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Chromosome spatial order in human cells: evidence for early origin and faithful propagation

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Abstract. We have investigated the origin and nature of chromosome spatial order in human cells by analyzing and comparing chromosome distribution patterns of normal cells with cells showing specific chromosome numerical anomalies known to arise early in development. Results show that all chromosomes in normal diploid cells, triploid cells and in cells exhibiting nondisjunction trisomy 21 are incorporated into a single, radial array (rosette) throughout mitosis. Analysis of cells using fluorescence in situ hybridization, digital imaging and computer-assisted image analysis suggests that chromosomes within rosettes are segregated into tandemly linked "haploid sets" containing 23 chromosomes each. In cells exhibiting nondisjunction trisomy 21, the distribution of chromosome 21 homologs in rosettes was such that two of the three homologs were closely juxtaposed, a pattern consistent with our current understanding of the mechanism of chromosomal nondisjunction. Rosettes of cells derived from triploid individuals contained chromosomes segregated into three, tandemly linked haploid sets in which chromosome spatial order was preserved, but with chromosome positional order in one haploid set inverted with respect to the other two sets. The spatial separation of homologs in triploid cells was chromosome specific, providing evidence that chromosomes occupy preferred positions within the haploid sets. Since both triploidy and nondisjunction trisomy 21 are chromosome numerical anomalies that arise extremely early in development (e.g., during meiosis or during the first few mitoses), our results support the idea that normal and abnormal chromosome distribution patterns in mitotic human cells are established early in development, and are propagated faithfully by mitosis throughout development and into adult life. Furthermore, our observations suggest that segregation of chromosome homologs into two haploid sets in normal diploid cells is a remnant of fertilization and, in normal diploid cells, reflects segregation of maternal and paternal chromosomes.

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Introduction

There has been considerable interest in the global organization of the cell nucleus – in particular how chromosomes are arranged. The possibility that chromosomes occupy preferred locations relative to one another in nuclei is an attractive, but controversial, concept that has resurfaced repeatedly over many years. Thus far, common principles that dictate nuclear organization have remained elusive (Heslop-Harrison and Bennett 1984; Manuelidis 1990; Haaf and Schmid 1991; Spector 1993; Xing et al. 1995). Nuclear polymorphism associated with variations in cell type and cell cycle phase has very likely hindered resolution of this issue, since common patterns of chromosome positioning and movements in interphase cells may be difficult to detect against a background of widespread variations in nuclear morphology.

During mitosis, chromosomes undergo a well-orchestrated, highly characterized series of movements that represents the epitome of evolutionary conservation at both structural and molecular levels. Since all human cells, regardless of cell type, exhibit nearly identical patterns of chromosome movements during mitosis, we have recently focused our search for common patterns of chromosome spatial positioning in mitotic cells. Scattered throughout the literature are numerous discriptions of chromosomes in mitotic cells assembled into ring-like arrays (Welter and Hodge 1985; Chaly and Brown 1989; Mosgoller et al. 1991; Leitch et al. 1994). In our previous study, chromosomes in mitotic human cells were shown to be arranged into wheel-like rosettes from late prometaphase through completion of mitosis (Nagele et al. 1995). Within these rosettes, chromosome homologs were consistently positioned on opposite sides, and heterologs exhibited a reproducible spatial interrelationship. This chromosome distribution pattern has raised the possibility that rosettes in normal human cells are composed of two spatially distinct groups ("haploid sets") of 23 chromosomes, and that each haploid set may represent a cohesive, ordered group of homologs inherited from one parent at the time of fertilization (Nagele et al. 1995). Evidence

for similar rosette-like chromosome arrays has also been described in some plant cells (Heslop-Harrison and Bennett 1984; Leitch et al. 1991; Schwarzacher et al. 1992). The common chromosome distribution pattern in several cell lines derived from different adult tissues and different individuals suggests that the observed chromosome distribution in human cells is species specific. Furthermore, it implies that chromosome spatial order is established early in development and transmitted faithfully into adult life. To explore this possibility, we have investigated chromosome spatial arrangements in mitotic cells derived from individuals exhibiting two different types of chromosomal numerical anomalies known to have their origin quite early in development: nondisjunction trisomy 21 (Down syndrome), and triploidy. Cells with nondisjunction trisomy 21 possess an extra chromosome 21 homolog, whereas those with triploidy have an extra full complement of 23 additional chromosomes. The relative spatial positioning of selected chromosomes in rosettes of these cells at mitosis was analyzed by fluorescence in situ hybridization using chromosome-specific DNA probes and digital image analysis.

