Size-Dependent Positioning of Human Chromosomes in Interphase Nuclei

Hui Bin Sun, Jin Shen, and Hiroki Yokota
Biomedical Engineering Program, Indiana University Purdue University at Indianapolis, Indianapolis, Indiana 46202 USA

ABSTRACT By using a fluorescence in situ hybridization technique we revealed that for nine different q-arm telomere markers the positioning of chromosomes in human G_1 interphase nuclei was chromosome size-dependent. The q-arm telomeres of large chromosomes are more peripherally located than telomeres on small chromosomes. This highly organized arrangement of chromatin within the human nucleus was discovered by determining the *x* and *y* coordinates of the hybridization sites and calculating the root-mean-square radial distance to the nuclear centers in human fibroblasts. We demonstrate here that global organization within the G_1 interphase nucleus is affected by one of the most fundamental physical quantities—chromosome size or mass—and propose two biophysical models, a volume exclusion model and a mitotic preset model, to explain our finding.

INTRODUCTION

Eukaryotic chromosomes, each composed of a linear DNA molecule and DNA-binding proteins, are confined within the nucleus. A human nucleus is ~10 μm in diameter and contains 46 chromosomes consisting of ~2 m of DNA in total. In the late 19th century biologists raised the question as to how well chromosomes are organized inside each nucleus (Rabl, 1885). The anatomy of a mammalian interphase nucleus, especially at G_1 interphase where chromosomes are de-condensed and many genes are actively transcribed, has been found to be a partially organized mixture (Gasser and Laemmli, 1987; Manuelidis, 1990; Haaf and Schmid, 1991; Cook, 1995; Spector, 1996). An individual chromosome is locally confined, occupying a discrete space often called a chromosome domain or chromosome territory (Lichter et al., 1988; Pinkel et al., 1988). Each chromosome domain or territory is irregularly shaped and largely immobile at the submicron level (Abney et al., 1997; Marshall et al., 1997). We have shown previously that the relative positioning of sister chromosomes in a pair of daughter cells is correlated with each other (Sun and Yokota, 1999). Despite these organizational features, large variations in the anatomy of the mammalian nucleus exist from one cell to another, unlike the orderly aligned chromosomes observed in *Drosophila* embryos (Hiraoka et al., 1990; Fung et al., 1998).

In this study we examined the chromosomal organization of the mammalian nucleus to see if any order would emerge from a seemingly random positioning of chromosomes. Our working hypothesis was a size-dependent positioning of chromosomes based on the following observation and reasoning. Because of a volume exclusion among irregularly shaped chromosomes, the positioning of any particular region of chromosomes would exhibit a wide variation in a population of cells. This positional variation could be modeled by using an inertial parameter such as chromosome size because the excluded volume should be correlated to chromosome size. Alternatively, because the divisional segregation of chromosomes is, at least in part, a regulated process mediated by microtubule and nuclear matrix-associated proteins, the wide positional variation could result from a geometric configuration of chromosomes at mitosis. Mitotic chromosomes are configured in a wheel-like rosette and all centromeres are clustered inside the rosette. Internal positioning of small chromosomes during mitosis would maintain their central localization in G_1 interphase nuclei. Human chromosomes were originally labeled according to their approximate DNA content. Chromosome 1 is estimated to consist of 263 megabase (Mb) pairs of nucleotides and is approximately five times longer, and therefore fivefold more massive, than chromosome 21 (Morton, 1991). We postulated that significant differences in size (and therefore mass) among human chromosomes would allow us to examine a size-dependent distribution of chromosomes within the G_1 interphase nucleus.

Fluorescence in situ hybridization provides a powerful tool for visualizing the localization of specific regions of chromosomes within G_1 interphase nuclei (Rappold et al., 1984; Emmerich et al., 1989; Popp et al., 1990; Hofers et al., 1993a, b; Chandley et al., 1996). The location of specific chromosomal sites can be detected in whole mounts of fibroblast cells by direct microscopic examination. In this report nine unique DNA probes, each corresponding to a specific subtelomeric region of a different q-arm, were used to establish the position of related chromosomes inside the G_1 nucleus. Because chromosomes are organized as partially de-condensed flexible polymers at G_1 interphase, any single measurement could not be used to accurately reveal a physical principle underlying chromosome positioning. A

Received for publication 13 May 1999 and in final form 2 February 2000.
Address reprint requests to Hiroki Yokota, Ph.D., Department of Anatomy and Cell Biology, Indiana University School of Medicine, 635 Barnhill Drive, MS504, Indianapolis, IN 46202. Tel.: 317-274-2448; Fax: 317-278-2040; E-mail: hiroki@anatomy.iupui.edu.

0006-3495/00/07/184/07 $2.00

statistical approach was therefore undertaken. Radial distances (from the center of the nucleus to the hybridization site of the DNA probe on a plane) were measured, and the root-mean-square (rms) radial distance was determined. Each of the nine probes used herein marked a subtelomeric region on a different chromosome q-arm. Although chromosome painting probes could show the global positioning of chromosome domain, these specific probes in this study facilitated to define radial distances.

