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Analysis of centromeric activity in Robertsonian translocations: implications for a functional acrocentric hierarchy

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Abstract. Approximately 90% of human Robertsonian translocations occur between nonhomologous acrocentric chromosomes, producing dicentric elements which are stable in meiosis and mitosis, implying that one centromere is functionally inactivated or suppressed. To determine if this suppression is random, centromeric activity in 48 human dicentric Robertsonian translocations was assigned by assessment of the primary constrictions using dual color fluorescence in situ hybridzation (FISH). Preferential activity/constriction of one centromere was observed in all except three different rearrangements. The activity is meiotically stable since intrafamilial consistency of a preferentially active centromere existed in members of six families. These results support evidence for nonrandom centromeric activity in humans and, more importantly, suggest a functional hierarchy in Robertsonian translocations with the chromosome 14 centromere most often active and the chromosome 15 centromere least often active.

Introduction

Robertsonian translocations are the most common structural chromosome abnormalities in humans with a general population incidence of 1 in 1000 (Hamerton et al. 1975). By classical definition, these rearrangements are the products of whole-arm exchanges between two acrocentric chromosomes (Robertson 1916). Approximately 90% of all Robertsonian translocations result in nonhomologous dicentric chromosomes (Niebuhr 1972; Dittes et al. 1975; Daniel and Lam-Po-Tang 1976; Mattei et al. 1979; Gosden et al. 1981; Cheung et al. 1990; Gravholt et al. 1992; Wolff and Schwartz 1992), which are meiotically and mitotically stable, as evidenced by phenotypically normal carriers. In some cases, this stability has been attributed to the proximity of the two centromeres so that they function cooperatively (John and Freeman 1975). However, when further apart, one of the centromeres is most likely inactivated or suppressed, and the dicentric chromosome behaves essentially as a monocentric (Sears and Camara 1952; Therman et al. 1974, 1986; Hsu et al. 1975; Daniel and Lam-Po-Tang 1976; Daniel 1979). While there is no a priori reason for nonrandom centromeric activity, cytogenetic data suggest that in some dicentric chromosomes the selection of the functional centromere is not random (Sears and Camara 1952; Nakagome et al. 1976; Dewald et al. 1979; Gravholt et al. 1992).

Although the necessary components of a functional centromere have yet to be fully described (Willard 1990; Tyler-Smith and Willard 1993), for this project, a centromeric region was defined by the α-satellite DNA, a 171 bp tandemly repeated monomeric unit which constitutes the majority of the primary constriction (Mitchell et al. 1985; Vissel and Choo 1987; Waye and Willard 1987; Willard 1991). Several studies have indicated a major role for α-satellite DNA in centromere function, while little is known about the contributions of the other repetitive DNA families located at or around the centromere. Haaf et al. (1992) showed that over 50 kb of chromosome 17-specific α-satellite DNA transfected into African Green Monkey cells confers some, if not all, aspects of centromeric structure and/or function. Binding of centromere proteins (CENPs) at both the integration site and the native centromere, as well as disruption of normal chromosomal segregation as expected of two functional centromeres on one chromosome, were demonstrated. Sequences necessary for Y centromere function have been localized to an interval containing approximately 200 kb of α-satellite and 300 kb of short-arm sequences (Tyler-Smith et al. 1993), and it appears that relatively small amounts of this Y-specific α-satellite DNA (200 kb) can confer the characteristics of a centromere, such as formation of a primary constriction, binding of CENPs, and abnormal mitotic segregation (Larin et al. 1994). It is unclear if or how other pericentromeric DNA sequences contribute to normal centromere behavior.