Materials and methods

Cells. The following cell lines used in this study were obtained from the Coriell Cell Repository, Camden, N.J.: normal human skin fibroblasts derived from a 31 year old female (AG07715, karyotype 46, XX, at PDL 8); normal human fetal fibroblasts (GM00011 A, karyotype 46, XY obtained at passage 6); AG05025 and GM01322 (both triploid with karyotype 69, XXY), the former derived from a conceptus exhibiting congenital malformations typical of triploidy, and the latter from a 2 year old individual with meningomyelocele and a female phenotype; AGO4823 (obtained at PDL 8) and AGO5024 (obtained at PDL 6), both nondisjunction trisomy 21 skin fibroblasts with karyotype 47, XX, +21. Cells were grown to subconfluence in plastic flasks in Dulbecco's Modified Eagle medium (Gibco-BRL) containing 10% fetal calf serum (Gibco-BRL) and 1% penicillin/streptomycin at 37°C in a 5% CO_2 atmosphere. Mitotic cells were obtained by gentle shake-off and quickly cytospun (Shandon Southern Instruments) at 500 *g* for 5 min onto SuperFrost Plus microslides (Fisher Scientific). Cells were immediately fixed in 4% paraformaldehyde in phosphate-buffered saline (PBS; 137 mM NaCl, 3 mM KCl, 16 mM Na_2HPO_4, 2 mM KH_2PO_4, pH 7.3) for 20 min at room temperature. After a brief rinse in PBS, they were then dehydrated in a graded series of increasing concentrations of cold (4°C) ethanol (30%, 50%, 70%, and 95% ethanol, 5 min each) and stored in 95% ethanol at –20°C until use.

Fluorescence in situ hybridization (FISH) and digital imaging. FISH was carried out on cells obtained by mitotic shake-off and cytospinning as described above. Fixed cells attached to microslides were air-dried from 95% ethanol at 37° C. Cells were rehydrated in 2×SSC (20×SSC is 3 M NaCl 0.3 M trisodium citrate, pH 7.0) for 10 min at room temperature. Digoxigenin-labeled DNA probes included a whole chromosome 7 painting probe, probes specific for alpha-satellite regions of chromosomes 1, 3, 7, 10, 11, 15, 16, 17, 20 and 22, and a digoxigenin-labeled DNA probe specific for the DZ701 locus of chromosome 21. All chromosome-specific probes were obtained from Oncor and prepared for hybridization following the recommendations of the manufacturer. Coverslips were mounted on slides containing attached cells and 15 µl DNA probe solution and sealed with rubber cement. Alpha-satellite probes and cellular DNA were co-denatured for 10 min on a heat block set to 95°C and hybridized overnight at 37°C in a humid environment. For chromosome painting, probes and cellular DNA were denatured separately. Posthybridization wash conditions for each chromosome-specific probe were as recommended by the manufacturer. For detection, specimens were incubated in 3% BSA in 4×SSC containing 0.05% Tween-20 (SSC-Tween buffer) for 10 min at room temperature to block nonspecific binding of detection reagents. Detection was performed with fluorescein isothiocyanate (FITC)-conjugated sheep anti-digoxigenin antibodies (200 µg/ml; Boehringer Mannheim) for 20 min at 37°C. After three washes in SSC-Tween buffer, cells were mounted in Vectashield mounting solution (Vector Laboratories) containing 4',6-diamidino-2-phenylindole (DAPI) or propidium iodide as a DNA counterstain. Controls for FISH included hybridization without labeled probe, and omission of the detection reagent that binds to the probe. Specimens were examined with a Nikon Optiphot or Nikon FXA microscope equipped with epifluorescence optics, a Princeton Instruments CCD camera, and a Nikon K2BIO spinning disk confocal attachment. The DAPI or propidium iodide image was used to define the morphological boundary of rosettes and nuclei. Separate digital images of the FITC signals and the DAPI-counterstained rosettes were acquired and processed using commercially available imaging software, Metamorph (Universal Imaging), ImagePro Plus (Phase 3 Imaging Systems) and Corel Draw 6 (Corel), to permit overlay of images recorded with different wavelength filters. False color was added to fluorescence images. For dual hybridization reactions using contrasting fluorochromes, posthybridization conditions were for the probe requiring the least stringent conditions. Images were stored on hard disks and printed using a Sony dye-sublimation printer.

Measurement of the spatial separation of chromosome homologs in rosettes. Measuring the angular separation between homologs was preferable to measuring actual distances because rosettes vary in diameter, presumably reflecting individual differences in the packing density of chromosomes during rosette assembly. To ensure the accuracy of our measurements, we applied selection criteria for rosettes that included an intact, radial and symmetrical chromosome array lacking obvious structural distortion and possessing the expected number of fluorescent signals (one for each homolog). Measurements of the angular separation of chromosome homologs in circular rosettes satisfying the selection criteria were carried out directly on printed images as well as on prepared templates. The center of the rosette was used as the reference point from which the angular separation between homologs was measured. With this method, homologs positioned on exactly opposite sides of the rosette, representing maximal spatial separation, would be 180° apart. In specimens where whole chromosome painting probes were used and fluorescent signals were relatively large and diffuse, the line drawn from the central reference point of the rosette that also passes through the exact center of the fluorescent chromosome territory was taken to represent the position of that chromosome homolog. Fluorescent signals from chromosome-specific alpha-satellites were small and punctate and considered to represent the position of the chromosome homolog. Since early prometaphase cells of the types used in the present study do not readily detach from the culture substratum during mitotic shake-off, the majority of rosettes available for examination and analysis in the present study were from cells at late prometaphase, metaphase, anaphase, and telophase. Some early G1-phase daughter cells that were still connected by the mitotic spindle remnant were also observed. Actual data were compared statistically with those generated by random simulations using the *t*-test. In these simulations, random relative homolog positions were dictated by a random number generator and placed on mock rosettes. Homolog separation angles were measured as described above.

