# BEHAVIOR OF NUCLEOLAR ORGANIZER REGIONS OF CHROMOSOMES IN POLYKARYOCYTES CONSISTING OF MICRONUCLEI

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UDC 612.014.24:576.315.45

KEY WORDS: chromosome, nucleolar organizer region; cells with micronuclei; Chinese hamster cells

Colcemid-induced multinuclear cells consisting of micronuclei are of definite interest in connection with the study of the physiology of the cell nucleus. In these cells mitotic condensation of the chromosomes follows an abnormal course, as is shown by the appearance of chromosomes with regions of delayed spiralization [1]. If 5-bromodeoxyuridine is administered simultaneously with colcemid, many dicentric chromosomes, whose formation is linked with delayed rupture of telomeric bonds [4], are found in the first division metaphases of the polykaryocytes. Both phenomena are readily reproduced in Chinese hamster cells (clone 237, line $BII^d$-ii-FAF28).

The aim of this investigation was to study the nucleolar organizer regions (NOR) of chromosomes by differential staining with silver nitrate in intact and colcemid-induced polykarocytes of clone 237 of line $BII^d$-ii-FAF28.

## EXPERIMENTAL METHOD

Cells were grown in rectangular flasks with a capacity of 0.5 liter on Eagle's medium with 10% bovine serum. To induce polykarocytes colcemid was added in a dose of 0.1 μg/ml to an actively growing culture for 40 h. During investigation of an intact culture, colcemid was added in the same dose for 2 h. Mild hypotonic treatment with 0.65% KCl solution for 5 min was used. The material was fixed with a mixture of ethanol and acetic acid (4:1) for 1 h. Chromosome preparations were obtained by the standard air-drying method. Differential staining of the NOR of the chromosomes was carried out in 50% silver nitrate solution at 37°C C for 24 h [2]. The chromosome preparations were then counterstained with a 2% solution of Romanovsky–Giemsa stain for 4 min. Altogether 100 metaphases each were analyzed in the experiment and control.

## EXPERIMENTAL RESULTS

The modal number of chromosomes of cells of clone 237 of line $BII^d$-ii-FAF28 is 18. On impregnation with silver NOR were found in 4 chromosomes (Fig. 1a). Since the karyotype of this line differs from the normal Chinese hamster karyotype, the chromosomes with NOR were conventionally identified as 1, 2, 3, and 4 (Fig. 1b). Four chromosomes with NOR were found in 8 metaphases of a normal culture. Stained NOR only of chromosomes 1, 2, and 3 were found in 45 cells. In the remaining cells NOR were most frequently identified in chromosome 1. NOR of the first two chromosomes and of one or two of chromosome 2 and 3 were stained in 4 tetraploid metaphases. NOR of chromosomes in tetraploids did not take part in association. Associations of NOR of chromosomes 1 and 2 were found in 3 cells (Fig. 1c).

On analysis of first division metaphases of polykaryocytes consisting of micronuclei, tetraploid metaphases and also hypotetraploid, and even hypodiploid metaphases, having micronuclei around them, were investigated. Of 100 metaphases analyzed only two did not contain nucleolar-organizing chromosomes, although metaphases lying side by side had stained NOR. As the writers showed previously, mitotic condensation in polykaryocytes consisting of micronuclei can take place sometimes in one or several micronuclei [3]. In our view, these two hypodiploid metaphases arose from a micronucleus not containing chromosomes with NOR.

Laboratory of Tissue Culture, Institute of Poliomyelitis and Viral Encephalitis, Academy of Medical Sciences of the USSR, Moscow. (Presented by Academician of the Academy of Medical Sciences of the USSR S. G. Drozdov.) Translated from Byulletin' Éksperimental'noi Biologii i Meditsiny, Vol. 103, No. 3, pp. 346-347, March, 1987. Original article submitted May 16, 1986.

