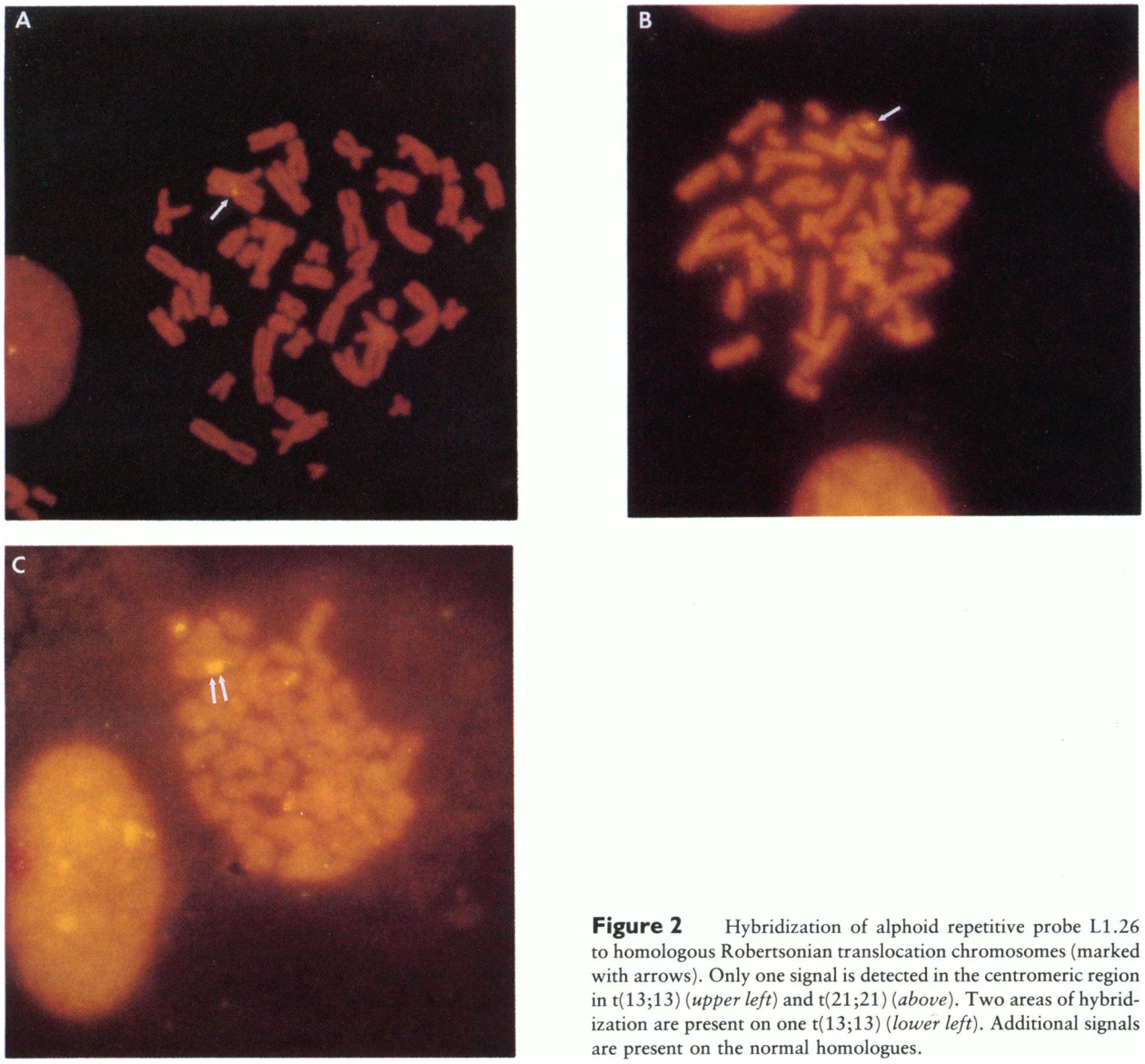

Figure 2 Hybridization of alphoid repetitive probe L1.26 to homologous Robertsonian translocation chromosomes (marked with arrows). Only one signal is detected in the centromeric region in t(13;13) (*upper left*) and t(21;21) (*above*). Two areas of hybridization are present on one t(13;13) (*lower left*). Additional signals are present on the normal homologues.