Results

Chromosome arrangement in rosettes of mitotic human cells

Despite having different numbers of chromosomes, the mitotic diploid cells, triploid cells and nondisjunction tri-

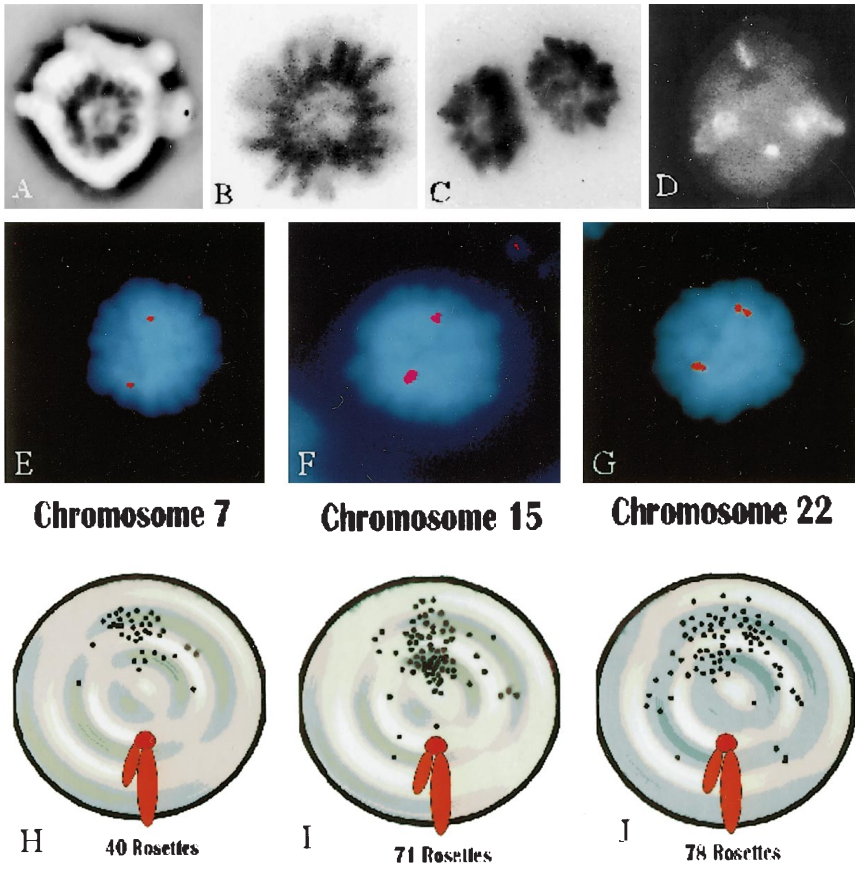

Fig. 1A–J. Chromosome distribution in rosettes of normal human diploid cells. **A, B** Chromosomes are organized into a radial array (a rosette) as shown in a reverse-contrast, phase image (**A**) and a brightfield image of a Feulgen-stained cell (**B**). **C** Feulgen-stained daughter rosettes at telophase showing that the rosette arrangement of chromosomes persists throughout mitosis. **D** Dual fluorescence in situ hybridization (FISH) with a whole-chromosome painting probe for chromosome 7 (larger signal) and an alpha-satellite probe for chromosome 16 (smaller signal) showing that both homologs and heterologs exhibit consistent spatial positioning. **E–G** FISH images showing the strong tendency for homologs of chromosomes 7, 15 and 22 to be situated on opposite sides of the cosette in normal diploid cells. In these images, the digoxigenin-labeled probe is pseudocolored red against the blue background counterstaining with 4'-6-diamidino-2-phenylindole. **H–J** Schematic representations of rosettes showing the relative spatial distribution of chromosome 7, 15 and 22 homologs. In each case, using one randomly selected homolog (the red chromosome) as a point of reference, the other homolog was restricted to the opposite side of the rosette. *Bar* represents 10 μm

somy 21 cells examined in the present study were found to have all of their chromosomes arranged into a single, wheel-shaped rosette with most centromeric domains positioned centrally and chromosome arms projecting radially (Fig. 1A–C). This rosette configuration first appears during prometaphase and lasts through completion of mitosis (Fig. 1A–C). Chromosome rosettes are structurally robust, which allows them rather easily to be laid flat on the surface of microslides by cytospinning. In this orientation, most fluorescent signals can be viewed and recorded photographically within a single focal plane, which greatly facilitates analysis and interpretation of fluorescent signal distribution patterns and direct examination of radially arranged chromosomes in large numbers of cells. Chromosomes in diploid cells were positioned nonrandomly, with homologs showing a strong tendency to be situated on opposite sides of rosettes (Fig. 1D–G, Table 1) and heterologs exhibiting a consistent relative spatial positioning (Fig. 1D). Quantitation of the spatial separation of chromosome homologs within circular rosettes was accomplished by measuring their angular separation, with each circular rosette encompassing 360° and the exact center of the rosette used as the reference point as described previously (Nagele et al. 1995) (Table 1). To ensure that valid information on relative chromosome position was obtained, we applied selection criteria for rosettes that included an intact, radial and symmetrical chromosome array that is free from obvious structural distortion and the presence of the expected number of fluorescent signals (one for each homolog). Figure 1H–J provides schematic representations of rosettes of diploid cells showing the relative spatial distribution of chromosome homologs, using one of the homologs as a point of reference. Most chromosome 7, 15 and 22 homologs were restricted to relatively small regions situated on opposite sides of the rosette (Fig. 1H–J, Table 1). Examination of DAPI- and Feulgen-stained rosettes has shown clearly that, although the rosette appears as a flattened, circular chromosome array, chromosomes are not in a simple