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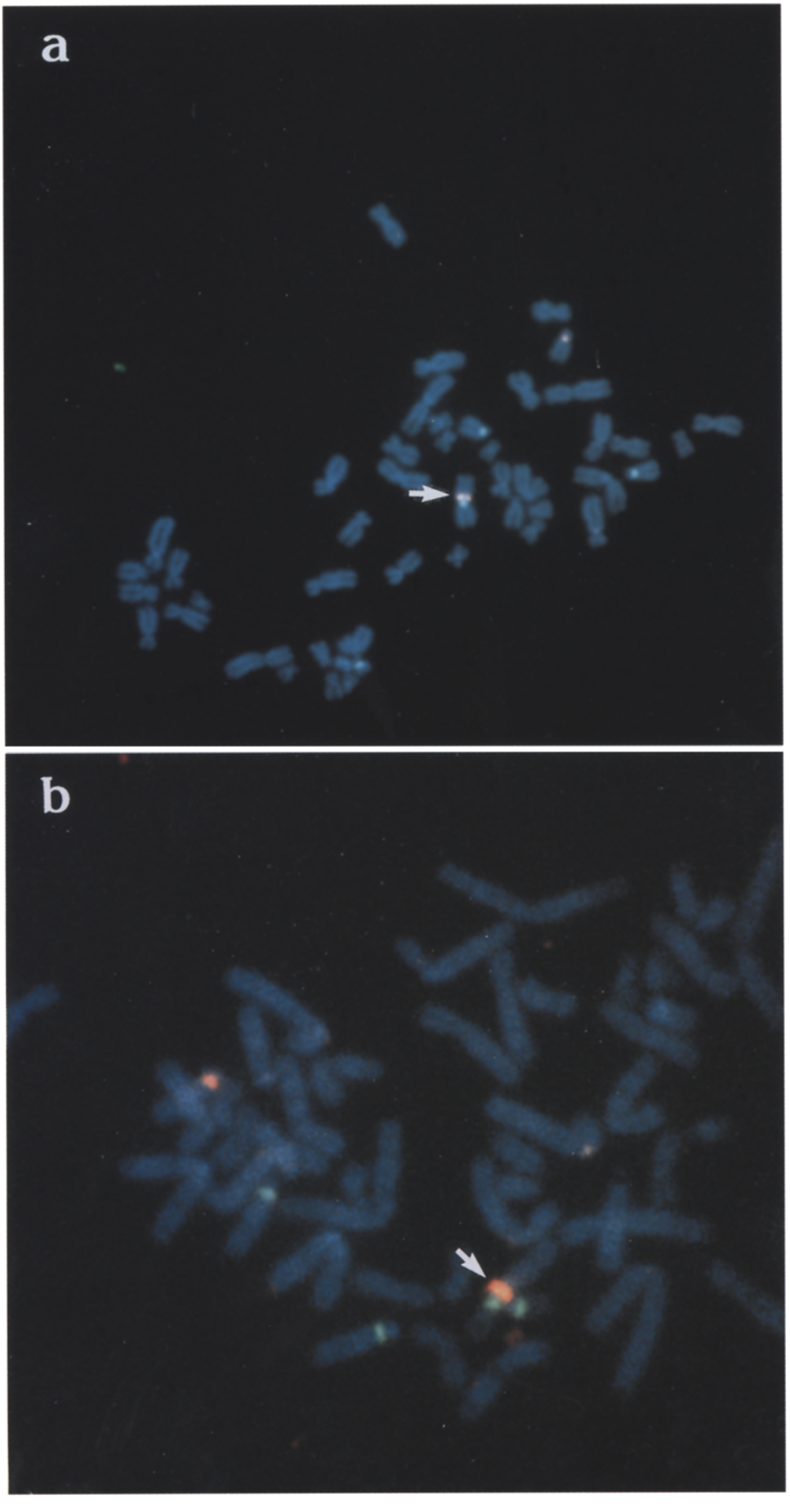

Fig. 1a, b. Assignment of centromeric activity using dual color in situ hybridization of α-satellite DNA probes. An inactive centromere (denoted by an *arrow*) is defined as a split (**a**) or decondensed (**b**) fluorescent signal not located at the primary constriction. **a** Shows a t(14q15q) in which the centromere of chromosome 14 is active. Biotin-labeled chromosomes 14/22 α-satellite is detected with fluorescein (*green*) and digoxygenin-labeled chromosome 15 α-satellite is detected with rhodamine (*red*). **b** Shows a t(15q22q) in which the chromosome 22 centromere is active. Biotinylated chromosomes 14/22 α-satellite is detected with fluorescein (*green*) and digoxygenin-labeled chromosome 15 α-satellite is detected with rhodamine (*red*)

To extend these previous observations, this study has used molecular cytogenetic technique to assign the active centromere in human dicentric Robertsonian translocations and to determine if preferential activity or inactivity of acrocentric centromeres occurs. Our results support the hypothesis of nonrandom centromeric activity in Robertsonian translocations and suggest that a specific hierarchy of acrocentric centromeric activity exists in these human chromosomal rearrangements.

