dipin molecule leads to more rapid alkylation of DNA, and as a result of this the level of alkylated products capable of activating AR is achieved after a shorter exposure time.

LITERATURE CITED

1. N. P. Dubinin, General Genetics [in Russian], 3rd edition, Moscow (1986), p. 309.
2. L. G. Dubinina, Z. I. Kurashova, and S. P. Sergievskaya, Genetika, 22, No. 12, 2305 (1986).

SPECIFICITY OF ARRANGEMENT OF HUMAN CHROMOSOMES IN THE NUCLEUS OF A MOVING CELL

I. V. Gar'kavtsev, T. G. Tsvetkova,
S. M. Terekhov, I. A. Aleksandrov,
and S. P. Mitkevich

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The study of the arrangement of chromosomes in the interphase nucleus is currently being intensively pursued. However, the difficulty of direct demonstration of chromosomes during interphase has hitherto prevented its being proved by direct experiments that they occupy a stable position in the nucleus. Attempts have therefore been made to obtain information about the arrangement of chromosomes in the nucleus by indirect methods [7, 12, 14, 15].

Electron microscopy has enabled information on the chromosomal organization of the interphase nucleus to be obtained directly [1]. The nonrandom nature of the chromosome distribution in the interphase nucleus has been demonstrated in cells of the Indian muntjac [6]. Nevertheless, the fundamental question of specificity of the chromosomes distribution in the interphase nucleus of human cells remains unexplained. Two conditions determine the possibility of obtaining an answer: 1) the availability of a model system for evaluating the localization of chromosomes in a system with assigned coordinates; 2) the use of the most promising approach to determine the position of chromosomes in the interphase nucleus with the aid of highly specific molecular probes in separate regions of the chromosome. The aim of the investigation described below was accordingly to determine the arrangement of the centromere of one chromosome in the interphase nucleus by the use of a molecular probe.

EXPERIMENTAL METHOD

Diploid skin fibroblasts from an 8-12-week male human fetus, obtained at therapeutic abortion, were used. The cells were cultured on Eagle's medium with 10% bovine serum and 5% human umbilical cord serum. DNA was labeled by the nick translation method [11]. In situ hybridization on interphase nuclei was carried out by the method in [8], including denaturation of the preparations in 0.07 N NaOH for 2 min, hybridization for 18 h, washing under standard conditions, and exposure under emulsion for 21 days.

EXPERIMENTAL RESULTS

The problem was tackled by the use of a cloned fragment of human alphasatellite DNA, specific for the centromeric region of the X chromosome. The use of human male fetal

Institute of Medical Genetics, Academy of Medical Sciences of the USSR, Moscow. All-Union Mental Health Research Center, Academy of Medical Sciences of the USSR, Moscow.
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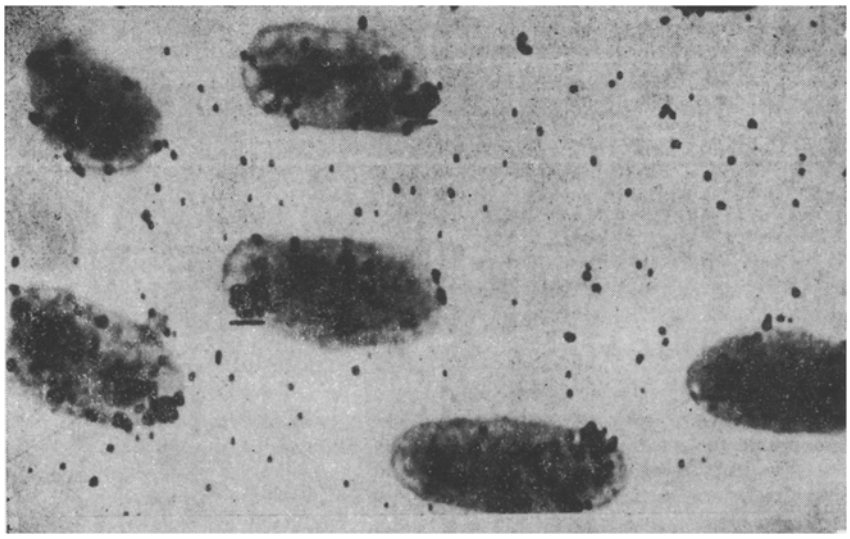