Table 1. Relative positioning of chromosome homologs in diploid rosettes.

Chromosome	Angular separation (degrees)	*n*
7	152.56±29.95	54
11	127.70±48.63	36
21	162.10+16.80	47
15	159.16±36.68	72
22	145.43±35.75	41

Quantitation of the relative positions of chromosome homologs in mitotic rosettes of normal human diploid fibroblasts. The angular separation of homologs on each circular rosette was measured in degrees. The exact center of the rosette was used as a reference point. Maximal separation of two homologs (i.e., situated on opposite sides of the rosette) is 180°. Data for each chromosome differed significantly ($P<0.001$) from random simulations as determined by the *t*-test.

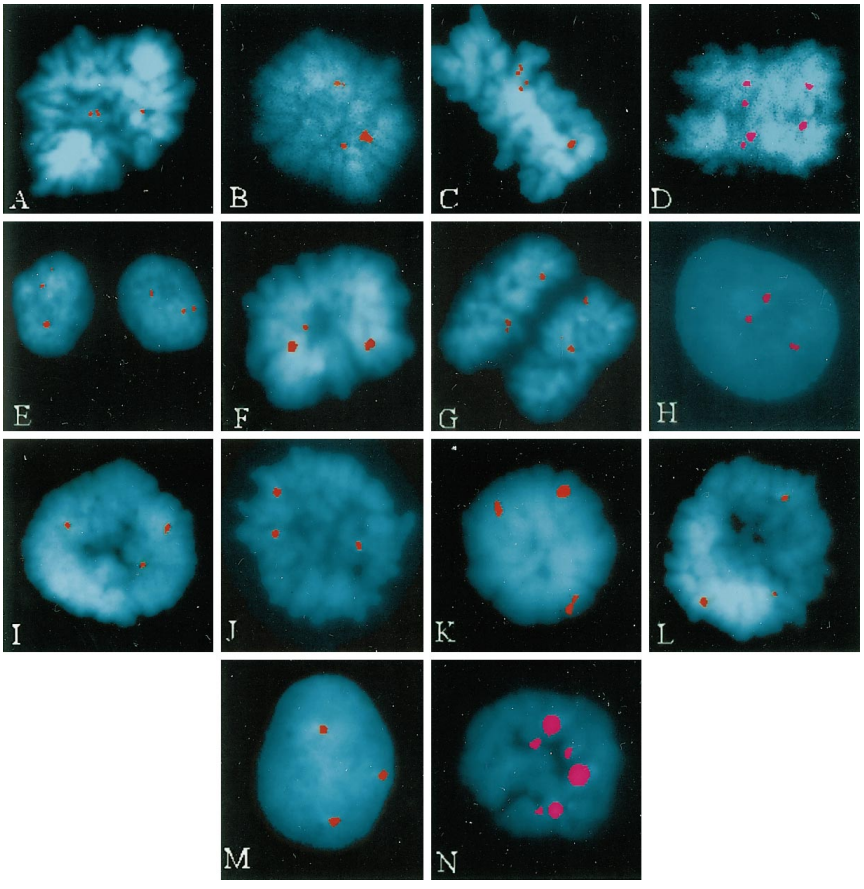

Fig. 2A–N. FISH images showing the relative distribution of chomosome homologs in cells exhibiting nondisjunction trisomy 21 or triploidy. **A–H** In trisomy 21 cells, all 47 chromosomes were incorporated into a single rosette. In both cell lines, two chromosomes 21 homologs were closely juxtaposed on one side of the rosette, while the remaining homolog was consistently positioned on the opposite side of the rosette (**A**–**D**, **F**, **G**). This chromosome distribution pattern persisted throughout mitosis as shown in FISH images of metaphase (**C**) and anaphase (**D**, **G**) rosettes, and was also observed in interphase cells (**E**, **H**). **I–N** FISH images showing the relative positioning of chromosome homologs in triploid cells. All 69 chromosomes were arranged into a single triploid rosette. In triploid rosettes hybridized with alpha-satellite probes specific for chromosomes 1, 3, 10 and 17 (**I**–**L**, respectively) two of the three homologs were in relatively close proximity on one side of the rosette, with the third homolog positioned on the opposite side. A similar homolog distribution pattern was observed in interphase cells (**M**). Dual FISH (**N**) with alpha-satellite probes for chromosomes 10 (larger spots) and 17 (smaller spots) showing chromosomes within rosettes arranged into three separate (presumably haploid) sets, one of which possesses an inverted order with respect to the other two (cf. Fig. 3). *Bar* represents 10 μm

monolayer, but rather are densely packed into a "best fit" staggered array, which gives the appearance of being two to three chromosomes thick (Fig. 1A, B). Overall, this remarkably consistent, chromosome spatial arrangement in normal human diploid cells suggests that rosettes are composed of two spatially distinct haploid sets of 23 chromosomes.