studied. This suggests that the breakpoints may lie at or near the alphoid sequences. The sequences of the centromeric alphoid DNA of chromosomes 13 and 21 share significant homology which may encourage pairing and exchange between these centromeres. Breakage and reunion at centromeric alphoid sequences would lead to the formation of a Robertsonian translocation with a hybrid centromere. However, breakpoints within monocentric t(13;21) chromosomes are not limited to locations at or near the alphoid repeats. Cheung et al. (1990) found their t(13;21) translocation chromosome to be monocentric, but the presence of silver-staining material indicated that a breakpoint was outside the centromeric region in at least one of the acrocentrics. Thus, the formation of even the same type of Robertsonian translocation may involve various breakpoints.

The other monocentric nonhomologous translocation was one of two t(21;22) chromosomes studied. The karyotype of both cases with t(21;22) contained an additional small marker chromosome which was thought to represent the reciprocal product of the translocation event (fig. 4). These markers were assumed to contain only short-arm and centromeric ma-

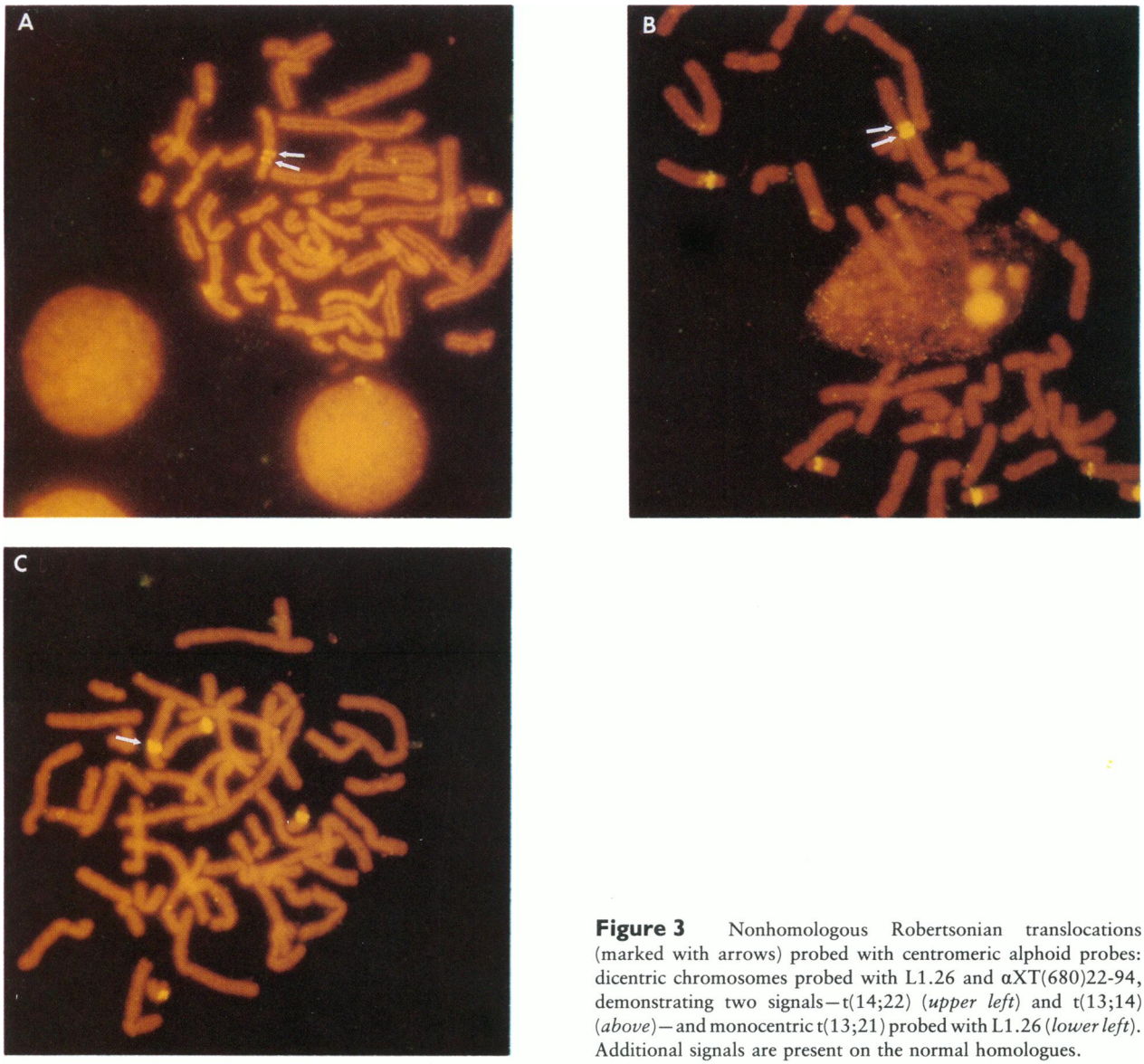