Materials and methods

Harvest techniques. Metaphase chromosomes from lymphocytes were harvested according to standard protocol with hypotonic treatment in 0.075 M KCl (8–10 min at 37° C). Chromosome undercondensation after exposure to Hoechst 33258 (50 μg/ml) (Verma and Babu 1989) allowed for better resolution of centromeres that were closely located. Additionally, fibroblast and lymphoblast cell lines were harvested using an alternate hypotonic treatment (0.8% sodium citrate, 10 min, 37° C) before fixation (3:1 methanol:acetic acid) (Moorhead et al. 1960). This treatment causes chromatids to separate from each other except for the tightly condensed centromeric DNA region. We expected the active centromere to remain constricted, while the inactive centromeric region would separate in the same manner as the chromatids.

DNA probes and hybridization conditions. Biotin- and digoxygenin-labeled α-satellite DNA probes for the acrocentric chromosomes were obtained from ONCOR, Inc (Gaithersburg, Md.). The normal homologues of the translocation chromosomes were used as internal hybridization controls. Hybridization and detection were performed according to manufacturer's protocols. Chromosomes were counterstained with DAPI and visualized on a Zeiss Axiophot fluorescence microscope, using a triple-band-pass filter. Photographs were taken using Kodak Ektachrome ASA 400 color slide film.

Designation of active and inactive centromeres. Analysis of centromeric constriction by FISH was performed blinded for the majority of translocations. In each case, two α-satellite probes, one biotin- and one digoxygenin-labeled, were hybridized simultaneously to metaphase chromosomes. Classical cytogenetic interpretation of inactive centromeres has been based on chromatid separation or a diffuse C-band at one centromere of a dicentric chromosome (Niebuhr 1972; Therman et al. 1974; Daniel and Lam-Po-Tang 1976; Daniel 1979). Likewise, for this study, an inactive, centromere was defined as an uncondensed or split fluorescent signal not located at the primary constriction (Fig. 1a, b). If sample material was sufficient, two independent hybridizations were performed for each translocation, using in the second hybridization the combination of α-satellite probes opposite that of the first hybridization. This was done to account for any properties of the individual DNA probes that might lead to misinterpretation in scoring (e.g., the digoxygenin-labeled chromosome 15 α-satellite sometimes demonstrates a split fluorescent signal even on a normal chromosome 15, which could be incorrectly scored as an inactive centromere on the translocation chromosome). Twenty-five to 100 metaphases were analyzed for each case, and active versus inactive centromere (constricted versus non-constricted fluorescent signal) was assigned for the Robertsonian translocation in each metaphase. Differences in morphology, size, and location (i.e. relative to the primary constriction) of the active versus inactive centromeres were also noted. Stringent scoring criteria only included cells in which hybridization signals were present at both centromeres of the translocation, as well as condensed fluorescent signals at the centromeres of normal homologues. Assuming that the designation of the active centromere is random, each centromere involved in the translocation should be active in approximately 50% of the cells scored. Chi-square tests were used to determine if the distribution of active centromeres in each translocation was significantly different from the expected 1:1 ratio. A centromere was ruled preferentially active if the chi-square value was greater than the critical value at 95% (α=0.05). When a chi-square failed to exceed the critical value, centromeric activity was considered random.

