PRINCIPLES OF SUPRAMOLECULAR CHROMATIN ORGANIZATION

K. N. Fedorova and I. É. Yudina

UDC 612.112.94.014.24:576.315.42:577.212.3]-088.3

KEY WORDS: supramolecular organization; deoxyribonucleoprotein complex; interphase chromatin

To study the structure of interphase chromatin of human cells in situ the method of thermal denaturation of cell deoxyribonucleoproteins (DNP) [14] was used in the author's modification [2], followed by cytofluorometric recording of changes in chromatin structure revealed by acridine orange staining [13].

Binding of acridine orange with chromatin DNA of human peripheral blood lymphocytes between temperatures of 20 and 100°C was found to be governed by precise rules and to be described by a curve with maxima within certain temperature intervals [7]. Computer analysis showed that normal subjects can be distributed into groups on the basis of identity of individual features (number and location of the maxima) of lymphocyte chromatin melting profiles. In 40% of cases (irrespective of sex) six maxima were obtained at certain temperatures (modal class), and in 60% different types of deviations (sex-dependent) were observed, according to the character of which a number of subgroups could be distinguished: five in the control group of women, seven subgroups in men (no fewer than five identical cases in each subgroup). In patients with congenital and acquired chromosomal anomalies specific differences were discovered in melting profiles [4, 8], which correlate with the similar characteristics of the curves for individual subgroups of normal subjects, indicating probable genetic predisposition.

Distribution into groups, or the possibility of "classifying" cells of healthy and sick individuals on the basis of chromatin melting profiles led to the conclusion that the supramolecular organization of the chromatin complex of interphase nuclei has a strictly specific conformation [3].

To understand the causes of differences in structural organization of the genomes of healthy and sick individuals the first essential is to study the nature of the maxima observed on the chromatin melting curve.

We know that physicochemical modifications both of the isolated nucleoprotein and of the chromatin complex of intact cells are mainly due to two factors: dissociation of protein or labilization of its bond with DNA and the state of the secondary structure of DNA.

Since the hyperchromic effect of chromatin DNA (A_{260}) of healthy human lymphocytes is observed from 78-80°C, it was concluded that the temperature-induced structural modifications of cell chromatin are linked mainly with dissociation or labilization of the bond between the protein component and DNA (in any case, up to 80°C) [5].

The aim of this investigation was to study the role of the protein component in the structural changes in the DNP complex of human cells.

EXPERIMENTAL METHOD

Cell chromatin from peripheral blood granulocytes and lymphocytes of 15 healthy people aged from 20 to 40 years was used. Changes in chromatin structure during heating of the cells from 20 to 95°C (at intervals of 2-3°C) were recorded as the quantity of bound luminescent label – acridine orange (AO) – with DNA [13]. Experiments were carried out on cells incubated for 1 h in Eagle's nutrient medium with the addition of 10% autologous serum. The intensity of luminescence of AO bound with cell chromatin DNA was measured on an MSP 0.5

Central Research Institute of Hematology and Blood Transfusion, Ministry of Health of the USSR, Moscow. (Presented by Academician of the Academy of Medical Sciences of the USSR S. S. Debov.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 104, No. 7, pp. 81-83, July, 1987. Original article submitted June 10, 1986.

 0007-4888/87/0007-0974$12.50

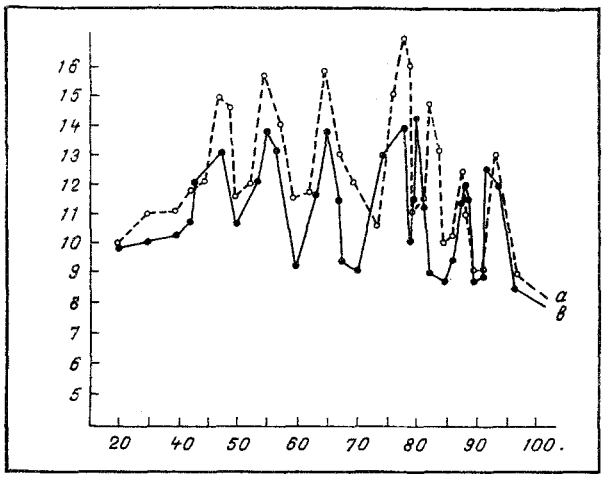

Fig. 1. Interphase chromatin melting profiles of healthy human peripheral blood granulocytes (a) and lymphocytes (b). Abscissa, temperature (in °C); ordinate, ratio of intensity of fluorescence of AO bound with human peripheral blood cell chromatin at T°C to its value at 20°C: $F_{530,T°}/F_{530,20°}$.

