The elucidation of these problems will be a task for future research.

LITERATURE CITED

1. L. A. Pevnitskii, L. Yu. Telegrin, and V. N. Bol'shev, Byull. Éksp. Biol. Med., No. 4, 438 (1977).
2. B. E. Domeyer and N. E. Sladek, Cancer Res., 40, 174 (1980).
3. N. K. Jerne and A. A. Nordin, Science, 140, 405 (1963).

INTERPHASE CHROMATIN OF HUMAN CELLS WITH DIPLOID AND HAPLOID GENOME

K. N. Fedorova and I. É. Yudina

UDC 616-055.3.07:616.155.32-075.5

KEY WORDS: chromatin; lymphocytes; polymorphism.

A previous analysis of formal denaturation of intracellular DNP by fluorescence microscopy and acridine orange staining in the writers' modification [4] showed that the structure of the interphase chromatin of normal human lymphocytes exhibits marked polymorphism under the influence of temperature: In about 40% of cases, irrespective of sex, the melting profiles of lymphocyte chromatin consist of a curve with six (or seven) maxima at definite temperatures; in 60% of the cases types of deviations, repeated regularly in different individuals, and whose character depended on sex, were found. The commonest type of deviation in men of the control group was absence of a maximum at 85°C [5].

In the present investigation the structural features of chromatin of human cells with equal quantities of genetic substrate, i.e., with a haploid set of chromosomes (and with the highest degree of condensation) were studied.

EXPERIMENTAL METHOD

The structure of chromatin of mature spermatozoa from 10 normal men aged from 25 to 40 years with normal spermatogenesis (the ejaculate was analyzed at the Family and Marriage Guidance Clinic, Sverdlovsk District Health Department) by comparative analysis of the melting curves of DNP from spermatozoa and lymphocytes of the same individual.

Changes in the structure of chromatin in response to temperature were tested relative to the quantity (F_{530}) of bound luminescent dye (acridine orange — AO). Tests were carried out on films of ejaculate stored in fixing solution (acetone:ethanol = 1:1) for not more than 72 h.

The intensity of luminescence of AO bound with DNA of chromatin from spermatozoa was measured on an MSP-0.5(P) microscope-photometer (from "Opton"). The excitation wavelength was λ = 365 nm. The intensity of luminescence was determined at λ = 530 nm. Thermal denaturation of chromatin in the spermatozoa was induced by Ringertz' method [9] in the writers' modification [4]. Values were read with an interval of 1-2°C.

As the control, melting profiles of lymphocyte chromatin from the same individual were tested in parallel experiments. The methods of taking blood, preparation of the films for spectrofluorometric analysis of the lymphocyte chromatin, the parameters of the apparatus, and calculation of the coefficient "α" were all described previously [6].

Melting profiles of chromatin from lymphocytes and spermatozoa of each individual were compared for all types on the melting curve with respect to mean intensity of fluorescence and the parallelness of the change in its intensity. Significance of differences were assessed by a special variant of two-factor dispersion analysis (F^2). The significance of differences in the mean level of the processes was determined as the significance of the

Institute of Medical Genetics, Academy of Medical Sciences of the USSR, Moscow.

(Presented by Academician of the Academy of Medical Sciences of the USSR N. A. Yudaev.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 95, No. 4, pp. 81-83, April, 1983. Original article submitted July 23, 1982.

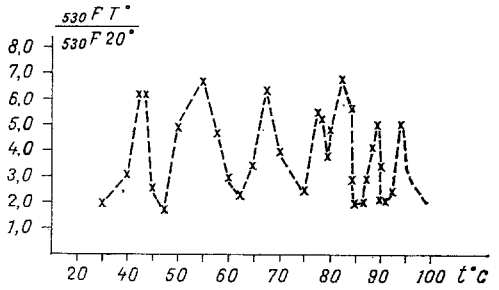

Fig. 1. Melting profile of chromatin from mature spermatozoa of normal men obtained by luminescence fluorometry with acridine orange (F_{530}) (averaged data). Here and in Fig. 2: abscissa, temperature (in °C); ordinate, ratio of intensity of fluorescence of AO bound with chromatin of diploid and haploid human cells (λ = 530 nm), at T° to intensity at 20°: $F_{530}T°/F_{530}20°$.

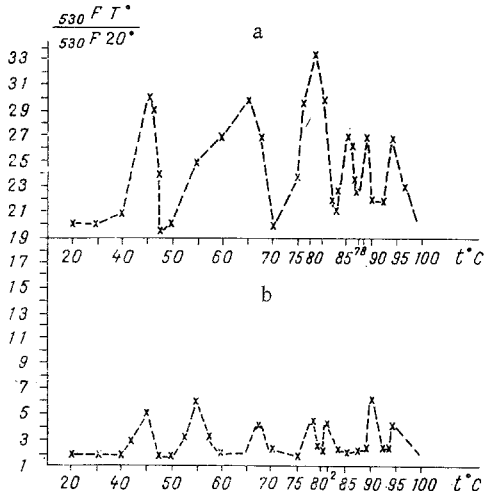

Fig. 2. Melting profiles of interphase chromatin of peripheral blood lymphocytes (a) and mature spermatozoa (b) of the same individual (separate case).

difference from zero of the mean difference of individual means, whereas the significance of divergence from parallel was assessed as the significance of the difference of variation of individual differences from random scatter [2].

