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Sex chromosome positions in human interphase nuclei as studied by in situ hybridization with chromosome specific DNA probes

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Summary. Two cloned repetitive DNA probes, pXBR and CY1, which bind preferentially to specific regions of the human X and Y chromosome, respectively, were used to study the distribution of the sex chromosomes in human lymphocyte nuclei by in situ hybridization experiments. Our data indicate a large variability of the distances between the sex chromosomes in male and female interphase nuclei. However, the mean distance observed between the X and Y chromosome was significantly smaller than the mean distance observed between the two X-chromosomes. The distribution of distances determined experimentally is compared with three model distributions of distances, and the question of a non-random distribution of sex chromosomes is discussed. Mathematical details of these model distributions are provided in an Appendix to this paper. In the case of a human translocation chromosome (Xqter→Xp22.2::Yq11→Yqter) contained in the Chinese hamster × human hybrid cell line 445×393, the binding sites of pXBR and CY1 were found close to each other in most interphase nuclei. These data demonstrate the potential use of chromosome-specific repetitive DNA probes to study the problem of interphase chromosome topography.

Introduction

Evidence that the arrangement of metaphase chromosomes does not occur at random has been obtained for a number of plant species and to a minor degree also for mammals including man (Bennett 1983; Comings 1980). In addition, it has been shown that such arrangements to a certain extent still reflect the arrangement of chromosome territories (also called chromosome domains) in the interphase nucleus (Cremer et al. 1984). In spite of this progress, the problem of chromosome topography in interphase nuclei and its possible functional significance (Lewin 1981; Vogel and Krüger 1983) have remained matters of great controversy and uncertainty (for reviews see Comings 1980; Avivi and Feldman 1980).

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Use of metaphase plates as a substitute for direct studies of interphase chromosome arrangements has two major disadvantages:

1. The extent to which chromosome arrangements at metaphase actually reflect their relative positions in the preceding interphase nucleus depends on numerous factors which are difficult to control. Such factors include chromosome condensation, natural movements of mitotic chromosomes and artifacts introduced by the various methods of preparation and evaluation of metaphase plates.

2. Studies of metaphase plates are principally limited to cycling cells and have to rely on the assumption that the evaluated sample of mitotic cells is representative for the whole cell population. This assumption seems especially questionable for cells of tissues in vivo, in which cell cycle transit times vary greatly in different types of differentiated cells, including terminally differentiated cells which do not cycle at all.

To overcome the first of the two disadvantages mentioned above, two approaches have been established in our institute. One approach makes use of a laser-UV-microbeam, which allows microirradiation of small parts of interphase nuclei. Microirradiated chromatin can be followed from interphase to metaphase and its distribution on metaphase chromosomes can be studied (Cremer et al. 1982a, b; Hens et al. 1983). The other approach is based on the analysis of chromosome exchange patterns which occur either spontaneously in lymphocytes from patients with Fanconi's anemia and Bloom's syndrome or can be induced experimentally in human lymphocytes or in Chinese hamster cells by mitomycin C or X-rays (Hager et al. 1982; Teltschik and Schroeder, unpublished data). The first approach is based on the assumption that the probability of microirradiated cells entering the first postirradiation mitosis is similar for all possible combinations of chromosomes damaged at the microirradiated nuclear sites. The second approach is based on the assumption that the frequency of exchanges between individual chromosomes is closely correlated with their proximity during interphase. The validity of these assumptions is difficult to prove. In particular, neither approach overcomes the second disadvantage involved in studies of metaphase chromosome arrangements. For this purpose we urgently need methods which allow the direct visualization of individual chromosomes or parts thereof in interphase

nuclei. Such methods are presently limited to a few heterochromatic chromosome regions which can be stained distinctly both in interphase and metaphase. For example, the positions of chromosomes No.9 in human interphase nuclei can be observed by means of the Giemsa-11-staining procedure (Gagné and Laberge 1972; Spaeter 1975). Another example concerns the position of the heterochromatic sex chromosomes forming large chromocenters in interphase nuclei of *Microtus agrestis* (Pera and Schwarzacher 1970), while the position of the human Y-heterochromatin can be identified by quinacrine mustard staining (Pearson et al. 1970; Schmid et al. 1981).

In this paper we describe a new approach by which we have visualized the positions of *both* sex chromosomes in male and female interphase nuclei of human lymphocytes simultaneously. This approach makes use of two cloned DNA probes CY1 (Müller et al. 1983) and pXBR (Yang et al. 1982) which hybridize in situ predominantly to the human Y and X chromosome, respectively. Distances between the two X-chromosomes in female nuclei and between the X and the Y-chromosome in male nuclei were determined and compared with each other and with three model distributions of distances, calculated under the assumption that two points (i.e., the labelled regions of the two sex chromosomes) were distributed independently from each other within a sphere (i.e., the human lymphocyte nucleus). Mathematical details of these models are described by Klar et al. in an Appendix to this paper. For comparison we have used a Chinese hamster x human cell line containing a human translocation chromosome (Xqter→Xp22.2:Yq11→Yqter) to study the distribution of the in situ hybridization sites of the two DNA probes in nuclei where the two sites are physically linked on one chromosome.

Materials and methods

Molecular probes. A tandemly repeated DNA sequence, pXBR, organized predominantly at or near the centromeric

region of the human X chromosome, was used (Yang et al. 1982). Female placental DNA was used as a source of DNA for cloning. After digestion with Bam H1 and fractionation on preparative agarose gels, a 2 Kb band was recovered from the gel, ligated to Bam H1-digested pBR 322 DNA and transfected into *Escherichia coli* HMS 175.