Results

Constriction of the chromosome 14 centromere

Chromosome 14 was involved in 35/47 (74%) of the translocations studied (Table 1), and its centromere was tightly condensed in the majority of cases studied (Tables 2, 3). Of sixteen t(13q14q)s, the chromosome 14 centromere was constricted in thirteen, decondensed in two and was condensed as well as the chromosome 13 centromere in only one translocation (Table 1). In the tables, „random" denotes the translocations in which centromeres were each constricted in a similar number of cells and for which chi-square values did not exceed the critical values for significance. In thirteen t(14q21q)s, the chromosome 14 centromere was constricted in eleven, the chromosome 21 centromere in one translocation, and both centromeres were condensed in two translocations (Table 3). In three of four t(14q15q)s and two t(14q22q)s, the chromosome 14 centromere was condensed. Overall, the centromere of chromosome 14 was preferentially constricted in 83% (29/35) of the Robertsonian translocations involving this chromosome.

Constriction of the chromosome 15 centromere

Chromosome 15 was involved in 12 Robertsonian translocations, and its centromere was never constricted in a majority of cells (Table 4). One maternally inherited t(13q15) (Table 4, case 5) appeared to be a true dicentric, since in some cells, constriction of either the chromosome 13 or chromosome 15 centromere was observed, while in other cells, both fluorescent α-satellite signals were condensed. Consistent with the segregation of a functional dicentric, a small proportion (6%) of the

Table 1. Nonhomologous dicentric Robertsonian translocations studied

Translocation	Number of individuals	Number of families
t(13q14q)	17	16
t(13q15q)	6	5
t(13q22q)	3	3
t(14q15q)	4	4
t(14q21q)	18	13
t(14q22q)	2	2
t(15q21q)	2	2
t(15q22q)	4	1
t(21q22q)	3	2
Total	59	48

Table 2. Centromeric constriction of t(13q14q)

Case	% Cells with constriction from:		Chi-square ($\alpha_{0.05}$=3.84)	Predominantly constricted centromere
	chromosome 13	Chromosome 14		
Individual 1A	20	80	34.6	14
Individual 1B	28	72	16.2	14
2	36	64	9.1	14
3	63	47	4.5[b]	13
4	20	80	35.6	14
5	35	65	9.8	14
6	18	82	15.1	14
7	17	83	20.6	14
8	26	74	22.8	14
9	44	53	0.3	random
10	23	77	8.5	14
11	21	79	8.2	14
12	12	88	18.9	14
13	3	97	29.1	14
14	94	6	36.8	13
15	18	82	19.6	14
16	0	100	25.0	14

[a] A, B, C, etc. represents different members of the same family
[b] At $\alpha_{0.01}$=6.63, this value is not significant and suggests random constriction rather than preferential constriction of the chromosome 13 centromere

Table 3. Centromeric constriction of t(14q21q)[a]

Case	% Cells with constriction from:		Chi-square ($\alpha_{0.05}$=3.84)	Predominantly constricted centromere
	Chromosome 14	Chromosome 21		
1	78	22	8.3	14
2	72	28	14.8	14
Individual 3A	92	8	85.3	14
Individual 3B	94	6	109.8	14
Individual 3C	90	10	64.0	14
Individual 3D	91	9	46.1	14
4	57	43	2.7	Random
5	73	27	7.8	14
6	91	9	65.3	14
7	26	74	10.3	21
8	81	19	17.8	14
9	61	39	2.2	Random
Individual 10A	90	10	32.0	14
Individual 10B	82	18	20.5	14
Individual 11A	95	5	36.4	14
Individual 11B	92	8	34.3	14
12	96	4	40.3	14
13	92	8	34.3	14

[a] A, B, C etc. represent different members of the same family

observed cells showed variant karyotypes, suggesting that the t(13q15q) had been lost or rearranged. Normal metaphases (46,XX) were found in lymphocytes and lymphoblasts of the child (4/132) and lymphocytes of the mother (3/54). Two of the child's lymphocytes demonstrated two chromosomes 15 and monosomy for chromosome 13 (45,XX,-13), and two additional cells each possessed a small marker chromosome, one of which was composed primarily of α-satellite DNA from chromosomes 13 and 15. One t(14q15q) may also be a true dicentric, since cells were observed where one or both centromeres appeared condensed at a primary constriction. The centromeres of this rearrangement were closely located, and in 35% of cells, a yellow fluorescent signal was observed, rather than discrete red and green signals. The chromosome 15 centromere was highly decondensed in the familial t(15q22q), and one of two t(15q21q)s. Thus, none of the Robertsonian translocations contained a preferentially constricted chromosome 15 centromere.