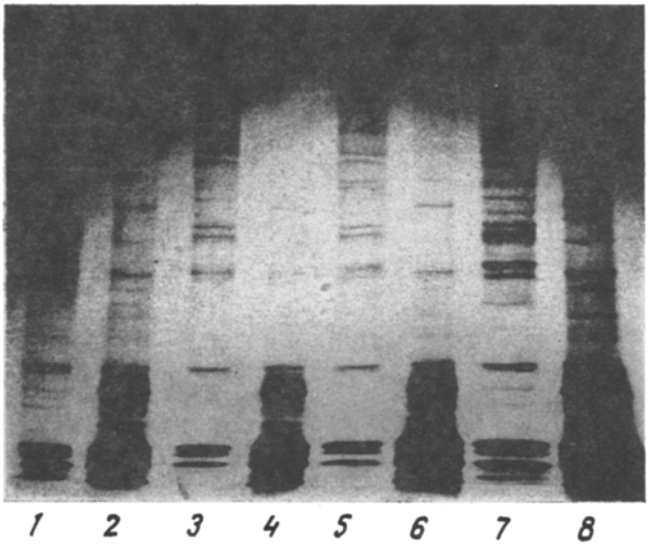

Fig. 2. Electrophoresis of nuclear proteins of human lymphocytes and granulocytes. 1, 3, 5, 7) Lysates of lymphocyte nuclei; 2, 4, 6, 8) lysates of granulocyte nuclei.

photometer-microscope (Opton). Details of the experiment were described previously [2]. Thermal denaturation was carried out as in [14], in the writers' modification [2]. Cell chromatin melting curves were compared for all points of the curve with respect to the average intensity of fluorescence and the parallel character of the change in its intensity. The significance of differences was estimated by one version of two-factor dispersion analysis [1].

Electrophoretic fractionation and isoelectric focusing of nuclear proteins of healthy human lymphocytes and granulocytes were carried out in parallel experiments. The nuclei of previously separated lymphocytes and granulocytes from heparinized peripheral blood were isolated by the method in [10] in a sucrose gradient (0.25 and 0.32 M), 3 mM $MgCl_2$, 0.01 M Tris-HCl, pH 7.3, preceded by homogenization in sucrose solution with the addition of 0.5% Triton X-100 and 1 μM phenylmethylsulfofluoride (PMSF).

One way polyacrylamide gel (PAG) electrophoresis of proteins in the presence of sodium dodecylsulfate (0.1%, SDS) was carried out by the method in [11] in a block with 7-15% acrylamide gradient. Samples for electrophoresis were dissolved in buffer containing 2% β-mercaptoethanol, 10% sucrose, 2% SDS, and PMSF, and heated for 2 min on a waterbath at 100°C.

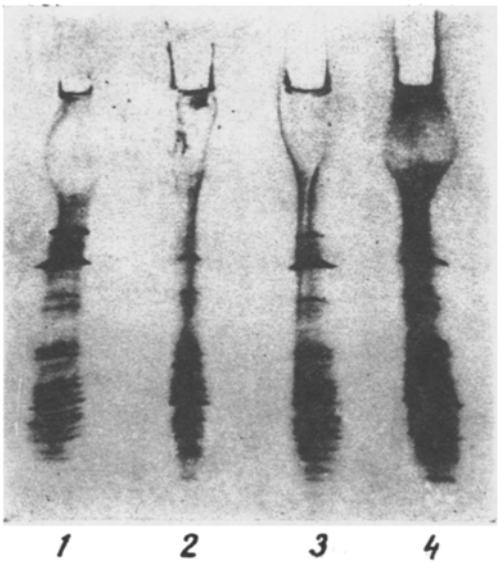

Fig. 3. IEF of nuclear proteins of human lymphocytes and granulocytes. Samples applied and direction of IEF from the cathode. 1, 3) Lymphocytes; 2, 4) granulocytes.

Isoelectric focusing (IEF) in PAG in the presence of 9.2 M urea and 2% Triton X-100 was carried out by the method [12] in a block with pH gradient 3.5-9.5. Samples for IEF were dissolved in buffer containing 9.5 M urea, 2% Triton X-100, 2% ampholines 3.5-9.5 (LKB, Sweden), and 5% β-mercaptoethanol. Samples were applied from the cathode.

The gel plates were stained in a mixture of isopropanol—acetic acid—water (25:10:65) with Coomassie R-250 in a concentration of 0.05%, followed by rinsing in 7% acetic acid.

EXPERIMENTAL RESULTS

Analysis of the intensity of fluorescence (F_{530}) of the DNA complex with AO between temperatures of 20 and 100°C (at intervals of 2-3°C) revealed no statistically significant differences between the chromatin melting profiles of the granulocytes and lymphocytes of the same individual, i.e., despite their different functions, the character of the supramolecular organization of the two cell populations is on the whole similar (Fig. 1).