Clone CY1 was derived by Benton-Davis plaque screening from a Y chromosome library (Müller et al. 1983; Davies, unpublished). It consists of two 3.4 and 2.6 Kb long Eco R1 inserts cloned in a λ gtWES λ B vector. With in situ hybridization, about 80% of all grains observed on metaphase plates were located exclusively on Yq with a second minor binding site in the centromeric region of chromosome 9. Thus CY1 represents a repetitive sequence which hybridizes predominantly to the Y chromosome. For in situ hybridization the two DNA probes were labelled to a specific activity of 2×10^7 cpm/ μ g DNA by nick translation (essentially according to Kunkel et al. 1979) with (3H)dTTP (100 Ci/mmol; New England Nuclear Corp.; 1 Ci = 3.7×10^{10} becquerels).

Cell cultures. For in situ hybridization experiments we used phytohaemagglutinin-stimulated human peripheral blood lymphocyte cultures (72h) and a Chinese hamster x human cell line 445 x 393 which contains a human translocation chromosome (Xqter→Xp22.2:Yq11→Yqter) and no normal X or Y chromosome. This cell line was kindly supplied by Dr. H.H. Ropers and described in detail by Goodfellow et al. (1983). Cells were grown under standard conditions (minimum essential medium, 10% fetal calf serum, 5% CO₂) in monolayer culture. Lymphocyte cultures were derived from probands with normal karyotypes 46,XY and 46,XX.

In situ hybridization. Slides were pre-coated by incubation in Denhardt's solution at 65°C (Gerhard et al. 1981), washed in water and air dried. Mitotic cells were arrested by colcemid (0.1 μ g/ml), treated by hypotonic shock (0.075 M KCl, 20–30 min), fixed in methanol/acetic acid (3:1) and spread on the pre-coated slides. Chromosome preparations were used within

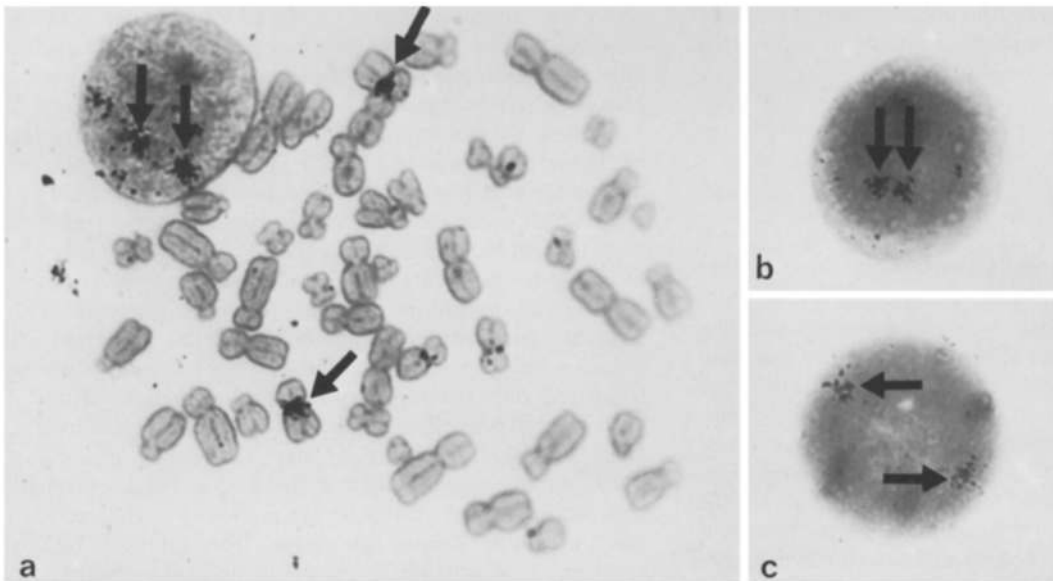


Fig. 1a–c. In situ hybridization of clone pXBR to female human lymphocytes. **a** Metaphase plate stained with Giemsa showing strong hybridization signals at the centromeric regions of the two X-chromosomes (arrows). Two major hybridization sites were also observed in interphase nuclei (arrows; **a**, upper left, **b** and **c**). The three nuclei demonstrate the large variation of the distances between Xcen in interphase nuclei of human lymphocytes

3 weeks. In situ hybridization was carried out as described in detail by Rappold and Vosberg (1983) and Rappold et al. (1984). Briefly, denaturation of chromosomal DNA was performed with 70% formamide/30% $2 \times$ SSC at 70°C for 2 min, followed by dehydration in ethanol. Denaturation of the (^3H)-labeled probe DNA was carried out at 100°C for 5 min. Hybridization of the probe under annealing conditions was carried out for 16 h at 40°C . After extensive washings in $2 \times$ SSC at 65°C and then in $0.1 \times$ SSC at room temperature, slides were dehydrated in an ethanol series and air dried. Slides were coated with Ilford L4 emulsion (1:1) and exposed up to 1 week. Exposed slides were developed in Ilford JD 19 developer. Autoradiographs were stained with Giemsa (Gurr) or quinacrine mustard (Sigma) and photographed using a Zeiss photomicroscope equipped with epifluorescence illumination.

Evaluation of cell nuclei. Nuclei showing two major binding sites following in situ hybridization of female lymphocytes with pXBR or of male lymphocytes and 445×393 cells respectively with the mixed probes CY1 and pXBR were evaluated from enlarged microphotographs. The distance between the centers of the two binding sites, i.e., spots of accumulated silver grains, was measured and divided by the diameter of the nucleus. In the case of non-circular nuclei the horizontal diameter was arbitrarily determined. The resulting normalized distances α between the two binding sites obtained in each sample of evaluated nuclei were grouped in 20 classes ranging from 0–0.05 to 0.95–1.0. The observed normalized mean distances were compared with their expectation derived from three model distributions (see Results and Appendix).