Fig. 1. In situ hybridization in interphase nuclei of alpha-satellite DNA clone on juxtacentromeric heterochromatin of X chromosome. Arrow indicates direction of movement of fibroblasts; short horizontal line indicates specific hybridization site.

TABLE 1. Distribution of Juxtacentromeric Heterochromatin of X Chromosome in Different Regions of Interphase Nucleus

No. of regions of nucleus	Number of nuclei with localization in the given region	Fraction and its error, % (P ± Sp)
1	58	13,3±1,6
2	46	10,5±1,5
3	41	9,4±1,4
4	74	17,0±1,8
5	65	14,9±1,7
6	43	9,9±1,4
7	47	10,8±1,5
8	62	14,2±1,7
1+8	120	27,5±2,1
4+5	139	31,9±2,3
1+4+5+8	259	59,4±2,3
2+3+6+8	177	40,6±2,3

fibroblasts enabled the arrangement of only the X chromosome to be studied in the interphase nucleus. To obtain oriented movement of the cells the method of wounding in a monolayer was used. In this way it is possible to obtain a large number of cells oriented in one direction.

The results of in situ hybridization of interphase nuclei of embryonic fibroblasts with the labeled clone are shown in Fig. 1. Concentration of grains of silver in the interphase nucleus reveals specific hybridization of the probe with the heterochromatic region of the X chromosome. The arrangement of this concentration reflects the location of the centromere in the interphase nucleus. To analyze the distribution of grains of silver the interphase nuclei were photographed and transferred, matching the negative exactly, to paper on the same scale.

Quantitative evaluation of the arrangement of the heterochromatic region of the X chromosome in the interphase nucleus was carried out as follows. Interphase nuclei, shaped like ellipses on the photographs, were divided into eight regions of equal area (Fig. 2). For this purpose the major and minor hemiaxes of the ellipse and two other straight lines parallel to the minor hemiaxis were drawn, together with another two straight lines parallel to the minor hemiaxis and at a distance from it of X_0, such that $X_0 = q \cdot a$, where a is the

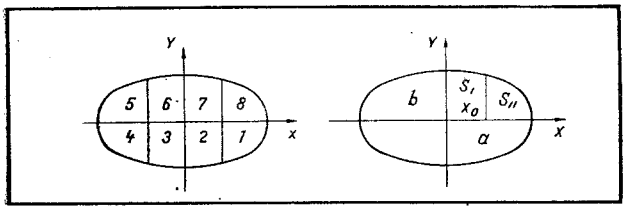

Fig. 2. Diagram showing division of ellipsoid interphase nuclei. a) Major hemiaxis of ellipse; b) minor hemiaxis of ellipse: $S_I = S_{II}$. Numbers indicate regions of equal area.

TABLE 2. Comparison of Frequencies of Finding Concentrations of Grains of Silver in Different Regions of Nucleus

Nos. of regions of nucleus compared	$P \pm Sp$		t	p
4 and 5	17,0±1,8	14,9±1,7	0,85	>0,05
1 and 8	13,3±1,6	14,2±1,7	0,38	>0,05
2 and 7	10,5±1,5	10,8±1,5	0,14	>0,05
3 and 6	9,4±1,4	9,9±1,4	0,25	>0,05
4,5 and 1,8	31,9±2,3	27,5±2,1	1,37	>0,05
2,7 and 3,6	20,3±1,9	19,3±1,9	0,37	>0,05
1,4, 5,8, and 2,3,6,7	59,4±2,3	40,6±2,3	5,78	<0,001