We demonstrate here that the q-arm telomeres on large chromosomes are more peripherally located than telomeres on small chromosomes, and that there exists a chromosome-size dependence for positioning in the human fibroblast G_1 interphase nucleus. The observed nuclear organization allowed us to propose two biophysical models that include a volume exclusion model and a mitotic preset model. The strengths and weaknesses of each model are evaluated in the Discussion.

MATERIALS AND METHODS

Cell preparation

Normal human fibroblasts from a male (HLF-CCL135, approximately passage 5; American Type Culture Collection, Rockville, MD) were grown on coverslips to 90–95% confluency in MEM supplemented with 20% fetal calf serum. Cells were then cross-linked by paraformaldehyde as described previously without freezing or thawing (Popp et al., 1990; Yokota et al., 1997). In brief, fibroblasts were rinsed in PBS (154 mM NaCl, 4 mM $MgCl_2$, 2.7 mM Na_2HPO_4, and 1.5 mM KH_2PO_4, pH 7.2) and fixed in 3% paraformaldehyde in PBS for 5 min at 25°C. The fixed cells were exposed to 0.01% Triton X for 10 min and 0.1 M Tris-HCl (pH 7.2) for 2 min, and equilibrated in 20% glycerol in PBS for 20 min. The cells were used immediately or stored in PBS containing 0.04% sodium azide at 4°C for up to two weeks. Although the fixation process used in this study is considered to preserve the cellular structure, the results are subject to perturbation by the fixation step.

Fluorescence in situ hybridization

Nine commercially available telomeric DNA probes that specifically label the q-arm termini of chromosomes 1, 2, 3, 7, 11, 12, 18, 19, and 21 were obtained (TelVysion telomere DNA probes, Vysis, Downers Grove, IL). Each of these telomeric probes contains chromosome-specific subtelomeric DNA sequences estimated to be located within 300 kb of the end of the chromosome (Reithman et al., 1989; National Institutes of Health and IMMC, 1996). Among nine chromosomes, chromosome 21 contains the nucleolus organizer region.

Fixed cells on coverslips were denatured at 90°C in 70% formamide/2XSSC for 5 min and quenched in cold 70% formamide/2XSSC. A 10 μl aliquot of hybridization mixture containing 0.5 μl spectrum orange-labeled DNA probe was applied to the denatured cells on the coverslip and incubated overnight. Hybridized nuclei were washed in 50% formamide/2XSSC for 20 min followed by in 2XSSC for 20 min. Nuclei were counterstained with 2 μM 4′,6-diamidino-2-phenylindole (DAPI) in an antifade solution containing 90% glycerol and 1 μg/μl phenyldiamine.

Determination of radial distance

Flat fibroblast nuclei were viewed using a cooled CCD camera (Pentamax, Princeton Instruments, Trenton, NJ) mounted on a Nikon Diaphot 300 fluorescence microscope (Fryer Co., Huntley, IL) with 100 × 1.4 NA Plan Apo oil objective. Spectrum orange signals were detected by a band-pass filter designed for detecting Texas Red (ChromaTechnology, VT). Randomly selected nuclei were imaged using the Metamorph imaging software (version 3.0, Universal Imaging, West Chester, PA). From a single nucleus a pair of radial distances between the nuclear center and hybridization sites of telomeric probes were measured on the projected focal plane. The center of a nucleus was determined as a midpoint on the major axis that passed through the longest axis of the nucleus (Fig. 1 *B*). The positioning of chromosomes was referred to the center of a nucleus defined on the projected plane. Rms distances were calculated together with standard deviation and standard error of the mean (SEM) from an average of 53 nuclei (range: 45–66). In order to estimate errors associated with the measurements, we measured R_1, R_2, θ_1, and θ_2 twice using a probe corresponding to the centromeric region of chromosome 1, and independently determined their mean value. The error, defined by $2(m_1 - m_2)/(m_1 + m_2)$, where m_1 = mean value from the first measurement and m_2 = mean value from the second measurement, was 1.6%, 1.1%, 0.8%, and 0.8% for R_1, R_2, θ_1, and θ_2, respectively.

Monte Carlo simulation

In order to simulate a uniform distribution whereby any chromosome is evenly placed in a nucleus, a numerical simulation was performed using the Monte Carlo method. We modeled the shape of a fibroblast nucleus as an ellipsoid. The major and the minor axes of the ellipsoid were defined by the mean nucleus dimension of the fibroblast cells used in the experiment. In