Figure 3 Nonhomologous Robertsonian translocations (marked with arrows) probed with centromeric alphoid probes: dicentric chromosomes probed with L1.26 and αXT(680)22-94, demonstrating two signals—t(14;22) (*upper left*) and t(13;14) (*above*)—and monocentric t(13;21) probed with L1.26 (*lower left*). Additional signals are present on the normal homologues.

terial from chromosomes 21 and 22. In the dicentric t(21;22), the translocation chromosome demonstrated alphoid repeat sequences from both chromosomes 21 and 22 (fig. 4*c*), but the marker contained only chromosome 22 sequences. The chromosome 22 probe showed consistently less hybridization signal on the translocation chromosome, in comparison with the marker. Therefore, the translocation event leading to this dicentric chromosome resulted from a break in the alphoid sequences of chromosome 22 and, most likely, from a break distal to the beta-satellite sequences of chromosome 21, since beta-satellite sequences were present on the translocation chromosome (fig. 4*a* and *b*). The monocentric t(21;22) chromosome contained the 22 alphoid sequences, while the 21 alphoid probe hybridized only to the marker chromosome (fig. 4*d*). No beta-satellite sequences were detectable on the translocation chromosome; thus, the breakpoints could be localized to the beta-satellite region distal to the pericentromeric region of chromosome 22 and proximal to the alphoid sequences on chromosome 21 (fig. 4*a* and *c*). Beta-satellite sequences were also present on both distal portions of each of the small marker chromosomes

