REACTION OF HUMAN HETEROCHROMATIN AND EUCHROMATIN TO SPERMINE

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The number of cell nuclei with X and Y chromatin and the number of nuclei with a diffuse and a coarse chromatin network were determined in diploid fibroblast cultures from persons with karyotypes of 47,XXY and 47,XYY after treatment with spermine. Spermine did not affect the number of nuclei with X and Y chromatin but led to a marked increase in the number of cells with a coarse chromatin pattern of their nucleus. After the action of spermin on peripheral blood lymphocyte cultures the number of metaphases with delayed chromosomal condensation caused by preliminary treatment with 5-bromodeoxyuridine was increased considerably, and the degree of this delay also was increased. The results indicate sensitivity of the euchromatin to the condensing action of spermine and inertia of the heterochromatin.

KEY WORDS: spermine; euchromatin; heterochromatin; fibroblast culture.

It was recently shown that strict correlation exists between the heterogeneity of staining of different parts of mitotic chromosomes and the presence of heterochromatin or euchromatin in those parts [1, 2, 4-6]. It has been postulated that subdivision of chromatin into euchromatin and heterochromatin, present in the interphase cell nucleus, must also persist in the metaphase chromosome [1]. From this it followed that the mechanisms of condensation and decondensation of heterochromatin and euchromatin may differ significantly.

To test this hypothesis the reaction of euchromatin and heterochromatin was studied with polyamines, which act in hybrid systems as inducers of premature condensation of chromosomes [3]. Preliminary observations showed that one polyamine – putrescine – had no condensing action on heterochromatin but condensed euchromatin. These observations were further developed in the present investigation in which the polyamine spermine was used to study the condensation of euchromatin and heterochromatin; chromatin both from interphase nuclei and from mitotic human chromosomes was studied.

EXPERIMENTAL METHOD

Human fibroblast-like cells with a chromosome set 47,XXY and 47,XYY and peripheral-blood lymphocyte cultures from persons with chromosome sets of 46,XX, 46,XY, and 47,XXY were used as the test material.

Fibroblasts were cultured in Petri dishes with coverslips for 48 h in an atmosphere with 5% CO_2, after which spermine (Serwa, West Germany) was added for 1 h to the experimental flasks in a dose of 400 μg/ml medium. After fixation, the preparation used for analysis of the X chromatin was stained with carbofuchsin and analyzed in the Ergobal (Carl Zeiss) microscope (ocular 10x, objective 100x). For analysis of the Y chromatin the preparations were stained with quinacrine and examined in the MBB-1 microscope with LSO-1 luminescent attachment (LOMO) (filters 4-SZS-14 and 3-SS-4, ocular 10x, water-immersion objective 85x).

The number of nuclei with two Y bodies (line 47,XYY), with one Y body or without Y chromatin was determined in the preparations stained with quinacrine (Fig. 1a, b). The relative percentages of nuclei with

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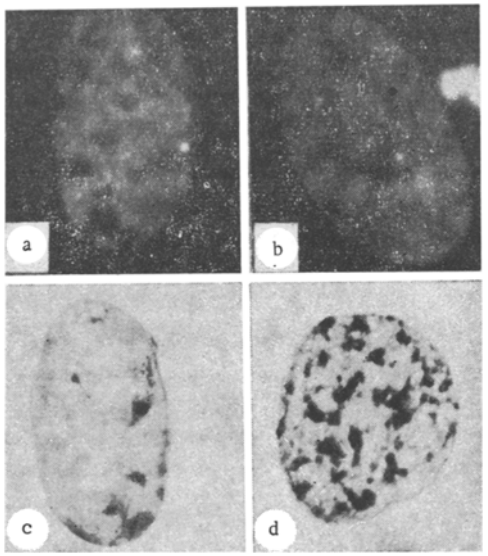

Fig. 1. Interphase nuclei of fibroblast cultures with karyotype 47,XXY and 47, XYY: a) nucleus with one Y body; b) nucleus with two Y bodies; c) nucleus with diffuse chromatin; d) nucleus with condensed chromatin.

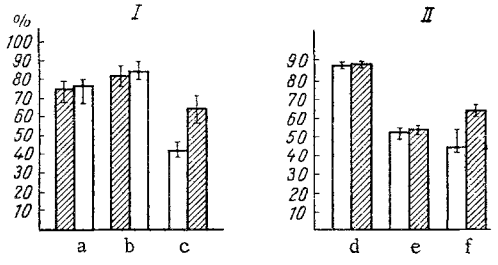

Fig. 2. Percentage of cells of fibroblast cultures with karyotypes 47,XXY (I) and 47,XYY (II) with nuclei containing X chromatin (a), Y chromatin (b), condensed nuclei (c and f), and one (d) and two (e) Y bodies. Unshaded columns – control; shaded columns – experiment.