 0007-4888/87/0003- 0390$12.50 

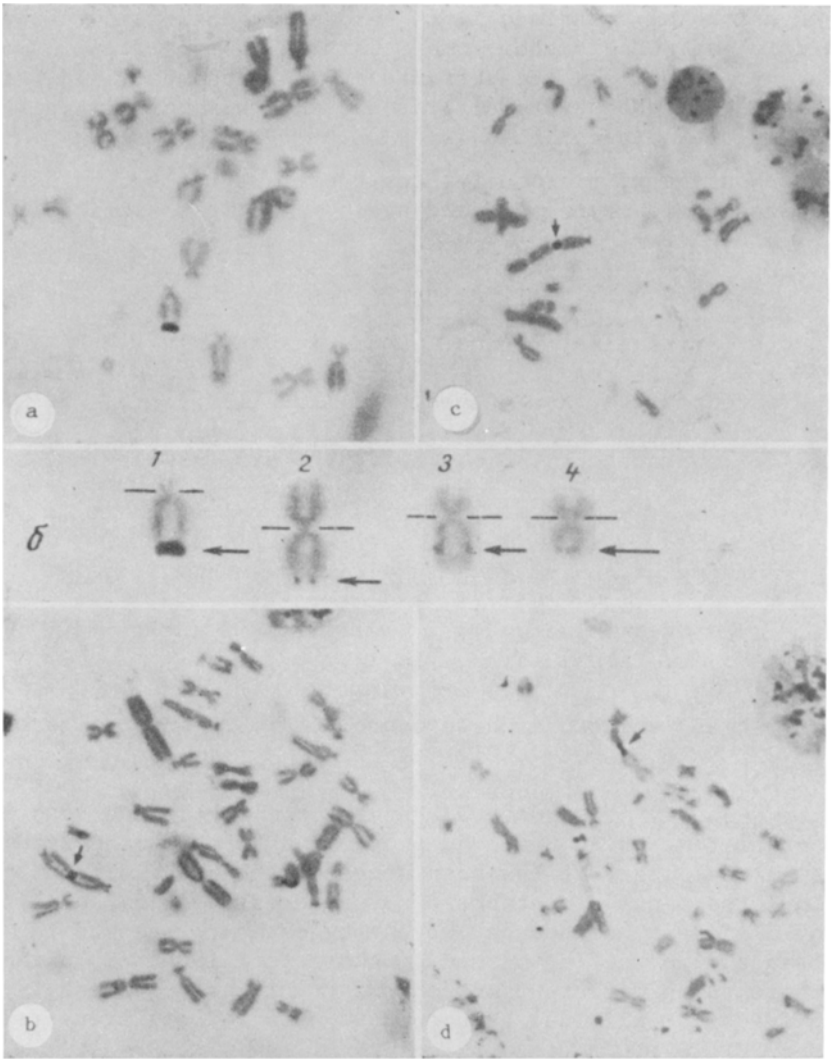


Fig. 1. NOR of chromosomes in mononuclear cells and cells with micronuclei. a) Metaphase of clone 237 of line $BII^{d}$-ii-FAF28 with nucleolar organizing chromosomes; b) nucleolar organizing chromosome 1, 2, 3, and 4; c) association between chromosomes 1 and 2 in a mononuclear cell; d) association between chromosome 1 in a cell with micronuclei, e) association between chromosome 2 in a cell with micronuclei. Impregnation with silver nitrate. 770 ×.

Analysis of 29 tetraploid metaphases showed that they all contained stained NOR of chromosomes 1, 2, and 3. In all metaphases chromosome 1 was present most frequently (88%). The frequency of chromosomes 2 and 3 was 62 and 31%. Chromosome 4 with NOR was found in 5 metaphases.

The problem of association of NOR of chromosomes in polykaryocytes is of the greatest interest. Since the formation of micronuclei from metaphase of mononuclear cells takes place arbitrarily, it can be tentatively suggested that both chromatids of a certain chromosome accidentally enter one micronucleus. Under these cirumstances, there will be two complete copies of a chromosome in the first-division metaphase of the polykaryocytes. If such chromosomes contain NOR, associating in mononuclear cells, with a high level of probability they must associate in metaphases of polykaryocytes.

By analyzing chromosomes of polykaryocytes we found that the first two chromosomes were found in 78 cases, in 40 of which they took part in association with one another (51.3%) (Fig. 1d). Chromosome 2 also was frequently present in these same metaphases. Meanwhile two second chromosomes were found in 40 metaphases, but their association was present in only 3 cases (7.5%). Chromosome 1 was absent in the hypotetraploid and in hypodiploid metaphases (Fig. 1e).

By using this experimental technique it is evidently possible to estimate quantitatively the powers of association of NOR of chromosomes, which cannot be done in mononuclear cells with respect to each chromosome. In the present case chromosomes 1 and 2 in mononuclear cells form associations with one another extremely rarely, and this makes it difficult to determine the associative activity of these chromosomes.

Polykaryocytes consisting of micronuclei can therefore be used to assess the powers of association of chromosomes carrying NOR and taking part in associations in mononuclear cells.

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# COMPARISON OF SOME METHODS OF MEASURING C SEGMENTS OF HUMAN CHROMOSOMES


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UDC 612.014.24:576.316.2]-086.3

KEY WORDS: chromosomes; structural heterochromatin; C segments; morphometry


The effect of the quantity of structural heterochromatin in the genome on formation of the human phenotype still remains an unsolved problem. To endow the results of investigations with an objective character, it has been suggested that the size of regions of structural heterochromatin (C segments of metaphase chromosomes 1, 9, 16, and Y) be estimated quantitatively. Many different methods have been suggested to measure the size of C segments [2-7]. The absence of a unified method of characterizing their size may be the reason why investigators using different methods of quantitative assessment of the size of C segments, studying identical clinical groups, have obtained different results.

The aim of this investigation was to study the comparability and reproducibility of the results of determination of the absolute dimensions of C segments, obtained by the use of known methods of quantitative measurement of their size.

## EXPERIMENTAL METHOD

Preparations of metaphase chromosomes of blood lymphocytes, obtained in the course of a double investigation of three healthy women, stained by the C method [11], were used. Altogether 120 metaphase plates (20 in each culture) were photographed on Mikrat-300 film. Negatives were projected on the screen of a "Mikrofot" instrument and the boundaries of the C segments and euchromatin regions of chromosomes 1, 2, 9, and 16 were outlined in accordance with the recommendations in [3]. The results of the measurements were estimated in microns, allowing for total magnification from the original size of the chromosome by 3000 times. Homologous chromosomes were studied separately. The absolute dimensions of the C segments of chromosomes 1, 9, and 16 were determined in 5 metaphase plates with a length of chromosome 2 of between 5 and 10 μ, by the method in [4] and by regression correction [2] in the modification described in [5, 7]. Method [3] was used on 5 metaphase plates with a length of chromosome 2 of 7-9 μ. Absolute dimensions of C segments obtained by the above methods, and in paired cultures, were compared by the t test collectively for chromosomes 1, 9, and 16 of each individual studied.

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(Presented by Academician of the Academy of Medical Sciences of the USSR N. P. Bochkov.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 103, No. 3, pp. 348-349, March, 1987. Original article submitted January 8, 1986.




0007-4888/87/0003-0392$12.50