Table 4. Centromeric constriction of chromosome 15[a]

	Case	% Cellswith constriction from:			Chi-square ($\alpha_{0.05}$=3.84)	Predominantly constricted centromere
		Chromosome 13	Chromosome 15	Both		
t(13q15q)	1	82	18	0	17.8	13
	2	96	4	0	62.5	13
	Individual 3A(CV)	53	47	0	0.1	Random
	Individual 3A(LY)	68	21	11	33.6	13/both
	Individual 3A(LC)	31	15	54	22.2	Both
	Individual 3B(LY)	82	12	6	28.6	13
	Individual 3B(LC)	35	25	40	21.1	Both
	4	83	17	0	12.4	13
	5	95	5	0	44.6	13
		Constriction from:				
		Chromosome 14	Chromosome 15	Both		
t(14q15q)	6	43	22	35	20.5	Random/both
	7	77	23	0	13.0	14
	8	77	23	0	8.5	14
	9	72	28	0	4.8[b]	14
		Constriction from:				
		Chromosome 15	Chromosome 21			
t(15q21q)	10	63	37		1.8	Random
	11	11	89		62.2	21
		constriction from:				
		Chromosome 15	Chromosome 22			
t(15q22q)	Individual 12A	10	90		19.2	22
	Individual 12B	33	67		5.7	22
	Individual 12C	15	85		44.3	22
	Individual 12D	4	96		42.3	22

LC=lymphoblast cells; CV=chorionic villus sample; LY=lymphocytes

[a] A, B, C etc. represent different members of the same family

[b] At α=0.01, this value is not significant and suggest random constriction rather than preferential constriction of the chromosome 14 centromere

Constriction of the chromosome 13 centromere

The chromosome 13 centromere was constricted in only two t(13q14q). It was the decondensed centromere in thirteen t(13q14q)s and all three t(13q22q)s. The only cases in which the chromosome 13 centromere was predominantly constricted were four of five t(13q15q)s (Table 4).

Centromeric constriction of chromosomes 21 and 22

In the D/G Robertsian translocations, the centromeres of chromosomes 21 or 22 were usually not located at constrictions. Chromosome 21 had a constricted centromere in only one of ten t(14q21q)s and one of two t(15q21q); the centromere of chromosome 22 was clearly condensed in all three t(13q22q)s and one familial t(15q22q). In three t(21q22q)s, the chromosome 21 centromere was constricted in a majority of cells.

Observation of "hybrid" centromeres

Assignment of centromeric activity, based on constriction, using fluorescence in situ hybridization was unequivocal in 95% of the Robertsonian translocations studied. In two t(14q15q)s (Table 4, cases 6 and 7), a

Table 5. Variety of cell types used for FISH analysis

Translocation	Tissues studied	Constricted centromere	% Cells with constricted centromere
t(14q21q)	Lymphoblasts;	14	95
	lymphocytes	14	85
t(14q21q)	Amniocytes;	14	95
	lymphocytes	14	92
t(15q22q)	Lymphoblasts;	22	92
	lymphocytes	22	83
t(21q22q)	Lymphoblasts;	21	78
	fibroblasts	21	88
t(13q15q)	Chorionic villus;	13/15	53/47
	lymphocytes;	13/15/both	68/21/11
	lymphoblasts	13/15/both	31/15/54
t(13q15q)	Lymphoblasts;	13	95
	lymphocytes	13	90

yellow fluorescent signal was observed rather than distinct red and green α-satellite signals. These centromeres were initially scored as both active due to proximity. Alternative harvesting techniques such as Hoechst 33258, which elongates heterochromatic regions, or sodium citrate hypotonic, which causes splaying of chromatids except for the centromere, allowed more confident assignment of active (constricted) and inactive (unconstricted) centromeres in case 7 (data not shown). Still, this approach did not clearly demonstrate distinct red and green α-satellite signals in the other t(14q15q) (case 6). Our interpretation is that the two α-satellite regions in this dicentric Robertsonian translocation are quite close together and, therefore, may function as a single centromeric unit.