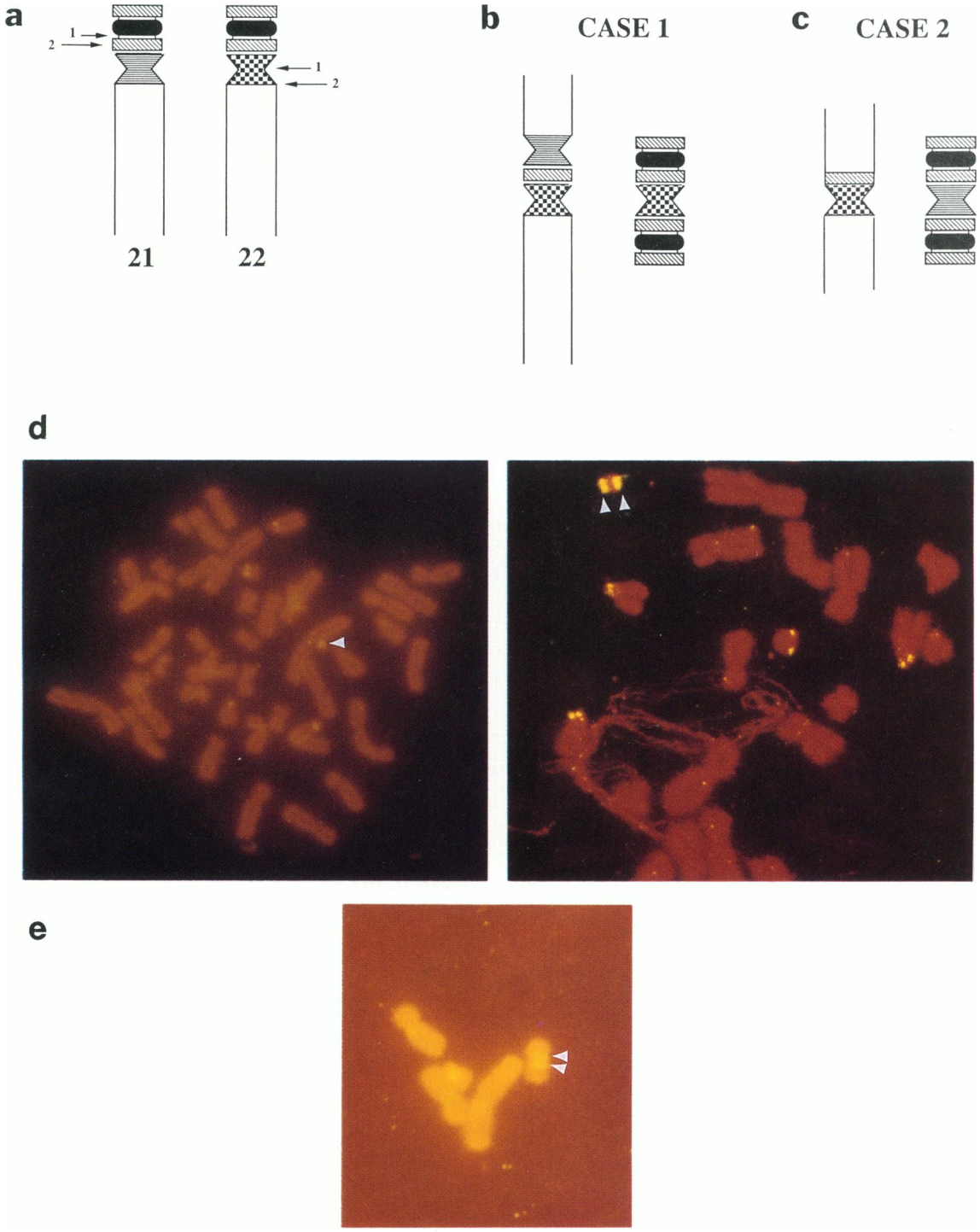

Figure 4 Schematic representation of centromeric and short-arm regions of normal chromosomes 21 and 22 (*a*) and both t(21;22) translocation chromosomes with accompanying marker chromosomes in cases 1 (*b*) and 2 (*c*). Proposed breakpoints are marked with arrows and are labeled with appropriate case numbers in panel *a*. In-situ hybridization data is shown in panels *d* and *e*. In panel *d*, two spreads from case 2 reveal that the marker chromosome (labeled with arrows) demonstrated signal with the L1.26 probe and that two signals were detected by using the beta probe. Both L1.26 and αXT(680)22-94 hybridized to the t(21;22) chromosome of case 1 (*e*).