Relative positioning of chromosome 21 homologs in rosettes of cells exhibiting nondisjunction trisomy 21

We have investigated the possibility that the common pattern of chromosome distribution observed in rosettes of mitotic human cells originates early in development and is transmitted faithfully through mitosis from one cell generation to the next. To test this hypothesis, we studied alterations in chromosome spatial order in cells that possess specific, well-defined, chromosome numerical abnormalities (i.e., nondisjunction trisomy 21 and triploidy) known to originate extremely early in development. For example, trisomy 21 cells (karyotype=46, XX, +21) were used to determine whether chromosome 21 nondisjunction results in stable insertion of the nondisjoined chromosome into the rosette in a specific location. Mitotic trisomy 21 cells were isolated by selective detachment, cytospun onto glass microslides, and subjected to FISH with digoxigenin-labeled DNA probes specific for the DZ701 locus of chromosome 21 (Fig. 2). As shown in Table 1, chromosome 21 homologs in normal human diploid cells, like all other chromosomes examined thus far, were consistently positioned on opposite sides of the rosette; the angular separation of chromosome 21 homologs was 162.1±16.8 degrees (n=47). In the nondisjunction trisomy 21 cell lines, all 47 chromosomes were incorporated into a single rosette (Fig. 2A–D, F, G). The relative distribution pattern of chromosome 21 homologs within rosettes derived from both trisomy 21 cell lines was found to be remarkably consistent. For example, two chromosome 21 homologs were found to be closely juxtaposed on one side of the rosette, while the remaining homolog occupied the more "typical" position on the opposite side of the rosette (Fig. 2A–D, F, G) . The spatial separation of the two closest homologs was 22.6±11.1 degrees (n=50). This chromosome distribution pattern persisted throughout subsequent phases of mitosis (Fig. 2C, D, G) and suggests that chromosome 21 nondisjunction results in a stable insertion of the extra chromosome into the rosette in a position closely juxtaposed with its homolog. In addition, the distribution of chromosome 21 homologs in the majority of interphase (early G1-phase) nuclei that accompany mitotic cells in selective detachment preparations was nearly identical to that in mitotic rosettes (Fig. 2E, H), with two of the three chromosome 21 homologs closely juxtaposed on one side of the nucleus and the remaining homolog on the other side of the nucleus.

Table 2. Relative positioning of chromosome homologs in triploid rosettes

Chromosome	Angular separation (degrees)		*n*
	Closest homolog	Next closest homolog	
1	60.5±19***	120.9±20.6**	28
3	84.7±21***	126.0±13.2***	30
7	57.5±24***	125.5±24.3***	30
7[a]	47.8±24.4	110.5±36.0	21
10	40.7±14.2	126.9±20.2***	31
11[a]	49.7±17.0*	119.0±25.6**	34
16	53.2±24.7**	110.9±33.8	33
17	62.9±30.3*	113.3±27.6**	47
17[a]	51.0±27.7	118.8±22.8***	29
20	52.2±32.5*	115.9±33.5*	41
20*	54.8±27.8**	109.9±23.7	35
21	31.0±21	133.7±23.8***	54

Quantitation of the relative positions of the closest and next closest chromosome homologs in rosettes of triploid measured in degrees. Each rosette encompassed 360° and the exact center of the rosette was used as a reference point. Values represent means±SD. The angular separation between the two closest homologs was remarkably consistent for each chromosome, but varied from one chromosome to the next. Separation angles of the next closest homologs approximate the expected 120° angle of separation for homologs in adjacent haploid sets with identical chromosome spatial ordering. *P* values refer to the level of significance of the difference between actual homolog separation angles and comparable data generated by random simulation as determined by the *t*-test. Data lacking a *p* value were not significantly different from the random simulation at $P<0.05$

* $P<0.05$; ** $P<0.01$; *** $P<0.001$

[a] Data obtained from triploid cell line GM01322; the remainder were from the AG00025 triploid cell line