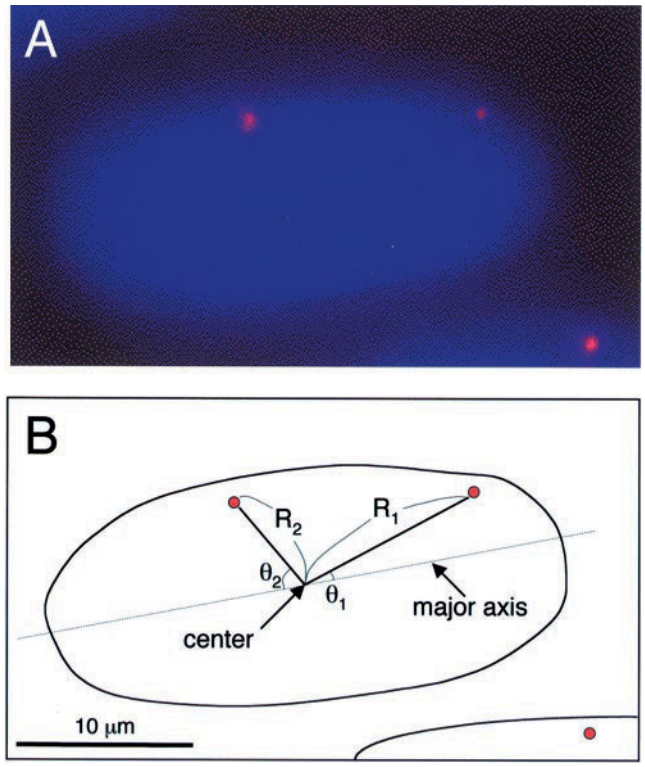

FIGURE 1 Typical fibroblast nucleus used in the study. (*A*) Fibroblast nucleus labeled with a DNA probe marking a subtelomeric region of the chromosome 1 q-arm (counterstained with DAPI). (*B*) Trace image of (*A*) where R_1 and R_2 are defined as radial distances, and θ_1 and θ_2 are defined as radial angles between 0 and 90°. The *x* and *y* coordinates were calculated as $R_i \cos \theta_i$ and $R_i \sin \theta_i$ (i = 1, 2), respectively. Bar = 10 μm.

the simulation two positions were randomly chosen corresponding to a homologous pair of hybridization sites within the ellipsoid. Rms radial distance was determined from 100,000 randomly assigned points. Because all distances were determined on the *xy*-plane, any non-zero value of nuclear thickness yielded the same rms distances in the Monte Carlo analysis.

RESULTS

Fibroblast nuclei were gently fixed with a cross-linker to better preserve three-dimensional nuclear structure for the resolution desired for this study. The majority of nuclei were determined to be in G_1 interphase based on the diploid number of hybridization signals. The nuclei were shaped like flattened ellipsoids and their mean shape was measured as a major axis of 32.2 $\pm$ 4.7 μm and a minor axis of 18.0 $\pm$ 1.9 μm (average of 111 nuclei). A typical nucleus hybridized with a probe marking the q-arm of chromosome 1 (counterstained with DAPI) is illustrated in Fig. 1. The nuclei of fibroblasts are well suited for the two-dimensional analysis because cells are flattened against the surface of the culture flask during interphase, and the nucleus appears nearly two-dimensional.

Cumulative hybridization sites exhibited a chromosome size-dependent pattern

To search for a general pattern of the positioning of chromosomes all hybridization sites were simultaneously plotted in one quadrant of the mean-sized nucleus for representative DNA probes (e.g., chromosomes 1, 2, 11, 12, 19, and 21) (Fig. 2, *A–F*). Clearly no chromosome showed a fixed localization pattern. However, there existed a chromosome size-dependent pattern of hybridization sites. In Fig. 2, the radius of the dotted quarter circle corresponds to the median of radial distances, where 50% of hybridization sites are located inside this circle and the other 50% outside the circle. The hybridization sites for large chromosomes (chromosomes 1 and 2) were scattered throughout the quadrant,