The similarity of the configuration of the cell chromatin melting curves (i.e., the presence of the same number of maxima in the same temperature intervals) was observed by the present writers also in other systems (lymphocytes and spermatozoa from the same individual), although we know that histone fractions in the nuclei of mature spermatozoa are replaced by cysteine-containing proteins [6, 9]. Suspensions of nuclei from granulocytes and lymphocytes (from the same individual) served as material for electrophoretic protein fractionation in parallel experiments. Under the denaturing conditions used, clear differences were found over the whole spectrum of total nuclear protein of cells of the myeloid and lymphoid series, which were basically quantitative in character (Fig. 2).

Suspensions of lymphocyte and granulocyte nuclei (from the same individual) served at the same time as material with which to study the composition of the nuclear proteins by isoelectric focusing in a pH gradient from 3.5 to 9.5. No significant differences (with this degree of resolution) could be obtained between cells of the lymphoid and myeloid series as regards the values of the isoelectric points (p_I) of the nonhistone proteins in the composition of the cell nuclei (Fig. 3).

CONCLUSIONS

1. Cell chromatin melting curves, which are considered to reflect the specific character of the supramolecular organization of the intracellular DNP complex, are independent of the quantity and molecular weight of the proteins composing it.

2. The absence of fundamental differences in the chromatin melting profiles and also in p_I of the proteins of granulocyte and lymphocyte nuclei of the same individual suggests that the specific character of the supramolecular organization of the cell DNP complex is determined by the charge of the proteins contained in the chromatin.

LITERATURE CITED

1. N. A. Plokhinskii, Biometric Methods [in Russian], Moscow (1975).
2. K. N. Fedorova, Usp. Sovrem. Biol., No. 4, 458 (1984).
3. K. N. Fedorova and I. É. Yudina, Byull. Éksp. Biol. Med., No. 4, 81 (1983).
4. K. N. Fedorova, I. E. Yudina, and E. B. Voronov, Byull. Eksp. Biol. Med., No. 4, 453 (1981).
5. K. N. Fedorova and I. É. Yudina, Byull. Éksp. Biol. Med., No. 2, 208 (1981)
6. K. N. Fedorova, "Structure of interphase chromatin of human lymphocytes with normal and abnormal karyotype," Author's Abstract of Dissertation for the Degree of Doctor of Medical Sciences, Moscow (1982).
7. K. N. Fedorova, Byull. Éksp. Biol. Med., No. 7, 878 (1976).
8. I. É. Yudina and K. N. Fedorova, Byull. Éksp. Biol. Med., No. 11, 586 (1984)
9. D. Bouvier, Cytobiologie, 15, 420 (1977).
10. A. Boyum, Scand. J. Clin. Lab. Invest., 21, Suppl. 97, 77.
11. U. K. Laemmli, Nature, 227, 680 (1970).
12. P. H. O'Farrell, J. Biol. Chem., 250, 4007 (1975).

5-IODODEOXYURIDINE, LIKE 5-BROMODEOXYURIDINE, INDUCES SPECIFIC DICENTRIC CHROMOSOME FORMATION IN CELLS WITH MICRONUCLEI

V. I. Stobetskii, Yu. Kh. Khapchaev, and L. L. Mironova

UDC 612.014.24:576.316.22].014.46:[615.31:577.113.3

KEY WORDS: 5-iododeoxyirodome; 5-bromodeoxyuridine; cells with micronuclei; dicentrics; chromosomal aberrations

Colcemid, in a dose blocking mitosis, if added to a culture of Chinese hamster cells of the B11d-ii-FAF28 line for 30-40 h, induces the formation of cells consisting of micronuclei. If simultaneous treatment with colcemid and 5-bromodeoxyuridine (BUdR) is given, many dicentric chromosomes are found in first division metaphases of the polykaryocytes. It was shown previously [3] that dicentric formation is connected with delayed disruption of telomeric links in the interphase nucleus. Besides in B11d-ii-FAF28 cells, this phenomenon has also been reproduced in hybrid human × Chinese hamster somatic cells [2].

The writers have studied the possibility of formation of specific dicentric chromosomes by the use of another halogen analog of thymidine instead of BUdR, namely 5-iododeoxyuridine (IUdR).

EXPERIMENTAL METHOD

Clone 237 of line B11d-ii-FAF28 was used. The cell line was generously provided by A. N. Chebotarev (Insitute of Medical Genetics, Academy of Medical Sciences of the USSR). The cells were cultured on Eagle's medium with 10% bovine serum in rectangular flasks with a capacity of 0.5 liter. Chromosome preparations were made by the standard air-dried method.

The following variants of the experiment were set up: 1) intact cell culture; 2) cells in culture treated with BUdR (20 µg/ml, from Serva) for 42 h; 3) cells in culture treated with IUdR (20µg/ml, Serva); 4) cells in culture treated with colcemid (0.1 µg/ml) for 42 h;

Laboratory of Tissue Culture, Institute of Poliomyelitis and Virus Encephalitides, 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 Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 104, No. 7, pp. 83-85, July, 1987. Original article submitted August 22, 1986.