Relative positioning of chromosome homologs in rosettes of triploid cells

As a separate test for the early origin and faithful propagation of chromosome spatial order in human cells, we examined the distribution of a number of selected chromosomes in rosettes obtained from two human triploid skin fibroblast cell lines (karyotype=69, XXY). As for the trisomy 21 cells, mitotic triploid cells were isolated by selective detachment and subjected to FISH with digoxigenin-labeled, chromosome-specific DNA probes, including a whole chromosome 7 painting probe, probes specific for the alpha-satellite regions of chromosomes 1, 3, 7, 10, 11, 16, 17, 20 and the Quint-Essential 21 probe (Oncor), which targets the DZ701 locus of chromosome 21 (e.g., Fig. 2I–N). Quantitation of the spatial separation of chromosome homologs within circular rosettes satisfying the selection criteria was accomplished by measuring their angular separation as described above. Angular separation distances between the two closest and next closest homologs in triploid rosettes were determined (Table 2). Based on the results described above for normal diploid fibroblasts, we expected that chromosomes in triploid rosettes would be arranged into three separate, but ordered, haploid sets, with homologs positioned equidistant from one another (i.e., roughly 120° apart within the rosette) (Fig. 3). In accord with our expectations, all 69 chromosomes in mitotic triploid cells were indeed invariably incorporated into a single rosette. However, despite the fact that the relative distribution of specific chromosome homologs in triploid rosettes was remarkably consistent and nonrandom, homologs were clearly not equidistant from one another. Figure 2I–L shows representative triploid rosettes illustrating the relative distribution of the homologs of chromosomes 1, 3, 10 and 17, respectively. Typically, two of the three chromosome homologs were found in relatively close proximity on one side of the rosette, with the third homolog situated on nearly the opposite side. Measurements of angular separation distances between the two closest homologs revealed significant chromosome-specific differences (Table 2). For example, the closest chromosome 3 homologs were positioned approximately four times more distant from one another than were those of chromosome 21. With all 69 chromosomes arranged into a single circular rosette encompassing 360°, each chromosome can thus be considered to occupy 5.2° of the 360° circle, thus making it possible also to express the separation distance between chromosome homologs in terms of the estimated number of interposed chromosomes. This estimate, of course, ignores the small variations in the observed width of individual mitotic chromosomes. As an example, the two closest chromosome 21 homologs are separated by a mean space equivalent to three chromosomes, whereas those of chromosome 3 are separated by a gap of approximately 16 chromosomes. Comparable data for the two closest homologs of chromosomes 1, 7, 10, 11, 16, 17 and 20 are presented in Table 2. Interestingly, measurements of the separation angles between the next closest homologs for the chromosomes examined in this study were found to closely approximate the expected 120° (Table 2), suggesting that chromosome spatial order in two of the three adjacent haploid sets is identical. The observed relative chromosome homolog positioning within triploid rosettes can readily be explained if one allows that chromosomes are arranged as three distinct, ordered haploid sets, and that the order of chromosomes within one of the haploid sets is inverted relative to the others (Fig. 3). Such an arrangement would cause chromosomes situated at or near the ends of this third haploid set to be brought into unusually close proximity to one of their homologs, whereas the remaining homolog is positioned at an angle of 120° from one of the two closest homologs. This arrangement means that chromosomes positioned more centrally within the haploid set would be less affected by its inversion, depending on how close the chromosome is to the center of the haploid set (Fig. 3). For the chromosomes examined in the present study, we have observed a mean separation angle between the closest homologs in triploid rosettes as high as 84.7° (e.g., chromosome 3), a value that begins to approach the expected maximal separation angle of 120° for chromosomes positioned at the exact center of each haploid set (Table 2). Comparison of the closest and next closest homolog separation distances for chromosomes 7, 17 and 20 revealed no significant differences between the two triploid cell lines. Results obtained using dual FISH with probes specific for chromosomes 10 and 17 (Fig. 2N) were consis-