Meiotic stability of preferential centromeric constriction

The inheritance of constricted centromeres was assessed in six families, and in all cases, the patterns of constriction were meiotically stable (Table 2, case 1; Table 3, cases 3, 10, and 11; Table 4, cases 3 and 11). While the percentage of cells with a certain condensed centromere varied from lymphoblasts to lymphocytes in one t(13q15q) (Table 4, case 3), the percentages among the same cell types were compared (i.e., lymphoblasts of Individual 3A versus lymphoblasts of Individual 3B).

Stability of centromeric constriction among various cell types

To examine whether preferential centromeric constriction was tissue-specific, multiple cell types (e.g., lymphocytes, fibroblasts, lymphoblasts, etc.) of several different Robertson translocation carriers were studied (Table 5). Preferential constriction of one centromere was consistent among tissues in all but one translocation. The familial t(13q15q) (Table 4, case 3) was the only case in which the percentages of cells with one or both centromeres at constrictions were dissimilar between cell types. The variation among the different tissues of the t(13q15q) (Table 4, case 3), yet similar proportions of cells with a particular constricted centromere within the same types of tissues of individuals 3A and 3B (Table 4) may reflect a selection for a monocentric configuration of the translocation in B-cells. In fact, the lymphoblasts of individuals 3A and 3B in which the chromosome 13 centromere was constricted had fewer karyotypically abnormal cells than lymphocytes in which both centromeres appeared constricted.

In general, the number of cells with a particular constricted centromere was often much higher in the lymphoblasts than in the lymphocytes of the same patient. Since lymphoblast cells are clonal, thereby constituting a more homogeneous cell population than lymphocytes, the high percentage of cells with the same centromere constricted suggests that the cellular assignment of active and inactive centromeres in dicentric chromosomes may be determined early and is consistently maintained. Any observed variability between unrelated carriers of the same type of Robertsonian translocation may reflect a individual-dependent, yet heritable, positive or negative cell selection for a given centromere. However, if the choice is random, the significantly skewed activity patterns in the lymphoblastoid cell lines may then represent a post-zygotic selection for or against cells with a certain constricted centromere. Overall, the fact that several tissues of four patients showed similar patterns of constriction implies that centromeric activity may be established early in embryogenesis and may be clonal.

Variability in proportions of cells with a constricted centromere

The proportion of cells exhibiting a particular constricted centromere varied among patients. For instance, the percentages of cells with a predominantly constricted chromosome 14 centromere ranged from 63 to 100% (Tables 2–4). The variability was observed among patients with the same type of translocation and preferentially constricted centromere (column 3 of Tables 2–4).

However, the total number of cells with a specific constricted centromere were very similar in the familiar translocations (Table 2, case 1; Table 3, cases 3 and 10; Table 4, cases 3 and 11), reflecting meiotic stability of the centromeric constriction. It is unclear why a preferentially constricted centromere was not consistently condensed in 100% of the cells, although occasional lapses in the as yet unidentified mechanism(s) which preferentially designates an active centromere by inducing or causing a constriction may be responsible for this observation.