Results

In situ hybridization of cloned DNA-probes CY1 and pXBR to male and female lymphocytes. Figure 1 shows examples of in situ hybridization experiments of clone pXBR to lymphocytes of two healthy females. Two major sites of binding were observed both in metaphase spreads over the centromeric regions of the two X chromosomes and over interphase nuclei indicating the sites of the centromeric regions of the two X chromosomes in interphase nuclei. Under the conditions used in the present experiment there was only minor hybridization

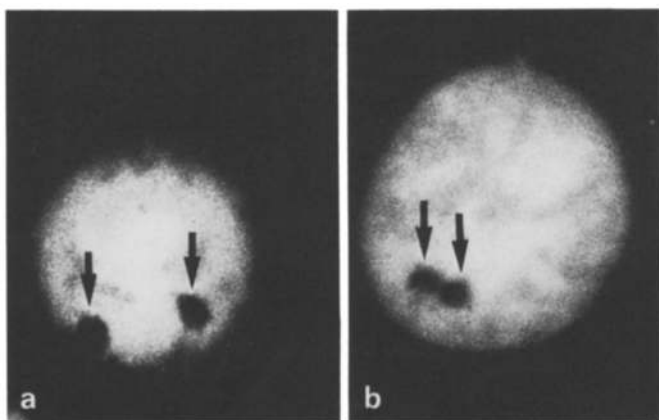


Fig. 2a and b. In situ hybridization with mixed probes pXBR and CY1 to male human lymphocytes. **a, b** Microphotographs of two nuclei stained with quinacrine mustard. Fluorescence was spared at the hybridization sites (arrows) as a result of silver grain accumulation over these sites which interfered with epifluorescence illumination

of pXBR and CY1 respectively to autosomal sites. Interphase nuclei where the distinction between minor hybridization sites (to autosomes) and major hybridization (to the sex chromosomes) was not clearly possible were excluded from further evaluation. In contrast, in situ hybridization of either pXBR or CY1 alone to male human lymphocyte nuclei generally resulted in an accumulation of silver grains at one major binding site only, representing the position of the labelled parts of either X or Y chromosome. Two distinctly separated spots were rarely seen in these experiments (1%).

In the case of in situ hybridization experiments of the mixed probes pXBR and CY1 to human lymphocytes of two healthy males, both the long arm of the Y chromosome and the centromeric region of the X chromosome appeared heavily labelled in metaphase spreads. Accordingly, interphase nuclei showed two major sites of hybridization indicating the relative positions

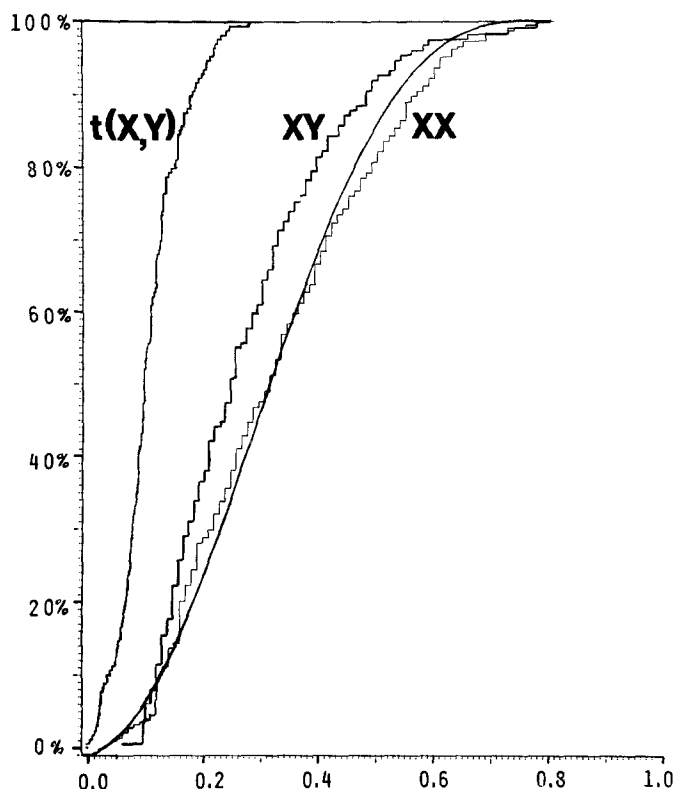


Fig. 3. *Abscissa:* Normalized distances β between two major binding sites (see Material and methods). *Ordinate:* Cumulative fraction of nuclei showing a normalized distance β equal to or smaller than the corresponding β on the abscissa. *XX:* Cumulative fraction of β derived for the two binding sites of pXBR in female human lymphocyte nuclei ($n = 280$). Data were pooled from two independent cases which gave a very similar distribution of distances. *XY:* Cumulative fraction of β obtained for the two binding sites of pXBR and CY1 in male human lymphocyte nuclei ($n = 399$). Data were pooled from two independent cases showing a very similar distribution of distances. *t(X;Y):* Cumulative fraction of β obtained for the two binding sites of pXBR and CY1 in nuclei ($n = 245$) of 445×393 hybrid cells indicating the normalized distances between the centromeric region of the human X and the translocated part of the short arm of the human Y in the translocation chromosome ($Xqter \rightarrow Xp22.2::Yq11 \rightarrow Yqter$). The smooth curve shows the cumulative fraction of β obtained in the case of model a described in detail in the Appendix [compare equation 11: $G_{2,0}$ (b)]. Two assumptions were made: (i) The lymphocyte nucleus is a sphere. (ii) Two points indicating the positions of the labelled chromosome segments are distributed uniformly and independently from each other within the sphere. (For further details see Results and Appendix)