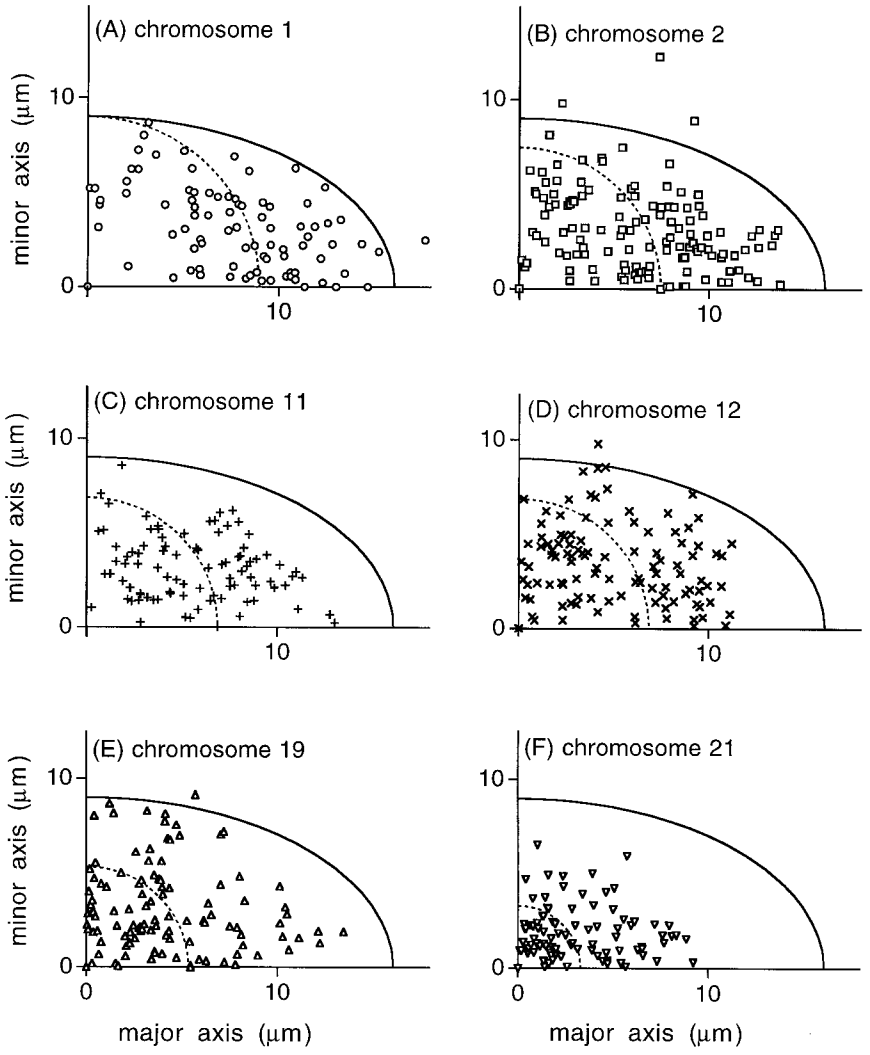

FIGURE 2 Cumulative plots of hybridization sites. All hybridization sites are plotted in a quadrant of the nucleus (16.1 μm $\times$ 9.0 μm). The dotted circle with radius equal to median value is illustrated where 50% of hybridization sites are located inside this circle. (*A*) Chromosome 1 hybridization sites (90 sites with median of 8.99 μm). (*B*) Chromosome 2 hybridization sites (132 sites with median of 7.46 μm). (*C*) Chromosome 11 hybridization sites (94 sites with median of 6.86 μm). (*D*) Chromosome 12 hybridization sites (104 sites with median of 6.86 μm). (*E*) Chromosome 19 hybridization sites (102 sites with median of 5.33 μm). (*F*) Chromosome 21 hybridization sites (100 sites with median of 3.31 μm).

while the sites for intermediate-sized chromosomes (chromosomes 11 and 12) were shifted toward the mid-nuclear region with few sites near the periphery. Furthermore, the sites for small chromosomes (chromosomes 19 and 21) were more clustered near the center of the nucleus than other chromosomes. The median radial distances were determined as 8.99, 7.46, 6.86, 6.86, 5.33, and 3.31 μm for chromosomes 1, 2, 11, 12, 19, and 21, respectively.

Rms radial distances were significantly smaller for q-arm probes of smaller chromosomes than for larger chromosomes

In order to quantify the chromosome size-dependent pattern of hybridization sites, the rms radial distances were plotted as a function of their cognate chromosome size, which ranged in size from 50 Mb (chromosome 21) to 263 Mb (chromosome 1) (Fig. 3 *A*). Chromosome 1's rms value was 9.6 $\pm$ 3.0 μm (n = 90), 2.1 times longer than that of chromosome 21 (4.5 $\pm$ 2.2 μm; n = 100). A positive correlation exists, therefore, between rms radial distance and chromosome size, with r^2 = 0.79 (r = correlation coefficient). Rms radial distances were also positively correlated to their size of q-arm (correlation coefficient of r^2 = 0.73) (Fig. 3 *B*). Using the Monte Carlo method, the hypothetical rms radial distance was simulated where a particular chromosome was evenly positioned at an equal frequency in any part of the nucleus. This simulated rms radial distance was 8.2 $\pm$ 3.3 μm (n = 100,000), close to the rms value of 8.1 $\pm$ 3.0 μm for chromosome 3.

Size and shape parameters of Weibull distributions best-fitted to radial distance distributions were chromosome size-dependent

Statistical variations of the chromosome size-dependent positioning of the G_1 interphase chromosomes were characterized. The distribution of radial distances was approximated by a Weibull distribution and two statistical parameters in the Weibull distribution, best-fitted to the fluorescence in situ hybridization results, were analyzed. Defining R as radial distance, the Weibull distribution function is defined as $1 - \exp\{-(R/\beta)^{\alpha}\}$ with two statistical parameters: α (shape parameter) and β (scale parameter). In general, a small α value makes the shape of the probability density function scattered, while a small β value decreases mean and median values. The Weibull distribution is a general form of the Rayleigh distribution (α = 2; two-dimensional Gaussian) that is used to characterize the random feature of interphase chromatin structure (Sachs et al., 1995; Yokota et al., 1995). For the distribution of radial distances in this study, α values varied depending on chromosomes.

Fig. 4, *A* and *B* illustrate two representative histograms corresponding to DNA probes marking the q-arm of chromosomes 1 and 21, respectively. The hypothetical histogram corresponding to the uniform distribution simulated by the Monte Carlo method is illustrated by dotted bars. The distribution of the chromosome 1 q-arm was skewed toward longer distances. The distribution of the chromosome 21 q-arm, in contrast, was skewed toward shorter distances.

The radial distance distributions obtained by fluorescence in situ hybridization were well-approximated by the Weibull distribution with r^2 = 0.995–0.999 (r, correlation coefficient). Fig. 4 *C* illustrates distribution functions of chromosomes 1, 11, and 21 together with the hypothetical uniform distribution. Shape and size parameters in the Weibull distributions were positively correlated to chromosome size (Fig. 4 *D*). The value of α ranged from 1.7 (chromosome 21) to 3.3 (chromosome 1), while the value of β ranged from 4.5 (chromosome 21) to 10.1 (chromosome 1). Thus, the statistical variations of radial distances are also chromosome size-dependent. Large chromosomes (e.g., chromosomes 1 and 2) have a larger-scaled, narrower-shaped distribution than small chromosomes (e.g., chromosomes 19 and 21).