EXPERIMENTAL RESULTS

Analysis of F_{530} data for the DNA–AO complex within the temperature range from 20 to 100°C showed that melting profiles or structural transitions of chromatin under the influence of temperature in normal healthy human spermatozoa in all cases studied were curves with seven maxima at particular temperatures: 45 (±2°), 55 (±1°), 67 (±2°), 77 (±1°), 82 (±0.5°), 89 (±1°), 92 (±2°)°C ($P < 0.01$). The intensity of luminescence of AO bound with spermatozoal DNP was 1.5-6.5 units (Fig. 1). Melting profiles of peripheral blood lymphocyte chromatin from these same individuals presented a polymorphic picture and could be divided into two subgroups: 1) six or seven maxima at particular temperatures: 45, 65, 78, 85, 88, and 92°C ($P < 0.01$), as we described previously for the standard, or classical, variant of normal (using the same test), irrespective of the subject's sex (seven cases); 2) cases with typical deviation from the standard variant of normal characteristic of men (for this test), namely absence of a maximum at 85°C and the appearance of one at 82°C (three cases).

The intensity of luminescence of AO bound with lymphocyte DNP ranged from 15 to 60 units (Fig. 2).

Melting profiles of chromatin from normal human spermatozoa are thus curves of uniform types (with this degree of resolution) and with maxima at particular temperatures, identical

to those obtained with lymphocytes from normal men with definite deviations from the standard variant of normal. Heteromorphism with respect to structure of spermatozoal chromatin was not revealed by this test.

It could be concluded from our previous investigations [3] on diploid cells with crossed induction of lymphocytes from healthy and sick individuals in homologous sera, and also from data in the literature [8, 10] on differential melting profiles of chromatins obtained from different tissues, the specific character of which depended on the presence of certain proteins, that each maximum in the melting profiles is a reflection of structural modifications of a definite region of the chromatin complex or of definite chromatin fractions differing from each other (according to our tests) in their degree of thermolability.

Considering data in the literature on absence of DNA renaturation at 65°C [1] and also our data showing no change in the red component (F_{640}) and in the coefficient "α" up to 88-90°C [6], changes in cell DNA detected up to 90°C evidently arise mainly on account of dissociation processes or labilization of the bond joining protein to DNA.

The present investigation revealed similarity of configuration of melting curves of chromatin from lymphocytes and spermatozoa, i.e., the presence of the same number of maxima within identical temperature ranges. These data suggest that there is no difference in principle between DNA—protein interaction in chromatin of diploid and haploid genomes, although we know that histone fractions in nuclei of mature spermatozoa are replaced by cysteine-containing proteins; this is connected with condensation of the chromatin and, as electron microscopy has shown, with changes in packing of chromatin fibrils [7]. Perhaps in this case the so-called "contradiction" is attributable to the use of different technical approaches, yielding basically different information while keeping to the same terminology.

The uniformity of chromatin melting profiles of spermatozoa led to the conclusion that marked polymorphism in the specific details of the structural organization of lymphocytes from different individuals is manifested only with a high level of organization of the genetic substrate, namely the diploid cell level. This is confirmed by cases in which different structural features are found in chromatin from lymphocytes and spermatozoa of the same individual (Fig. 2).

The decrease in intensity of fluorescence of the dye (F_{530}) bound with spermatozoal DNP to one-tenth of its value in identical investigations of lymphocyte chromatin from the same donor, although the quantity of test substrate in the haploid cells is reduced only by half, must be noted.

This fact confirms the data showing that nuclear chromatin of spermatozoa is condensed compared with that in lymphocytes.

LITERATURE CITED

1. G. P. Zlobina, "Temperature-dependent changes in lymphocyte chromatin of normal individuals and schizophrenics," Author's Abstract of Candidate's Dissertation, Moscow (1974).
2. N. A. Plokhinskii, Biometric Methods [in Russian], Moscow (1975).
3. K. N. Fedorova, Byull. Eksp. Biol. Med., No. 7, 878 (1976).
4. K. N. Fedorova and D. M. Spitkovskii, Byull. Eksp. Biol. Med., No. 6, 672 (1976).
5. K. N. Fedorova, I. É. Yudina, and E. B. Voronov, Byull. Eksp. Biol. Med., No. 4, 453 (1981).
6. K. N. Fedorova, I. É. Yudina, and V. N. Shchepilov, Byull. Eksp. Biol. Med., No. 12, 717 (1981).
7. D. Bouvier, Cytobiologie, 15, 420 (1977).
8. A. Kurtz, A. Russel, and F. Sinex, Mech. Ageing Dev., 3, 37 (1974).
9. N. Ringertz and B. Gledhill, Exp. Cell Res., 62, 204 (1970).
10. L. Tsai and A. Ansevin, Biochemistry (Washington), 14, 1257 (1975).