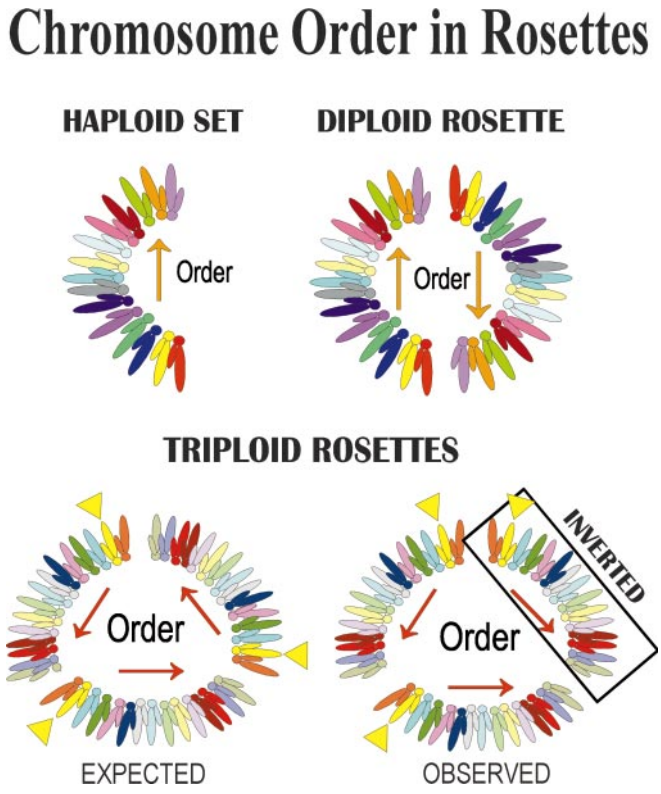

Fig. 3. A model for the arrangement of chromosomes in diploid and triploid rosettes of human cells. Chromosomes are organized into haploid sets, each containing 23 different chromosomes in a specific order inherited from one parent. The diploid rosette is assembled at the first metaphase after fertilization by end-to-end fusion of the two ordered haploid sets contributed by the parents. The relative order of chromosomes within each haploid set (*arrows*) is identical, and haploid sets are oriented such that homologs are consistently positioned on opposite sides of the diploid rosette. This spatial arrangement of chromosomes is propagated faithfully by mitosis throughout subsequent cell generations in normal cells. In triploid cells, all 69 chromosomes are assembled into a single rosette formed by three haploid sets. The spatial order of chromosomes in each haploid set is identical. The direction or orientation of chromosome order within each haploid set was expected to be maintained in triploid rosettes, resulting in homologs (*yellow arrowheads*) positioned at 120° angles from one another (*EXPECTED*). Instead, in the two triploid cell lines used in the present study, the order of chromosomes in one of the three haploid sets is inverted with respect to the other two (*OBSERVED*). This inversion causes homologs of chromosomes positioned at or near the ends of the haploid set to be brought into unusually close proximity, whereas those positioned more centrally within haploid sets are less affected

tent with the idea of three haploid sets, one of which is inverted with respect to the other two sets as shown schematically in Fig. 3.

Discussion

The issue of whether or not chromosomes are preferentially arranged relative to one another in interphase and mitotic cells remains unclear, and abundant evidence both for and against a nonrandom chromosome organization exists (Comings 1980; Heslop-Harrison and Bennett 1984; Manuelidis 1990; Haaf and Schmid 1991; Spector 1993). Here, we have taken advantage of the fact that chromosome rosettes appear to be a common feature of mitotic human cells and have utilized them to explore the possibility that different human cell types have a consistent pattern of chromosome spatial organization that originates very early in development. In our previous study, we showed that the chromosome 7, 8, 16 and X homologs were positioned on opposite sides of rosettes, and that these chromosomes exhibited a consistent relative spatial relationship within each half-rosette (Nagele et al. 1995). The present study confirms these results and shows that the chromosome 7, 11, 21 and 22 homologs are also positioned on opposite sides of rosettes in normal diploid fibroblasts. These findings agree with earlier observations that close association of chromosome homologs in normal mitotic cells of mammals is not normally observed (Emmerich et al. 1989; Fergusson and Ward 1992; Leitch et al. 1994), although in *Drosophila* and other dipteran insects it is a well-established phenomenon (Tartof and Henikoff 1991; Chandley et al.1996). Taken together, this work strengthens our proposal that rosettes are composed of two spatially distinct, tandemly linked, groups of 23 chromosomes (haploid sets), reminiscent of the organization that is now thought to exist in some plant cells (Heslop-Harrison and Bennett 1984; Schwarzacher et al. 1992; Leitch et al. 1991). It also raises the interesting possibility that each haploid set represents a cohesive, ordered group of homologs inherited from one parent.

Trisomy 21 and triploid cells provide evidence for an early origin for chromosome spatial order

To examine the possibility of an early origin for chromosome spatial order in rosettes of mitotic human cells, we compared chromosome distribution patterns in rosettes of normal cells with cells showing specific, well-characterized chromosome numerical anomalies known to arise early in development. First, we analyzed rosettes in two early passage cell lines derived from nondisjunction trisomy 21 (Down syndrome) patients. Trisomy is the most prevalent chromosome abnormality in humans, and has been found in at least 4% of all clinically recognized pregnancies and in nearly 10% of preimplantation embryos (Hassold and Jacobs 1984). Despite the high incidence and clinical importance of nondisjunction trisomy 21, relatively little is known about mechanisms that cause chromosome nondisjunction except that it is strongly correlated with maternal age and reduced recombination (Hassold and Jacobs 1984; Dagna-Bricarelli et al. 1989; Gaulden 1992; Sherman et al. 1994; Angell 1995; Hassold et al. 1995a, b). Evidence is accumulating that the majority of trisomies arise during the first maternal meiotic division (Sherman et al. 1994; Hassold et al. 1995b). Our results show that, in both trisomy 21 cell lines, chromosome 21 homologs were distributed in rosettes such that two of the three chromosome 21 homologs were closely juxtaposed, while the remaining chromosome 21 was positioned on the opposite side of the rosette. This suggests that the nondisjoined chromosome 21 is inserted into

the rosette adjacent to another chromosome 21 homolog, which is consistent with our current understanding of the mechanics of chromosomal nondisjunction (Dagna-Bricarelli et al. 1989; Gaulden 1992; Sherman et al. 1994; Angell 1995; Hassold et al. 1995a). If this proves to be the case, a similar pattern of relative homolog positioning would be expected in rosettes of cells with trisomies involving other chromosomes. Thus, our results suggest that the specific distribution pattern of chromosome 21 homologs seen here is a structural remnant of the nondisjunction event, and that the additional chromosome was incorporated into the chromosome rosette during the formation of the first mitotic rosette after fertilization. The fact that both cell lines derived from Down syndrome patients exhibited nearly identical chromosome 21 distribution patterns indicates that this chromosome spatial order is faithfully transmitted via mitosis from gestation into adulthood. Interestingly, the distribution of chromosome 21 homologs in the majority of interphase (early G1-phase) nuclei in mitotic shake-off preparations was nearly identical to that of rosettes, i.e., two of the three chromosome 21 homologs were closely juxtaposed, suggesting that the specific chromosome arrangement within rosettes influences the relative positions of chromosomes at interphase, or vice versa.