DISCUSSION

This study reveals that human chromosomes are positioned in G_1 interphase nuclei according to size. This was established by determining rms radial distances as well as two

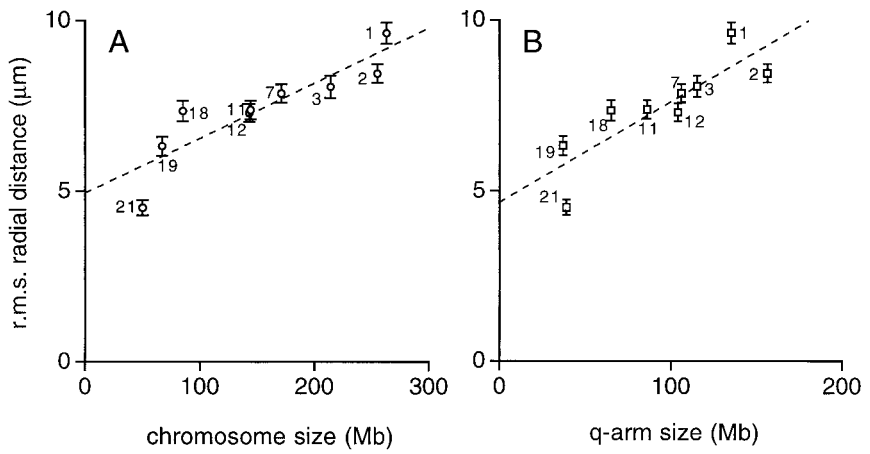

FIGURE 3 The relationship of rms radial distance to chromosome size and q-arm size. Rms radial distance for probes marking a subtelomeric region of q-arm on chromosomes 1, 2, 3, 7, 11, 12, 18, 19, and 21 are plotted as a function of chromosome size and q-arm size. Each data point represents an average of 106 radial distance measurements (range: 90–132) with standard error of the mean (SEM) of 0.22–0.32. The number next to the data point indicates the chromosome identification number. (*A*) Rms radial distance and chromosome size. The dashed line is the best-fit linear regression line with r^2 = 0.79 (r, correlation coefficient). (*B*) Rms radial distance and q-arm size. The dashed line is the best-fit linear regression line with r^2 = 0.75.

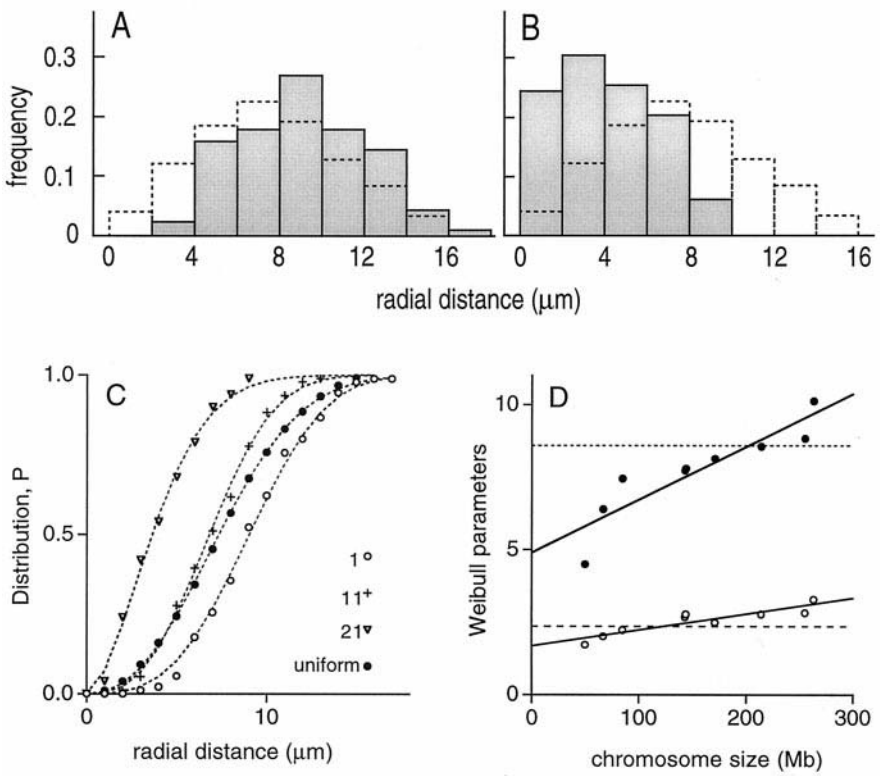

FIGURE 4 Representative histograms and Weibull approximation of radial distance distributions. (*A*) Histogram of radial distance for chromosome 1 q-arm (90 hybridization sites). The dotted histogram corresponds to the uniform distribution simulated by the Monte Carlo method. (*B*) Histogram of radial distance for chromosome 21 q-arm (100 hybridization sites). The dotted histogram is the same as (*A*). (*C*) Weibull approximation of radial distributions for chromosomes 1, 11, 21, and the uniform distribution obtained by the Monte Carlo simulation. Median is estimated as $\beta(\ln 2)^{1/\alpha}$. (*D*) Statistical parameters (α and β) in the Weibull approximation as a function of chromosome size. The open circle represents α (shape parameter) and the filled circle shows β (size parameter). The dashed and dotted lines indicate α and β for the uniform distribution. The best-fit linear regression gave an r^2 value of 0.83 for α and 0.82 for β.