Discussion

It is universally accepted that the primary constriction of a chromosome marks the centromere. Traditionally defined by decondensed C-bands or heterochromatic regions which are relaxed or separated, suppressed or inactive centromeres have been observed in various types of dicentric and multicentric chromosomes (Niebuhr 1972; Therman et al. 1974; Hsu et al. 1975; Daniel and Lam-Po-Tang 1976; Nakagome et al. 1976; Daniel 1979; Dewald et al. 1979; Zuffardi et al. 1980; Ing and Smith 1983; Earnshaw and Migeon 1985; Merry et al. 1985; Therman et al. 1986). Nonrandom as well as apparently random centromeric function has been reported in dicentric Robertsonian and other translocations based primarily on cytogenetic banding and light microscopy (Angell et al. 1970; Warburton et al. 1973; Daniel and Lam-Po-Tang 1976; Roberts et al. 1977; Daniel 1979; Dewald et al. 1979; Wandall 1989). One more recent study examined 21 t(13q14q)s and 3 t(14q21q) using single color in situ hybridization and reported the centromere of chromosome 14 as active in 16 of 20 unrelated t(13q14q)s and 2 of 3 t(14q21q)s (Gravholt et al. 1992). Our results, which include almost every type of Robertsonian translocation, combined with data from the two types of translocations studied by Gravholt et al., indicate that the chromosome 14 centromere is nonrandomly active when involved in human dicentric Robertsonian translocations. We further conclude that the chromosome 15 centromere is rarely preferentially active in these translocations. The results of our study provide the first evidence for a hierarchy of centromeric activity among the chromosomes involved in dicentric Robertsonian translocations. Based on the observation that the centromere of chromosome 14 was usually constricted, and therefore interpreted as active in 83% of the Robertsonian translocations, it is most often the active centromere in this hierarchy. Data from the remaining dicentric translocations indicate that the chromosomes 21, 22, and 13 centromeres are also more often active than the chromosome 15 centromere.

The active centromere of a dicentric chromosome may be accurately identified by and its function be dependent on its location at the primary constriction. There is support for the contribution of α-satellite DNA to centromere function (Haaf et al. 1992; Tyler-Smith and Willard 1993; Tyler-Smith et al. 1993; Larin et al. 1994). However, it is important to consider the differences between induced (α -satellite integrated into chromosomes) and naturally occurring dicentric chromosomes. The chromosomes into which α-satellite was transfected, while instrumental to the understanding of centromere function, may not reflect centromeric activity in stable dicentric Robertsonian translocations. These recent studies have shown that when transfected α-satellite DNA integrates into chromosomes, it forms or induces a primary constriction and disrupts normal chromosome segregation. The majority of Robertsonian translocations possess two α -satellite regions, form only one constriction, and segregate faithfully in meiosis and mitosis. Since our results indicate there is preferentially activity of one centromere in Robertsonian translocations, it is possible that unique features of centromeric DNA or its relationship to associated proteins which functionally distinguish between centromeric regions on the same chromosome. Despite remarkable sequence similarities, as in the cases of chromosomes 13 and 21 and chromosomes 14 and 22 α-satellite DNA, certain acrocentric centromeres must have functional advantages, perhaps due to the number of chromosome-specific repeat subfamilies, centromere and short-arm sequences, or repeat array-size differences. More intriguingly, there might even be unique structural features of Robertsonian translocations that control centromeric activity.

Rearrangements such as t(14q22q) reflect this paradox to the hypothesis of α-satellite DNA as the major component of an active centromere, primarily because the α -satellite DNA sequences of chromosomes 14 and 22 are virtually identical. The t(14q22q)s in our study demonstrated preferential activity of the chromosome 14 centromere. The α-satellite DNA is organized into higher-order repeat units (arrays) and displays chromosome-specific restriction site periodicities, which result from array size and basic nucleotide sequence differences (reviewed by Waye and Willard 1987). Variations in array-size length, modification of array organization, or the contribution of additional DNA sequences may confer or influence preferential centromeric activity in Robertsonian translocations. Differences in the specific binding of a centromeric protein, CENP-B, to α-satellite DNA arrays have been recently observed. CENP-B binds within the α-satellite monomer to a 17 bp region called the CENP-B box (Masumoto et al. 1989). Haaf and Ward (1994) showed that two chromosome 7-specific α-satellite arrays, D7Z1 and D7Z2, differ tenfold in the number of CENP-B boxes, with increased binding of CREST antibodies at D7Z1, which contains a greater number of CENP-B boxes. In Robertsonian translocations, the binding of some or all CENPs may occur competitively, if at all. Therefore, preferential centromeric activity may be due in part to array sizes which encode a greater number of CENP-B boxes or specific target DNA sequences, such as the CENP-B box, which are more accessible to protein complexes.