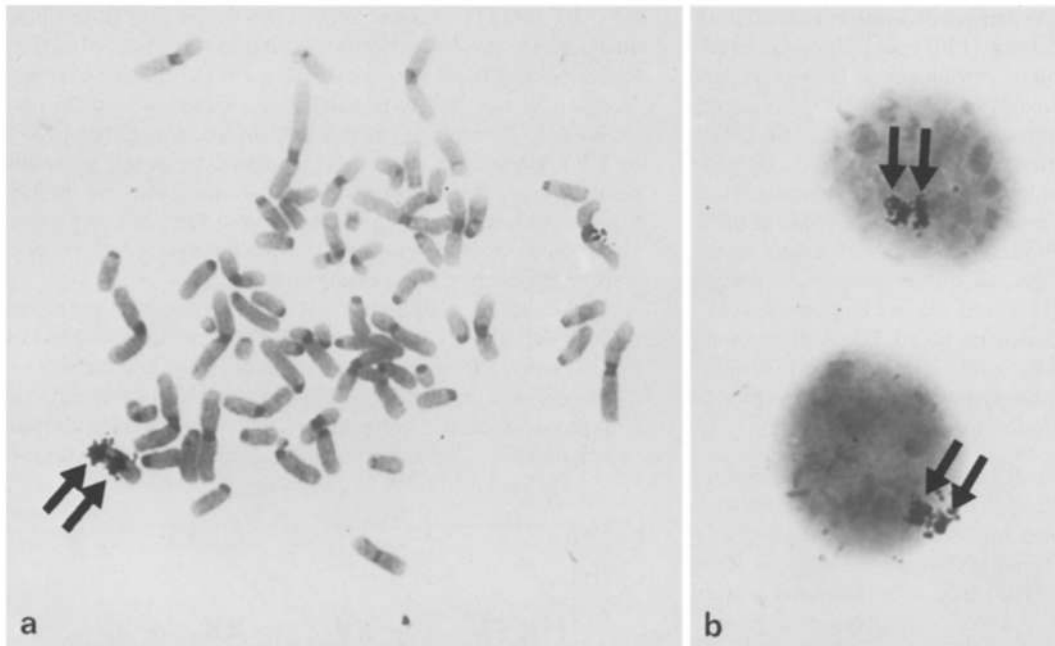


Fig. 4a and b. In situ hybridization with mixed probes pXBR and CY1 to a metaphase spread (a) and two interphase nuclei (b) of the Chinese hamster \times human cell hybrid line 445 \times 393, containing a human translocation chromosome (Xqter \rightarrow Xp22.2::Yq11 \rightarrow Yqter). In this chromosome the centromeric region of the X and the translocated part of Yq could be simultaneously labelled with the two probes (a, arrows). The distances between the two labelled sites observed in interphase nuclei reflect the extent of elongation of the investigated chromosome arm during interphase (b, arrows)

of the labelled parts of the X and Y chromosomes. When interphase nuclei were counterstained with quinacrine mustard, fluorescence could not be observed at the hybridization sites due to the accumulation of silver grains over these sites, which interferes with epifluorescence illumination (Fig. 2).

For quantitative evaluation, samples of interphase nuclei showing two clear sites of hybridization were randomly taken. Normalized distances α (see Materials and methods) determined between X,X and X,Y positions in female and male human lymphocyte nuclei are shown in Fig. 3. For comparison Fig. 3 shows a model curve of distances [see Appendix; model a, equation (11)] obtained under the assumptions that (i) the human lymphocyte interphase nucleus has a spherical shape and (ii) two points indicating the positions of the respective chromosome segments are distributed uniformly and independently of each other within the sphere. Distances between each possible combination of two points were calculated after orthogonal projection onto a plane. Due to the method of evaluation, the maximum experimental distance was necessarily somewhat smaller than 1 even in cases where the positions of the two labelled sites were exactly opposite each other at the nuclear equator. Accordingly, the diameter of the model sphere was assumed to be equal to the maximum normalized distance β_{\max} determined experimentally. This was done in order to minimize a possible difference between the distribution of distances predicted by the model and the experimental distances. For $\alpha_{\max} = 0.85$ the mean distance $\bar{\alpha}$ obtained for the model curve was 0.34 as compared to $\bar{\alpha} = 0.40$ in case of $\alpha_{\max} = 1.0$ [see Appendix; model a, equation (12c)]. When compared with this model curve, cumulative distances obtained from lymphocyte nuclei of the two male individuals still showed a significantly smaller mean distance of the labelled X and Y chromosome segments (0.30). In contrast, no significant difference between this model curve of distances and the

experimental curve of distances was obtained for the X chromosomes in lymphocyte nuclei of the female individuals (0.34 observed as compared to 0.33 expected) (Fig. 3). In addition to model a, two other model distributions were also considered (see Discussion and Appendix, models b and c). These models take into account the possibility that the labeled region of one or both sex chromosomes is preferentially situated at the nuclear periphery, and notably predict a larger mean distance between the labeled segments as compared to model a. Accordingly, the mean distances obtained experimentally are significantly smaller than predicted by models b and c not only for the sex chromosomes in male but also in female nuclei.