statistical parameters in the best-fitted Weibull distribution. Using gently fixed fibroblast nuclei and nine DNA probes, each of which marks the tip of a chromosome's q-arm, quantitative data were collected. The statistical analysis validated the conclusion that size and position are correlated. Smaller chromosomes such as chromosomes 19 and 21 are more centrally positioned with respect to the center of a nucleus on the projected plane than the q-arms on larger chromosomes, such as chromosomes 1 and 2. Although telomeric probes represent only a small region of a chromosome, the positioning data are striking. A chromosome size-dependent positioning has also been observed using other non-telomeric probes located on chromosomes 4, 5, 7, 8, 14, 17, 19, 20, and X in human lymphoblast cells and fibroblast cells (H.Y., unpublished data). The central positioning of chromosome 21 can be explained by the positioning of the nucleolus. A nucleolus is a specialized structure for the synthesis of ribosomal RNAs. In the human nucleus, it is formed from chromosomes 13, 14, 15, 21, and 22. These chromosomes contain a nucleolus organizer region, and the organizer regions fuse to form a centrally located nucleolus in the G_1 nucleus. Therefore, the central positioning of these relatively small chromosomes (mean $\pm$ SD = 87 $\pm$ 31 Mb) may result from an active transport for the formation of a nucleolus on the nuclear center. However, even without nucleolus organizer chromosomes, there existed a clear chromosome size-dependence (r^2 = 0.79 including the chromosome 21 data and r^2 = 0.83 excluding the chromosome 21 data in Fig. 3 *A*).

Two biophysical models, i.e., a volume-exclusion model and a mitotic preset model, are proposed to account for the observed chromosome size-dependent distribution of human chromosomes (Fig. 5). Because of its finite, size any chromosome experiences steric hindrance and has a limited ability to co-occupy the primary domain of another chromosome. Such an excluded volume effect predicts a non-uniform, chromosome size-dependent spatial arrangement. Our ongoing analysis of inter-homolog distances (i.e., distance between a pair of homologous chromosomes) shows a positive correlation of rms inter-homolog distances to chromosome size (Sun and Yokota, 1999). This chromosome size-dependence of inter-homolog distances and the size-dependence of radial distances are qualitatively consistent with the volume exclusion model. The steric hindrance between chromosomes and a nuclear envelope, however, may qualitatively contradict the observed size-dependence of radial distances. This steric hindrance to the nuclear envelope maintains any chromosome inside the nucleus. Because of a difference in volume, an approach of large chromosomes to the envelope is more limited than small chromosomes.

An alternative scheme, i.e., a mitotic preset model, would explain the fluorescence in situ hybridization observation based on the positioning of chromosomes in mitosis. In this model, a centromere provides the size-dependent positioning during mitosis. That size-dependence during mitosis would be maintained during G_1 interphase. It has previously been reported that small chromosomes are centrally located

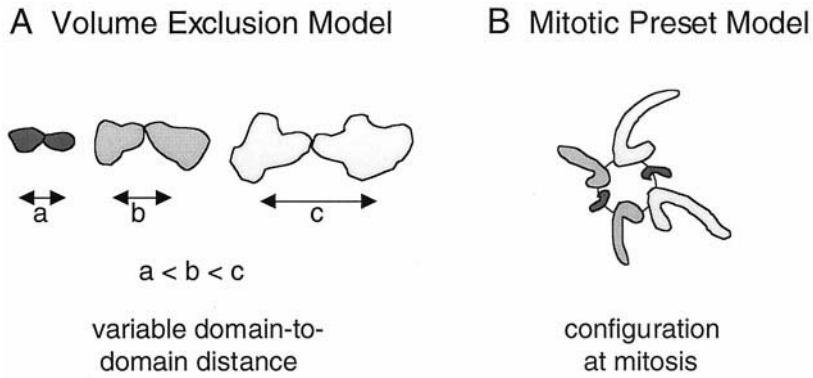

FIGURE 5 A schematic diagram explaining the volume exclusion model and the mitotic preset model. (*A*) Volume exclusion model. The mean distance between a pair of chromosome domains is affected by their domain sizes. According to the volume exclusion model, a chromosome size-dependence for the positioning of chromosomes originates in this differential steric effect among chromosomes. (*B*) Mitotic preset model. Mitotic chromosomes are configured as a wheel-shaped rosette. Because centromeres are positioned toward the inside of the rosette, small chromosomes are located centrally and large chromosomes extend outward. According to the mitotic preset model, the positioning of chromosomes at G_1 interphase results from the configuration of chromosomes at mitosis.

on a metaphase plate (Wollenberg et al., 1982; Mosgoller et al., 1991; Leitch et al., 1994). Centromere-containing micro-chromosomes have been demonstrated to be situated on the center of the metaphase plate (Jakobsson et al., 1989), consistent with the positioning in G_1 interphase observed in this report. This model predicts that DNA fragments without centromeres would not obey the size-dependent distribution either at mitosis or G_1 interphase. Indeed, acentric double minute chromosomes, despite their small size, have been reported to be peripherally localized at metaphase (Levan and Levan, 1978). Many acentric minute chromosomes in HeLa cells are also peripherally positioned in the G_1 interphase nuclei (H.Y., unpublished data).