While sizes, alterations, or other differences in the alphoid arrays might give a centromere a functional advantage, nonrandom centromeric activity in Robertsonian translocations might also be dependent on a specific area(s) of breakage and loss or retention of certain centromeric or short-arm DNAs. Consequently, individuals

demonstrating centromeric activity opposite the majority could reflect structural heterogeneity within the translocation chromosomes. Some α-satellite and satellite III DNA subfamilies of the centromere and short arm are shared among the acrocentrics (reviewed by Choo 1990; Vissel et al. 1992). The p11 region of chromosome 14 contains satellite III DNA subfamilies which are not shared by the other acrocentrics and in which the breakpoints of several t(14q21q)s have been localized (Choo et al. 1990, 1992; Early et al. 1992; Gravholt et al. 1992). Satellite III DNA, located primarily in acrocentric short arms, is composed of a highly conserved 5 bp repeat and can be interspersed within minor α-satellite arrays (Trowell et al. 1993). Chromosomes 13, 14, and 21 contain highly conserved satellite III DNA and α-satellite DNA, which are separated by an equally conserved 9 bp direct repeat which is recognized by the protein pJα (Gaff et al. 1994). This nuclear protein effectively binds to at least one of the 9 bp repeats and more generally to typical α-satellite DNA. Disruption of the 9 bp repeat by specific mutations may decrease or eliminate binding of pJα (Gaff et al. 1994). It is conceivable that any disturbance, as a result of a translocation breakpoint, of this conserved junction sequence, the surrounding α-satellite, or another functionally important sequence could disrupt normal centromere function, perhaps making the DNA unrecognizable to proteins or preventing the initiation step in the binding of a centromeric protein complex.

Lastly, the familial case of t(13q15q) raises the issue of truly dicentric, rather than pseudodicentric, Robertsonian translocations. It was the only translocation which demonstrated instability of not only the primary constriction but the chromosome itself. In addition to cells with the balanced translocation, the finding of normal cells (46, XX) in the mother, as well as 46,XX, 45,XX,-13 and 45,XX, +mar cells in the child, supports the existence of a functionally dicentric translocation chromosome, at least in a fraction of the cell population. Variant karyotypes associated with other types of dicentric chromosomes have been reported (Daniel et al. 1980; Ing and Smith 1983; Schwartz et al. 1983; Haaf and Schmid 1990), and it is presumed that the abnormal chromosomes are the consequences of breakage caused by two active centromeres moving the chromosomes toward opposite spindle poles. Our t(13q15q) is stably inherited, since most cells demonstrated an intact translocation chromosome. For normal transmission of the dicentric from mother to daughter to occur, either one of the centromeres is inactivated and undetectable by the usual observation of chromatin decondensation, or the dicentric is stable while having two active and distinctly located centromeres. Based on staining with anti-kinetochore antibodies, the formation of two kinetochores at both centromeres of dicentric chromosomes associated with mosaic karyotypes can occur (Haaf and Schmid 1990). More substantial evidence for retained activity of suppressed centromeres and the existence of essentially stable, true discentrics comes from a recent study of a t(5;13) in which both centromeres, irrespective of activity, developed kinetochores, attached to microtubules, and were equally or cooperatively transported along the mitotic spindle (Wandall 1994). It appears, then, that while centromeres of some dicentric chromosomes may be suppressed, they maintain the necessarily elements for activity and can be reactivated (Hsu et al. 1975). It is worth mentioning that the normal cells (46, XX) in our t(13q15q) might have resulted not from the instability of the dicentric chromosome, but from "normal" fission events, which can occur in Robertsonian translocations (Holmquist and Dancis 1980; Imai 1988; Schubert et al. 1992).

Our approach to assigning centromeric function in dicentric Robertsonian translocations is based on location of α-satellite DNA probes at the primary constrictions of these dicentric chromosomes and the observation of morphological differences at the two centromeric regions of metaphase chromosomes. Because particular CENPs are associated with the active centromeres in dicentric chromosomes (Earnshaw and Migeon 1985; Earnshaw et al. 1989), immunolocalizing centromere-associated proteins (CENPs) to Robertsonian translocations should be useful in more accurately identifying active centromeres, corroborating the in situ hybridization data, and substantiating the hierarchy for centromeric activity in Robertsonian translocations.

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