In situ hybridization of CY1 and pXBR to the Chinese hamster \times human hybrid cell 445 \times 393. In situ hybridization experiments ($n = 4$) with the mixed probes pXBR and CY1 were also performed in the Chinese hamster \times human cell hybrid line 445 \times 393 containing a human translocation chromosome (Xqter \rightarrow Xp22.2::Yq11 \rightarrow Yqter). In this chromosome the centromeric region of the X and the translocated part of Yq could be simultaneously labelled by hybridization with the two probes (Fig. 4). The distances between the two labelled sites as observed in interphase nuclei then reflect the extent of elongation of the investigated chromosome arm during interphase. In most cases the major binding sites of the two probes were found in close proximity (Figs. 3, 4), suggesting that this chromosome arm was generally retained within a rather compact nuclear domain.

Discussion

Two cloned DNA probes, pXBR and CY1, which bind to specific regions of the human X and Y chromosome, were used in the present investigation to study the relative positions of the

two sex chromosomes directly within interphase nuclei of female and male human lymphocytes. Data obtained for two normal female and two normal male individuals show a large variability of the distances between the two sex chromosomes in interphase nuclei (Fig. 3). Yet our data do not exclude the possibility that each sex chromosome might occur at a preferential position in a haploid set or subset of chromosomes. In such a case, however, these data indicate that the relative arrangements of these ordered sets would vary largely from cell to cell.

For several plant species evidence has been presented for a highly ordered arrangement of chromosomes in haploid sets as predicted by a model proposed by Bennett (1983). If an ordered arrangement of haploid chromosome sets exists in mammalian interphase nuclei, one can predict differences in the distribution of distances between different pairs of non-homologous chromosomes. In situ hybridization with mixed probes of chromosome specific repetitive DNAs obtained from different chromosomes, when compared with the in situ hybridization pattern of each probe alone, could be used to study this question. Interestingly, the mean distance between the X and the Y chromosome was significantly smaller than the mean distance between the two X chromosomes. The feasibility of this method is further demonstrated by the fact that the mean distance between the hybridization sites of CY1 and pXBR was very small in interphase nuclei of the Chinese hamster \times human cell line 445 \times 393. At present such investigations are limited by the number of probes available (Gosden et al. 1981; Law et al. 1982; Kanda et al. 1982).

The choice of the model distribution of distances used for comparison with the experimentally measured distances causes particular problems (see Appendix). The distribution of distances between the two major binding sites of pXBR in female human lymphocyte nuclei closely follows the distribution predicted by model a (see Appendix). In this case the centromeres of the two X-chromosomes are considered to be two points distributed uniformly and independently from each other within a sphere (Fig. 3). Since it is well established that the inactivated X in many nuclei is located at the nuclear periphery, model b in the Appendix considers one point to be distributed uniformly at the surface of a sphere, while the other point is distributed within the sphere. A third model c considers both points to be distributed at the surface of the sphere in a random manner. Notably, the mean distances $\bar{\alpha}$ between the two points obtained for model b [$\bar{\alpha} = 0.47$; see Appendix, equation (15c)] and model c [$\bar{\alpha} = 0.52$; see Appendix, equation (19c)] were considerably larger than $\bar{\alpha}$ for model a [0.40; see Appendix, equation (12c)]. In comparison to models b and c the experimentally determined distances between the centromeric regions of the two X-chromosomes were significantly smaller than the model distances. In other words, if the centromeric regions of one X or both X-chromosomes in female nuclei were indeed located close to or at the nuclear periphery, the experimental data would suggest a distribution significantly closer than expected at random. The mean distance obtained between the X and Y chromosomes in male human lymphocyte nuclei was significantly smaller than the mean distances obtained for all three model distributions.

Difficulties in choosing an adequate model distribution of distances can in part be eliminated by a direct comparison of distances between different pairs of chromosomes in the interphase nucleus. Independent of the chosen model, significant

differences between the mean distances of different pairs of chromosomes would indicate a non-random pattern of chromosome distribution.

A possible interpretation of the difference found between the distribution of the sex chromosomes in male and female nuclei could be that the small Y chromosome was located preferentially at a more central region of the investigated nuclei, while the larger X chromosomes were generally distributed closer to the nuclear periphery (Hens et al. 1982). Further information on the three-dimensional position of the sex chromosomes within interphase nuclei is necessary to prove or disprove such an idea. In situ hybridization of probes pXBR and CY1 to serially sectioned nuclei might provide such information.

Our data provide the first example of the potential use of cloned DNA probes to study the problem of interphase chromosome arrangements. It is a particular advantage of this approach that it can be extended to studies of interphase chromosome arrangements of cycling and non-cycling cells in vivo. Further studies are intended to clarify two questions: (i) Do other somatic cell types show the same variability in the arrangement of sex chromosomes as found in human lymphocytes? (ii) Does the arrangement of sex chromosomes in premeiotic stages of germ line cells differ from that found in somatic cell types?

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Appendix

Models for the distribution of distance of specific chromosome regions in interphase nuclei

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1. The problem

Rappold et al. (1984) have investigated the distribution of distance between specific regions of the sex chromosomes in male and female human lymphocyte nuclei by in situ hybridization experiments with two chromosome specific DNA probes CY1 and pXBR (see above). In this appendix three distinct model distributions of distances will be considered:

- The labelled regions of both chromosomes lie in the interior of a cell nucleus;
- One labelled region lies in the interior and the other at the boundary of the nucleus;
- Both labelled regions lie at the boundary of the nucleus.

Each model is based on the following assumptions:

- The cell nucleus of human lymphocytes can be considered as the interior of a sphere;
- The position of the labelled region of one chromosome either in the interior of the cell nucleus (region 1) or at its boundary (region 2) is random and independent of the position of the other labelled chromosome segment;

- Microphotographs used for measurements of distances represent an orthogonal projection of the cell nucleus onto a plane.