length of the major hemiaxis and, as can easily be shown from the condition of equality of areas, $q\sqrt{1 - q^2} \arcsin q = (\pi/4)$ and, consequently, $q \approx 0.4$. After division of the ellipse in this way the frequencies of discovery of centromeric regions of the X chromosome in each of the regions of the ellipse were determined. The point at the center of a concentration of grains of silver on the autoradiograph was taken to be the centromeric region. Several interphase nuclei in which the center of such a concentration lay on the boundary dividing the regions were excluded from analysis. Patterns of distribution of centromeric heterochromatin of the X chromosome in 436 interphase nuclei are shown in Tables 1 and 2. It follows from these results that the centromeric heterochromatin of the X chromosome is mainly located in the terminal parts of the interphase nucleus. Differences observed between regions 1, 4, 5, and 8 in this case were not statistically significant, but the arrangement of labeled material in the terminal regions differed significantly from that in the central regions. It can thus be concluded: a) that the centromeric heterochromatin of the X chromosome in moving fibroblasts is located in the terminal regions of the interphase nucleus; b) that a preferred localization of the centromeric region of the X chromosome in the anterior or posterior regions of the nucleus (sectors 4 + 5 and 1 + 8) as regards direction of movement was not observed.

In experiments in which wounding of a monolayer of endothelial cells or fibroblasts was used, several workers found that the center of organization of the microtubules was oriented in the direction of movement [5, 9]. However, our own results on distribution of heterochromatin of the X chromosome showed that no preferred localization of the marker used in this case in the anterior or posterior regions of the nuclei was present. On the other hand, there is evidence that chromosomes are connected to the nuclear membrane [2, 3]. It can thus be postulated that anteroposterior polarity of the interphase nucleus relative to the cytoplasm is evidently absent during movement of the cell.

There is evidence that individual chromosomes in the interphase nucleus occupy relatively compact domains [4, 10, 13]. Accordingly, our results for the distribution of juxtacentromeric heterochromatin of the X chromosome probably reflect also the localization of the whole chromosome. Thus by using molecular probes on single human chromosomes it is possible to determine the precise mutual arrangement of all the chromosomes in the nucleus and to establish relations between individual regions of the chromosomes and other nuclear structures.

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LITERATURE CITED

1. C. A. Bourgeois, F. Laquerriere, and D. Hemon, Hum. Genet., 69, 122 (1985).
2. S. Brenner, Exp. Cell Res., 5, 257 (1953).
3. D. E. Comings and T. A. Okada, Exp. Cell Res., 62, 293 (1970).
4. T. Cremer, C. Cremer, and H. Baumann, Hum. Genet., 60, 46 (1982).
5. A. I. Gotlieb, L. M. May, L. Subrahmanyan, et al., J. Cell Biol., 91, 589 (1981).
6. G. Hadlaczky, M. Went, and N. R. Ringertz, Exp. Cell Res., 167, 1 (1986).
7. H. D. Hager, T. M. Schroeder-Kurth, and F. Vogel, Hum. Genet., 61, 242 (1982).
8. M. E. Harper and G. F. Saunders, Chromosoma, 81, 431 (1982).
9. A. Kupfer, D. Louvard, and S. J. Singer, Proc. Natl. Acad. Sci. USA, 72, 2603 (1982).
10. L. Manuelidis, Hum. Genet., 71, 288 (1985).
11. P. W. J. Rigby, H. Dickmann, C. Rhodes, and P. Berg. J. Mol. Biol., 113, 237 (1977).
12. T. C. Rodman, B. J. Flehinger, and F. J. Rohlf, Cytogenet. Cell Genet., 27, 98 (1980).
13. M. Schardin, T. Cremer, H. D. Hager, et al., Hum. Genet., 71, 281 (1985).
14. M. Schmid, W. Vogel, and W. Krone, Cytogenet. Cell Genet., 15, 66 (1975).
15. F. Vogel and T. M. Schroeder, Hum. Genet., 25, 265 (1974).