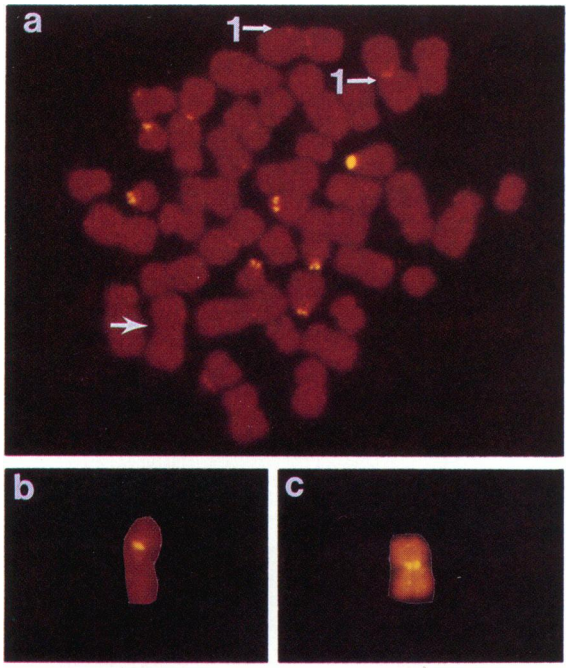

Figure 5 Beta-satellite probe hybridized to metaphase spread containing t(13;14) translocation (*a*). The translocation is marked by an arrow. The two chromosomes 1 are labeled because they exhibit cross-hybridization with the probe. Beta-satellite sequences were detected on only 2 of 26 translocations studied: t(13;22) (*b*) and t(21;22) (*c*).

found in the t(21;22) cell lines (fig. 4*d*), revealing that a majority of these sequences had been translocated to the markers in both cases. These cases provide evidence that different mechanisms may give rise to the same type of Robertsonian translocation.

The biotin-labeled probe specific for beta sequences (pβ4) was used to study 26 of the translocations, including three monocentric homologous translocations (one t(13;13) and two t(21;21)), three nonhomologous monocentric translocations (two t(13;21), and one t(21;22)), and at least one representative dicentric translocation from each family unit (four t(13;14), two t(13;15), two t(13;22), one t(14;15), eight t(14;21), two t(14;22), and one t(21;22)) (fig. 5). Beta-satellite sequences were detected in only two translocation chromosomes: one dicentric t(13;22) and, as mentioned previously, the dicentric t(21;22) (figs. 4 and 5). This analysis revealed that the breakpoints involved in the formation of the dicentric t(13;22) were distal to the beta sequences in at least one of the acrocentrics. It is important to note that, although, under less stringent conditions, the beta-satellite probe hybridizes to both regions surrounding the NOR area, increasing the stringency results in hybridization to only the more distal sequences (H. Willard, personal communication). For our analysis, we relaxed the stringency of wash conditions, to allow hybridization in both regions.

Results from using the ribosomal gene probe (5B) revealed that rDNA sequences were absent in 10 translocations studied, including one t(13;15), one t(13;21), one t(14;22), three t(13;14), and four t(14;21). In-situ hybridization with 5B is advantageous over conventional NOR banding, because the probe binds to the DNA directly and because the signal may be detected independent of NOR activity. Our results confirm previous cytogenetic studies which found that the majority of Robertsonian translocation chromosomes did not contain rDNA sequences (Brasch and Smyth 1979; Mattei et al. 1979; Mikkelsen et al. 1980). Only 10 of the translocation chromosomes were studied using 5B, because the beta probe provided a more complete analysis of the sequences involved in translocation formation.

Two individuals were studied from each of nine families, and three individuals were studied in a 10th family. The translocations were structurally identical in each individual within a family, indicating that the Robertsonian translocations were stably inherited. These family studies also revealed that the beta-probe polymorphisms (Willard 1991) were seemingly stably inherited as well.

The results of our analysis using DNA probes specific for alphoid and short-arm repeat sequences of the acrocentric chromosomes indicate that various breakpoints are involved in Robertsonian translocation formation. It appears that the breakpoints in these chromosomes are concentrated in the region distal to the alphoid DNA at the centromere but proximal to the rDNA and beta-satellite DNA repeat sequences, resulting in dicentric chromosomes (17/22 of our sample; homologous "translocations" were omitted because of uncertainty as to isochromosome formation). Only five of the translocations analyzed (two 13;21, two 21;22, and one 13;22) appeared to have breakpoints in other regions (see discussion above). A majority of the acrocentric breakpoints lie within a region which may constitute a "fragile region" that has a unique DNA sequence that predisposes to recombination events, similar to the "inverted sequence" mechanism proposed by Choo et al. (1988).

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