2. The mathematical model

Consider a pair of independently and uniformly distributed random points, P_1 and P_2 , of which i are in the interior and j are on the boundary (surface) of a sphere of radius R ($0 \leq i, j \leq 2$, $i+j=2$), i.e., for each of the two points the probability that it lies in any region of the interior of the sphere (resp. of its surface) shall be proportional to the volume (resp. area) of this region, independently from the position of the other point. Let r ($0 \leq r \leq 2R$) be the distance between P_1 and P_2 and $f_{i,j}(r)$ the probability density function (p.d.f.) of r . Then it is well known that:

$$(1a) \quad f_{2,0}(r) = \frac{3}{R} \left(\frac{r}{R}\right)^2 \left[1 - \frac{3}{4} \left(\frac{r}{R}\right) + \frac{1}{16} \left(\frac{r}{R}\right)^3\right],$$

$$(1b) \quad f_{1,1}(r) = \frac{3}{4R} \left(\frac{r}{R}\right)^2 \cdot \left(2 - \frac{r}{R}\right),$$

$$(1c) \quad f_{0,2}(r) = \frac{1}{2R} \left(\frac{r}{R} \right)$$

[for (1a) see, e.g., Hammersley 1950, for (1b) and (1c) see Miles 1971].

In order to obtain the distribution of the distance b between the images of P_1 and P_2 by orthogonal projection onto a plane, which apparently has not been derived up to now, one has first to go back to the distribution of the vector $\mathbf{r} = \mathbf{r}_1 - \mathbf{r}_2$, where \mathbf{r}_1 and \mathbf{r}_2 are the radius vectors from the centre of sphere to P_1 and P_2 , respectively. Because of the spherical symmetry of the model, the distribution of \mathbf{r} depends only on $r = |\mathbf{r}|$ ($|\mathbf{r}|$ = length of \mathbf{r}), and as the set of all vectors with fixed length r drawn from the same origin describes a sphere of radius r , the p.d.f. of the vector \mathbf{r} is:

$$(2) \quad f_{ij}^*(\mathbf{r}) = \frac{1}{4\pi r^2} f_{i,j}(r)$$

We now decompose \mathbf{r} into the sum of a vector \mathbf{h} situated in the plane of projection ("projection vector") and a vector \mathbf{z} perpendicular to this plane. To any vector \mathbf{r} with the same projection vector \mathbf{h} there belongs exactly one vector \mathbf{z} with

$$\mathbf{z} = z\mathbf{z}_0, \quad -\sqrt{4R^2 - b^2} \leq z \leq \sqrt{4R^2 - b^2}$$

and inversely, where \mathbf{z}_0 is a constant unit vector and $b = |\mathbf{h}|$ (cf. Fig. 1). To obtain the p.d.f. of \mathbf{h} one has to integrate $f_{ij}^*(\mathbf{r})$ over z :

$$(3) \quad g_{ij}^*(\mathbf{h}) = \frac{1}{2\pi} \int_0^{\sqrt{4R^2 - b^2}} [f_{i,j}(\sqrt{z^2 + b^2}) / (z^2 + b^2)] dz$$

Finally the p.d.f. of b is obtained by integrating (3) over all vectors \mathbf{h} with the same length b

$$(4) \quad g_{i,j}(b) = b \int_0^{\sqrt{4R^2 - b^2}} [f_{i,j}(\sqrt{z^2 + b^2}) / (z^2 + b^2)] dz$$

For simplification we introduce $b = 2R\beta$ in the following derivations.

Case a: $(i, j) = (2, 0)$, i.e., both points P_1 and P_2 lie in the interior of the sphere.

From (1a) and (4), using the integral formulas

$$(5) \quad \int \sqrt{z^2 + \beta^2} dz = \frac{z}{2} \sqrt{z^2 + \beta^2} + \frac{\beta^2}{2} \ln(z + \sqrt{z^2 + \beta^2}) + \text{const},$$

$$(6) \quad \int z^2 \sqrt{z^2 + \beta^2} dz = \frac{z}{8} (2z^2 + \beta^2) \sqrt{z^2 + \beta^2} - \frac{\beta^2}{8} \ln(z + \sqrt{z^2 + \beta^2}) + \text{const}$$

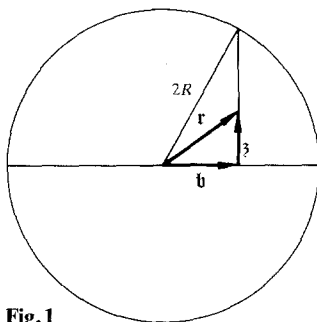


Fig. 1

(see, e.g., Gröbner and Hofreiter 1961), we get

$$(7) \quad g_{2,0}(b) = \frac{3b}{2R^2} \int_0^{\sqrt{4R^2 - b^2}} \left[2 - \frac{3}{2R} (z^2 + b^2)^{\frac{1}{2}} + \frac{1}{8R^3} (z^2 + b^2)^{\frac{3}{2}} \right] dz$$

$$= \frac{6\beta}{R} \int_0^{\sqrt{1 - \beta^2}} \left[2 + (\beta^2 - 3) \sqrt{z^2 + \beta^2} + z^2 \sqrt{z^2 + \beta^2} \right] dz$$

$$= \frac{9\beta}{4R} \left[(\beta^2 + 2) \sqrt{1 - \beta^2} + \beta^2 (\beta^2 - 4) \ln \frac{1 + \sqrt{1 - \beta^2}}{\beta} \right]$$