In investigating the molecular mechanism behind the size-dependent positioning, mobility of chromosome domains in the G_1 interphase nucleus needs to be considered. Chromosome domains with low mobility favor the mitotic preset model, because chromosome domains are immobile and the positioning of chromosomes set in mitosis is preserved during the G_1 interphase. High mobility, in contrast, is inconsistent with the mitotic preset model, and high fluidity may facilitate chromosome rearrangement based on volume exclusion. Previous photobleaching and fluorescence-tagging studies indicate that chromosomes in the G_1 interphase nucleus are immobile on a large scale, such as chromosome domains. In photobleaching experiments interphase chromatin was immobile over distances of >0.4 μm, as indicated by small spots bleached by a laser persisting for over an hour (Abney et al., 1997). The study of tagging specific chromosome sites in living cells has revealed that chromatin is free to undergo substantial Brownian motion, but any given chromatin segment is confined to a subregion of the nucleus (Marshall et al., 1997). This immobility of chromosome domains favors the mitotic preset model for the positioning of chromosomes in the G_1 interphase nucleus.

Understanding the molecular principles governing the positioning of chromatin loops or inter-chromosomal domains helps evaluate biophysical models that adequately describe the size-dependent chromosome positioning observed in this study. Chromatin is modeled to consist of 20–200 kb random-coil loops attached to scaffold associated regions (Milkovitch et al., 1984; Gerdes et al., 1994) or megabase-sized giant loops that are attached to a flexible backbone (Sachs et al., 1995; Yokota et al., 1995). Furthermore, chromatin is considered to be packaged into many compartments where the space between compartments forms inter-chromosomal domains (Cremer et al., 1993; Kurz et al., 1996). Investigating the relationship of these organized features in chromatin structure to the positioning of chromosomes is one of our future research areas. In conclusion, we demonstrate that large-scale organization within the G_1 interphase nucleus is affected by the most fundamental physical quantity—chromosome size or mass—and that the statistical methods developed in this report are useful for investigating the physical principle governing complex cellular organization.

We thank James McAteer, George Malacinski, and David Mack (Indiana University, IN) and Barbara J. Trask and Ger van den Engh (University of Washington, WA) for valuable suggestions.

This work was supported in part by the Whitaker Foundation.

REFERENCES

Abney, J. R., B. Cutler, M. L. Fillbach, D. Axelrod, and B. A. Scalettar. 1997. Chromatin dynamics in interphase nuclei and its implications for nuclear structure. *J. Cell Biol.* 137:1459–1468.

Chandley, A. C., R. M. Speed, and A. R. Leitch. 1996. Different distributions of homologous chromosomes in adult human Sertoli cells and in lymphocytes signify nuclear differentiation. *J. Cell Sci.* 109:773–776.

Cook, P. R. 1995. A chromomeric model for nuclear and chromosome structure. *J. Cell Sci.* 108:2927–2935.

Cremer, T., A. Kurz, R. Zirbel, S. Dietzel, B. Rnike, E. Schrock, M. R. Speicher, U. Mathieu, A. Jauch, P. Emmerich, H. Scherthan, T. Ried, C. Creme, and P. Lichter. 1993. Role of chromosome territories in the functional compartmentalization of the cell nucleus. *Cold Spring Harbor Symp. Quant. Biol*. LVIII:777–792.

Emmerich, P., P. Loos, A. S. Jauch, A. H. N. Hopman, J. Wiegant, M. J. Higgins, B. N. White, M. van der Ploeg, C. Cremer, and T. Cremer. 1989. Double in situ hybridization in combination with digital image analysis: a new approach to study interphase chromosome topography. *Exp. Cell Res.* 181:126–140.

Fung, J. C., W. F. Marshall, A. Dernburg, D. A. Agard, and J. W. Sedat. 1998. Homolog chromosome pairing in *Drosophila melanogaster* proceeds through multiple independent initiations. *J. Cell Biol.* 141:5–20.

Gasser, S. M., and U. K. Laemmli. 1987. A glimpse at chromosome order. *TIG.* 3:16–22.

Gerdes, M. G., K. C. Carter, P. T. Moen, and J. B. Lawrence. 1994. Dynamic changes in the higher-level chromatin organization of specific sequences revealed by in situ hybridization to nuclear halos. *J. Cell Biol.* 126:289–304.

Haaf, T., and H. Schmid. 1991. Chromosome topology in mammalian interphase nuclei. *Exp. Cell Res.* 192:325–332.

Hiraoka, Y., D. A. Agard, and J. W. Sedat. 1990. Temporal and spatial coordination of chromosome movement, spindle formation, and nuclear envelope breakdown during prometaphase in *Drosophila melanogaster* embryos. *J. Cell Biol.* 111:2815–2828.

Hofers, C., P. Baumann, G. Hummer, T. M. Jovin, and D. A. Arndt-Jovin. 1993a. The localization of chromosome domains in human interphase nuclei. Three-dimensional distance determinations of fluorescence in situ hybridization signals from confocal laser scanning microscopy. *Bioimaging.* 1:96–106.