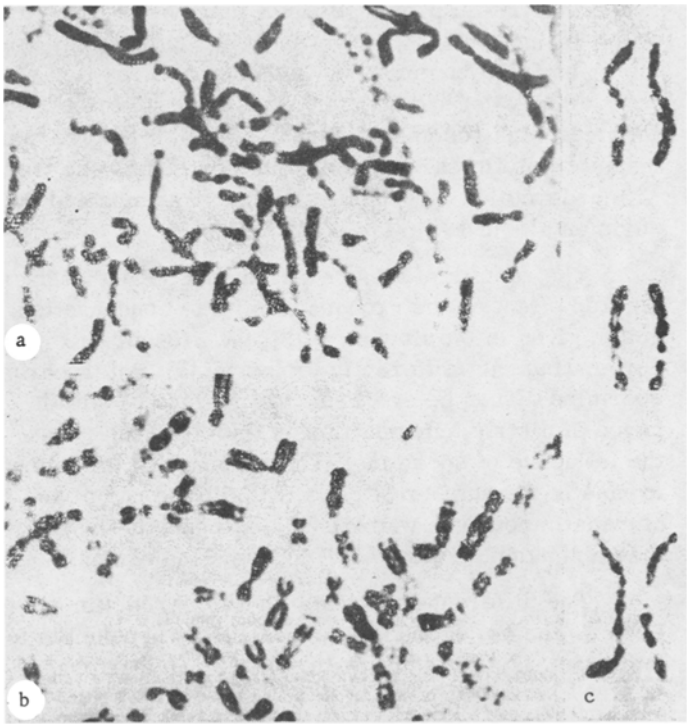

Fig. 3. Human metaphase chromosomes delayed in condensation by 5-bromodeoxyuridine, after additional treatment with spermine: a, b) fragments of metaphase plates; c) individual chromosome with sharply defined longitudinal differentiation.

a diffuse and a condensed chromatin network were studied in the same preparations stained with carbolfuchsin (Fig. 1c, d).

Peripheral venous blood obtained from persons with chromosome sets 46,XX, 46,XY, and 47,XXY, was cultivated in the usual way. 5-Bromodeoxyuridine (BDU) was added in a dose of 100 μg/ml culture medium to the control and experimental flasks 6 h before fixation. Spermine was added in a dose of 100 μg/ml medium to the experimental flask 3 h before fixation and colchicine was added in a dose of 0.5 μg/ml medium 2 h before fixation. Fixation was carried out in the usual way and air-dried preparations were stained with azure-eosin. During analysis of the chromosomal preparation attention was concentrated on the frequency of metaphases with good segmentation of the chromosomes in the control and experimental series, and also on the ratio between the number of prophases and the total number of metaphases in the control and experimental series.

EXPERIMENTAL RESULTS

The results of experiments to study the reaction of chromatin of the interphase nuclei to spermine are given in Fig. 2. The frequency of discovery of nuclei with X and Y chromatin was unchanged after the treatment with spermine and no difference was found between the control and experimental groups (Fig. 2a, b). The relative percentage of nuclei with diffuse and condensed chromatin was considerably shifted toward predominance of the latter after treatment with spermine (Fig. 2c). No changes were observed in the numbers of nuclei with one and two Y bodies after treatment of the 47,XYY culture with spermine, but a statistically highly significant increase was observed in the number of nuclei with a coarse chromatin network and with chromocenters (Fig. 2 d, e, f).

Analysis of preparations of metaphase chromosomes showed an increase of 5-6 times in the frequency of metaphase plates with well-marked differential condensation of chromosomes after treatment with spermine plus BDU compared with the control, treated with BDU only. In the experimental series cells with very sharply defined segmentation of most chromosomes were occasionally observed (Fig. 3a, b). These chromosomes resembled chromomeres, a feature that can be used with success for drawing cytological maps of mitotic chromosomes and for identifying them (Fig. 3c). The ratio between the numbers of plates with undercoiled, prophase-like chromosomes and the total number of metaphase plates was 1:3 in the experimental and 1:9 in the control series.

The results of these experiments show that spermine, a biologically active natural polyamine, has a marked effect on the process of mitotic condensation; however, compared with the account of this condensing action given in the literature [3], the present writer showed for the first time that heterochromatin and euchromatin react differently to spermine. Heterochromatin does not respond to the condensing action of spermine. Even heterochromatin regions of mitotic chromosomes delayed artificially in condensation behaved similarly. By contrast to this, both interphase and mitotic euchromatin underwent condensation under the influence of spermine. The response of the interphase chromatin to spermine was expressed as a decrease in the number of cells with diffuse nuclei and an increase in the number of nuclei with a coarse chromatin network, whereas the response of the mitotic chromatin was expressed as an increase in the size and number of the heterochromatin segments of chromosomes stretched by the action of BDU [1].

The different response of heterochromatin and euchromatin to polyamines could perhaps be one stage in the as yet unknown mechanism responsible for differential condensation of the two types of chromatin that is a constant feature of the various phases of the cell cycle under natural conditions.

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