Using the likewise well-known integral formulas

$$(8) \quad \int x^k \sqrt{1 - x^2} dx = \frac{1}{k+1} \sin^{k+1} \varphi \cos \varphi + \frac{1}{k+1} \int \sin^{k+2} \varphi d\varphi$$

($k = 0, 1, 2, \dots$),

$$(9) \quad \int x^k \ln \frac{1 + \sqrt{1 - x^2}}{x} dx = \frac{x^{k+1}}{k+1} \ln \frac{1 + \sqrt{1 - x^2}}{x} + \frac{1}{k+1} \int \sin^k \varphi d\varphi$$

($k = 0, 1, 2, \dots$)

(with $\sin \varphi = x$), and

$$(10) \quad \int \sin^{2k+1} \varphi d\varphi = \sum_{v=0}^k (-1)^{v+1} \binom{k}{v} \frac{1}{2v+1} \cos^{2v+1} \varphi$$

($k = 0, 1, 2, \dots$)

we obtain the distribution function of b by integration of $g_{2,0}(b)$:

$$(11) \quad G_{2,0}(b) = \int_0^b g_{2,0}(x) dx = 2R \int_0^\beta g_{2,0}(2Rt) dt$$

$$= 1 + \frac{1}{4} (3\beta^4 + 16\beta^2 - 4) \sqrt{1 - \beta^2}$$

$$+ \frac{3}{4} \beta^4 (\beta^2 - 6) \ln \frac{1 + \sqrt{1 - \beta^2}}{\beta}$$

In the same way the moments of the distribution of b can be obtained:

$$m_k = \int_0^{2R} x^k g_{2,0}(x) dx = (2R)^k \int_0^1 t^k g_{2,0}(2Rt) dt$$

$$= 9(2R)^k \left[\frac{k+5}{(k+4)(k+6)} \int_0^{\pi/2} \sin^{k+5} \varphi d\varphi - \frac{k}{(k+2)(k+4)} \int_0^{\pi/2} \sin^{k+3} \varphi d\varphi \right]$$

and hence, using the product formula of Wallis

$$(12a) \quad m_{2s} = (2R)^{2s} \frac{9}{(s+1)(s+2)(2+3)} \prod_{v=1}^{s+1} \frac{2v}{2v+1} \quad (s = 1, 2, \dots),$$

$$(12b) \quad m_{2s+1} = (2R)^{2s+1} \frac{36\pi}{(2s+4)(2s+5)(2s+7)} \prod_{v=1}^{s+1} \frac{2v-1}{2v}$$

($s = 0, 1, 2, \dots$)

Especially:

$$E(b) = m_1 = 2R \frac{9\pi}{70} = 0.403919(2R),$$

$$\text{var}(b) = m_2 - m_1^2 = (2R)^2 \left[\frac{1}{5} - \left(\frac{9\pi}{70} \right)^2 \right]$$

$$= 0.036849(2R)^2.$$

Case b: $(i, j) = (1, 1)$, i.e., P_1 lies in the interior and P_2 on the surface of the sphere.

From (1b) and (4), again using (5) and (6), we get

$$(13) \quad g_{1,1}(b) = \frac{6\beta}{R} \int_0^{\sqrt{1-\beta^2}} (1 - \sqrt{z^2 + \beta^2}) dz$$

$$= \frac{3\beta}{R} (\sqrt{1-\beta^2} - \beta^2 \ln \frac{1 + \sqrt{1-\beta^2}}{\beta})$$

Hence, using (8)–(10),

$$(14) \quad G_{1,1}(b) = \int_0^b g_{1,1}(x) dx =$$

$$= 1 + \frac{1}{2} (5\beta^2 - 2) \sqrt{1-\beta^2} - \frac{3}{2} \beta^4 \ln \frac{1 + \sqrt{1-\beta^2}}{\beta}$$

and

$$(15a) \quad m_{2s} = (2R)^{2s} \frac{3}{(s+1)(s+2)} \prod_{v=1}^{s+1} \frac{2v}{2v+1} \quad (s = 1, 2, \dots),$$

$$(15b) \quad m_{2s+1} = (2R)^{2s+1} \frac{6\pi}{(2s+3)(2s+5)} \prod_{v=1}^{s+2} \frac{2v-1}{2v}$$

$$(s = 0, 1, 2, \dots)$$

Especially:

$$E(b) = m_1 = 2R \frac{3\pi}{20} = 0.471239(2R),$$

$$var(b) = m_2 - m_1^2 = (2R)^2 \left[\frac{4}{15} - \left(\frac{3\pi}{20} \right)^2 \right]$$

$$= 0.044601(2R)^2.$$

Case c: $(i, j) = (0, 2)$, i.e., both points P_1 and P_2 are situated on the surface of the sphere.

From (1c) and (4), using the integral formula

$$(16) \quad \int \frac{dz}{\sqrt{z^2 + \beta^2}} = \ln(z + \sqrt{z^2 + \beta^2}) + \text{const},$$

we obtain

$$(17) \quad g_{0,2}(b) = \frac{\beta}{R} \int_0^{\sqrt{1-\beta^2}} \frac{dz}{\sqrt{z^2 + \beta^2}} = \frac{\beta}{R} \ln \frac{1 + \sqrt{1-\beta^2}}{\beta}.$$

Hence, using (9) and (10),

$$(18) \quad G_{0,2}(b) = \int_0^b g_{0,2}(x) dx = 1 - \sqrt{1-\beta^2} + \beta^2 \ln \frac{1 + \sqrt{1-\beta^2}}{\beta}$$

and

$$(19a) \quad m_{2s} = (2R)^{2s} \frac{1}{s+1} \prod_{v=1}^s \frac{2v}{2v+1} \quad (s = 1, 2, \dots),$$

$$(19b) \quad m_{2s+1} = (2R)^{2s+1} \frac{\pi}{2s+3} \prod_{v=1}^{s+1} \frac{2v-1}{2} \quad (s = 0, 1, 2, \dots)$$