Hofers, C., T. M. Jovin, G. Hummer, and D. J. Arndt-Jovin. 1993b. The localization of chromosome domains in human interphase nuclei. Semi-automated two-dimensional image acquisition and analysis of fluorescence in situ hybridization signals. *Bioimaging.* 1:107–118.

Jakobsson, A. H., Y. Wettergren, and T. Haaf. 1989. Chromatin bodies in multidrug resistant hybrid cells have centromeres and originate from homogeneously staining regions. *Anticancer Res.* 9:267–272.

Kurz, A., S. Lampel, J. E. Nickolenko, J. Bradl, A. Benner, R. M. Zirbel, T. Cremer, and P. Lichter. 1996. Active and inactive genes localize preferentially in the periphery of chromosome territories. *J. Cell Biol.* 135:1195–1205.

Leitch, A. R., J. K. M. Brown, W. Mosgoller, T. Schwarzacher, and J. S. Heslop-Harrison. 1994. The spatial localization of homologous chromosomes in human fibroblasts at mitosis. *Hum. Genet.* 93:275–280.

Levan, A., and G. Levan. 1978. Have double minutes functioning centromeres? *Hereditas.* 88:81–92.

Lichter, P., T. Cremer, J. Borden, L. Manuelidis, and D. C. Ward. 1988. Delineation of individual human chromosomes in metaphase and interphase cells by in situ suppression hybridization using recombinant DNA libraries. *Hum. Genet.* 80:224–234.

Manuelidis, L. 1990. A view of interphase chromosomes. *Science.* 250: 1533–1540.

Marshall, W. F., A. Straight, J. F. Marko, J. Swedlow, A. Dernburg, A. Belmont, A. W. Murray, D. A. Agard, and J. W. Sedat. 1997. Interphase chromosomes undergo constrained diffusional motion in living cells. *Curr. Biol.* 7:930–939.

Milkovitch, J., M.-E. Mirault, and U. K. Laemmli. 1984. Organization of the higher-order chromatin loop: specific DNA attachment sites on nuclear scaffold. *Cell.* 39:223–232.

Morton, N. E. 1991. Parameters of the human genome. *Proc. Natl. Acad. Sci. U.S.A.* 88:7474–7476.

Mosgoller, W., A. R. Leitch, J. K. M. Brown, and J. S. Heslop-Harrison. 1991. Chromosome arrangements in human fibroblasts at mitosis. *Hum. Genet.* 88:27–33.

National Institutes of Health and Institute of Molecular Medicine Collaboration. 1996. A complete set of human telomeric probes and their clinical application. *Nat. Genet.* 14:86–89.

Pinkel, D., J. Landegent, C. Collins, J. Fuscoe, R. Seagraves, J. Lucas, and J. W. Gray. 1988. Fluorescence in situ hybridization with human chromosome-specific libraries: detection of trisomy 21 and translocations of chromosome 4. *Proc. Natl. Acad. Sci. U.S.A.* 85:9138–9142.

Popp, S., H. P. Scholl, P. Loos, A. Jauch, E. Stelzer, C. Cremer, and T. Cremer. 1990. Distribution of chromosome 18 and X centric heterochromatin in the interphase nucleus of cultured human cells. *Exp. Cell Res.* 189:1–12.

Rabl, C. 1885. Uber Zellteilung, *Morphologisches Jahrbuch.* 10:214–330.

Rappold, G. A., T. Cremer, H. D. Hager, K. E. Davies, C. R. Muller, and T. Yang. 1984. Sex chromosome positions in human interphase nuclei as studied by in situ hybridization with chromosome specific DNA probes. *Hum. Genet.* 67:317–325.

Reithman, H. C., R. K. Moyzis, J. Meyne, D. T. Burke, and M. V. Olson. 1989. Cloning human telomeric DNA fragments into *Saccharomyces cerevisiae* using a yeast-artificial-chromosome vector. *Proc. Natl. Acad. Sci. U.S.A.* 86:6240–6244.

Sachs, R. K., G. van den Engh, B. J. Trask, H. Yokota, and J. E. Hearst. 1995. A random-walk/giant-loop model for interphase chromosomes. *Proc. Natl. Acad. Sci. U.S.A.* 92:2710–2714.

Spector, D. L. 1996. Nuclear organization and gene expression. *Exp. Cell Res.* 229:189–197.

Sun, H. B., and H. Yokota. 1999. Correlated positioning of homologous chromosomes in daughter fibroblast cells. *Chromosome Res.* 7:603–610.

Wollenberg, C., M. P. Kiefaber, and K. D. Zang. 1982. Quantitative studies on the arrangement of human metaphase chromosomes. *Hum. Genet.* 60:239–248.

Yokota, H., G. van den Engh, J. E. Hearst, R. K. Sachs, and B. J. Trask. 1995. Evidence for the organization of chromatin in megabase pair-sized loops arranged along a random-walk path in the human G_0/G_1 interphase nucleus. *J. Cell Biol.* 130:1239–1249.

Yokota, H., M. J. Singer, G. J. van den Engh, and B. J. Trask. 1997. Regional differences in the compaction of chromatin in human G_0/G_1 interphase nuclei. *Chromosome Res.* 5:157–166.