To test further the possible early origin for chromosome spatial order within mitotic rosettes, we analyzed the chromosome distribution in rosettes of two cell lines derived from triploid individuals. Our results show that all 69 chromosomes are incorporated into a single rosette. Analysis with FISH suggests that triploid rosettes contain chromosomes that are segregated into three, tandemly linked, spatially ordered, haploid sets. In both triploid cell lines, chromosome positional order within one haploid sets was inverted with respect to the other two sets. In addition, we found that the magnitude of the spatial separation of the two closest homologs in triploid cells was chromosome specific, providing additional strong evidence that the location of specific chromosomes within each haploid set is not random. Since triploidy and nondisjunction trisomy 21 represent chromosome numerical anomalies that arise early in development (e.g., at meiosis or during the first few mitoses) (Kaufmann 1991; Miny et al. 1995), we conclude that normal and abnormal chromosome distribution patterns in mitotic human cells are established extremely early in development and are propagated faithfully by mitosis throughout embryonic development and into adult life. In view of this, we propose that the observed segregation of chromosome homologs into two haploid sets in normal diploid cells is a vestige of fertilization and reflects segregation of maternal and paternal chromosomes as shown in some plant cells (Leitch et al. 1991; Schwarzacher et al. 1992).

Functional significance of chromosome spatial order

The results of the present study also prompt speculation as to why chromosomes maintain such a consistent spatial order in mitotic human cells. First, it is conceivable that chromosome spatial order helps to ensure the remarkable high degree of accuracy of chromosome segregation during mitosis (Ault and Nicklas 1989; McIntosh and Koonce 1989; McIntosh 1994; Nicklas 1997). Mechanisms involved in assembly of the chromosome rosette and the basis for its structural integrity are not known. Current dogma posits that chomosomes move randomly and individually to the metaphase plate during congression as a result of a delicate balance between forces associated with the directional instabilities of sister kinetochores and "polar ejection" forces arising from the outgrowth of microtubules and their "impact" on chromosomes (Cassimeris et al. 1994; Rieder and Salmon 1994). However, a high degree of chromosome positional order within the rosette appears to be inconsistent with the idea of random and individual chromosome movements based on microtubule "capture" at kinetochores during congression. For example, it is difficult to reconcile how random and individual chromosome movements could generate the highly ordered chromosome distribution within rosettes. A possible explanation for this apparent discrepancy may be that much of the data on chromosome movements during mitosis has been derived from nonmammalian cell systems. A second possible reason for a consistent chromosome spatial order in rosettes and interphase nuclei may be that relative chromosome position is crucial for maintaining normal patterns of gene expression as discussed in numerous models proposed by others (Comings 1980; Heslop-Harrison and Bennett 1984; Manuelidis 1984; Blobel 1985; Gasser and Laemmli 1987; Manuelidis 1990; Haaf and Schmid 1991; Cremer et al. 1993; Dernberg et al. 1996). Indeed, changes in relative chromosome spatial order, such as those described here for nondisjunction trisomy 21 and triploidy, are associated with abnormalities of gene expression that may be causal of the associated congenital anomalies. Perhaps certain genes function properly only when operating within the appropriate chromosome environment or context, i.e., when they are surrounded by the correct chromosomes (Karpen 1994). Mechanisms associated with this putative chromosomal "cross-talk" have been proposed (Bestor et al. 1994; Monk 1995), but testing these models has proved challenging and solid evidence for such mechanisms is still lacking. However, such a mechanism could provide a straightforward explanation for the fact that human embryos with a single extra copy of most chromosomes (i.e., trisomies) either do not develop at all or abort early, whereas a triploid fetus with a full extra set of 23 chromosomes can develop and even go to full-term, albeit with severe congenital malformations. We propose that survival of triploid cells and embryos is due to the fact that, as shown here, the extra chromosomes are still able to function within their normal context because they are inserted into the chromosome rosette as a coherent group of 23 chromosomes in the correct relative spatial order. Thus, gene context on a chromosomal scale as well as gene dosage may be crucial for appropriate gene expression.

Insertion of chromosomes into rosettes

Finally, our results suggest that both normal and abnormal chromosome configurations can be transmitted faithfully to all cells throughout the life of the individual. Based on our data on triploid and trisomy 21 cells presented above, it is tempting to speculate that the evolution of aneuploidy in cancer cells involves a situation similar to that of triploidy and trisomy 21, where additional chromosomes are stably inserted into the rosette, generating a new, but inevitably abnormal, chromosome positional order. When this new chromosome order is not lethal, cells are likely to be phenotypically different from the parent cell. In cases where this new chromosome order imparts a selective growth advantage, a prolific cell clone may arise that carries this new chromosome configuration along with its associated aberrant phenotype, thereby completing a key step in the evolution of cancer.

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