Especially:

$$E(b) = m_1 = 2R \frac{\pi}{6} = 0.523599(2R),$$

$$var(b) = m_2 - m_1^2 = (2R)^2 \left[\frac{1}{3} - \left(\frac{\pi}{6} \right)^2 \right]$$

$$= 0.059178(2R)^2.$$

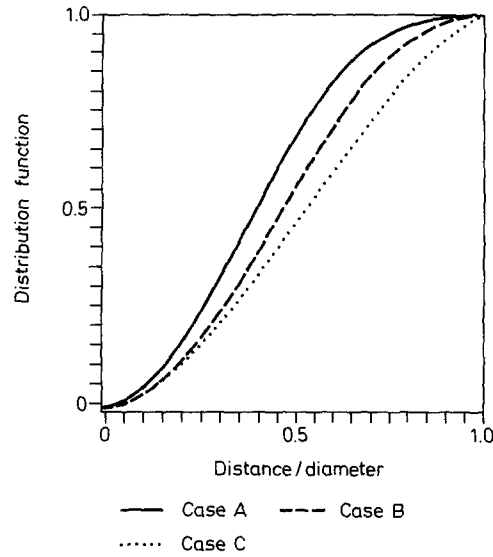


Fig. 2. Distribution function of distance b between the projections of two random points

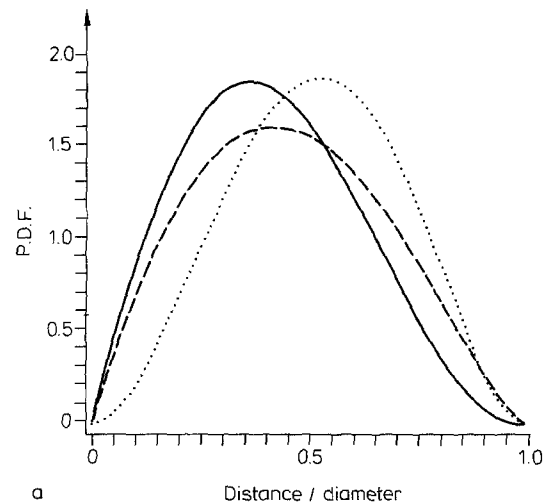


Fig. 3a. Comparison of probability density functions (p.d.f.) for the distributions of three distances in case a: ——— Distance between the projections of two random points in the sphere onto a plane. Distance between two random points in the sphere themselves. - - - Distance between two random points in the circle

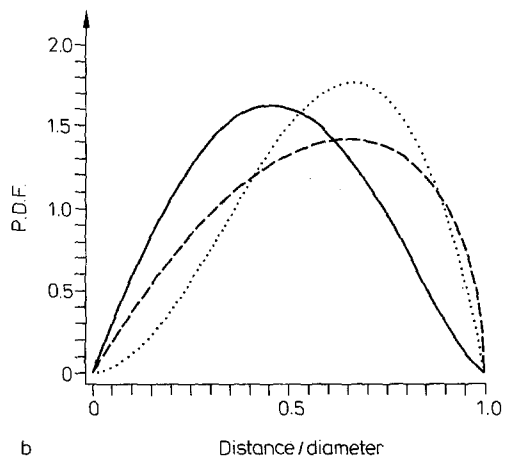


Fig. 3b. Comparison of the three p.d.f.'s in case b, for explanations of the curves cf. Fig. 3a

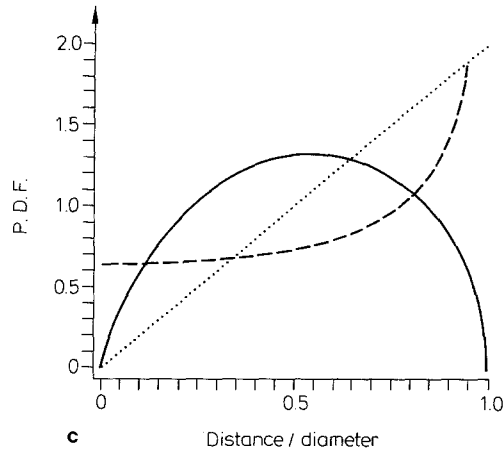


Fig.3c. Comparison of the three p.d.f.'s in case c, for explanation of the curves cf. Fig.3 a

Figure 2 shows the distribution functions of b for the three cases a–c together in one graphical representation. In Fig. 3a–c, the p.d.f. of b in each of the three cases is compared with the corresponding p.d.f. $f_{i,j}(r)$ of the distance between the points P_1 and P_2 themselves and with the corresponding p.d.f. $h_{i,j}(r)$ of distance for the analogous model in the plane: two random points of which i are in the interior and j are on the

boundary of a circle of radius R ($0 \leq i, j \leq 2, i+j=2$). The latter p.d.f. is given by the formulae (see Miles 1971):

$$(20a) \quad h_{2,0}(r) = \frac{8\beta}{\pi R} (\arccos \beta - \beta \sqrt{1-\beta^2}),$$

$$(20b) \quad h_{1,1}(r) = \frac{4\beta}{\pi R} \arccos \beta,$$

$$(20c) \quad h_{0,2}(r) = \frac{1}{\pi R} \frac{1}{\sqrt{1-\beta^2}},$$

were again $\beta = r/2R$.

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