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LATE CHANGES IN THE COURSE OF MITOSIS IN A SYNCHRONIZED CHINESE HAMSTER CELL CULTURE AFTER INHIBITION OF RNA AND PROTEIN SYNTHESIS

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The effect of inhibition of synthesis of various types of RNA and proteins at different periods of interphase on the course of late mitosis was studied in a synchronized culture of Chinese hamster cells. Analysis of the mitotic index and forms of pathology of division showed that the action of different doses of actinomycin D and puromycin in the first half of interphase produces an identical effect: C-mitoses in the immediate and late waves of cell division. Suppression of synthesis of total cell RNA and proteins in the second half of interphase was accompanied by delay of the cells in metaphase, with scattering of the chromosomes, evidence of a disturbance of the synthesis of the components of the division spindle. It is suggested that proteins (tubulins) and RNA participate as a reserve pool in the organization of the division spindle of late mitosis.

KEY WORDS: interphase; late mitosis; actinomycin D; puromycin; pathology of mitosis.

In continuously proliferating cell populations after division of the maternal cell the daughter cells, when commencing a new mitotic cycle, contain reserves of several structural proteins and enzymes, and also a set of different types of RNA synthesized in the preceding interphase, which provide for the passage of the cell through the initial and also, possibly, the late stages of the cycle. It was accordingly decided to use inhibitors of RNA and protein synthesis in an attempt to discover the relationship between synthetic processes in interphase and the course of the second mitosis after synchronization and to determine any functional relations between them.

EXPERIMENTAL METHOD

Experiments were carried out on a synchronized culture of Chinese hamster cells of strain B11du FAF-28, clone 237. The cells were synchronized by mitotic selection after preliminary treatment of the cells with colcemid [3, 6]. The harvested population of metaphase cells was seeded on penicillin flasks containing 2 ml culture medium at the rate of 100,000 cells to 1 ml. To inhibit rRNA synthesis actinomycin D was added to the cells in a dose of 0.1 μg/ml, and to inhibit the transcription of the total nuclear RNA (xRNA) the actinomycin D concentration was increased to 1 μg/ml. Puromycin (10 μg/ml) was used as inhibitor of protein synthesis. Incubation of the cells with the antibiotics began immediately after their emergence from the colcemid block. The inhibitors were added to the cells in the first half of interphase for 2 h (beginning of the G_1-period), for 4 h (G_1 and beginning of the S-period), for 6 h (G and first half of the S-period), and also in the second half of interphase for 6 h (second half of the S-period and G_2-period), for 4 h (end of the S-period and G_2-period), and for 2 h (principally the G_2-period). After incubation with the inhibitors the cells were thoroughly rinsed in Hanks' solution and transferred to fresh nutrient medium, in which incubation continued at 37°C until the cycle of the second wave of mitoses. The cells were fixed (alcohol : acetic acid 3 : 1) in the period of entry of the maximal number of synchronized cells into the second mitosis (23-24 h after emergence of the cells from

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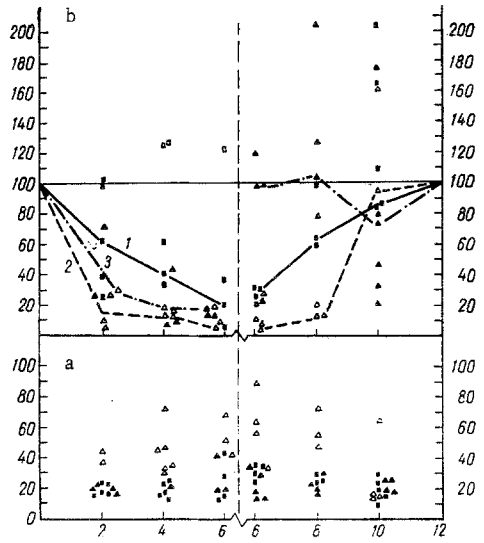

Fig. 1. Changes in MI and number of pathological mitoses after exposure to inhibitors in various periods of interphase in synchronized culture of Chinese hamster cells. Left half of graph shows action of inhibitors in first half of interphase, right half of graph – second half of interphase. a) Changes in number of pathological mitoses (in % of corresponding MI); b) changes in MI (in % of control). 1) Puromycin (10 μg/ml); 2) actinomycin D (1 μg/ml); 3) actinomycin D (0.1 μg/ml). Abscissa, here and in Figs. 2 and 3, time after emergence of cells from colcemid block (in h); ordinate: in a, changes in number of pathological mitoses; in b, changes in MI.

the colcemid block). The mitotic index (MI), phases of mitosis, and number of pathological mitoses and their types, calculated per 1000 cells on each slide, were determined. All the results were subjected to statistical analysis.

EXPERIMENTAL RESULTS

Analysis of the state of mitosis after the action of inhibitors of RNA and protein synthesis showed that in the second mitosis after synchronization some changes (after blocking of synthesis in the first half of interphase) were of the same character as in the first mitosis, whereas the other changes in mitotic activity and in the pathology of mitosis showed specific features (the second half of interphase).

Inhibition of transcription of total xRNA at all stages of the cycle in late mitosis led to a sharp decrease in mitotic activity. Inhibition of rRNA also caused delay in the entry of the cells into mitosis, but it was less marked, especially in the second half of interphase (Fig. 1b). The blocking of protein synthesis also led to a decrease in MI with division held up at the stage of prophase and telophase (only the G_1-period), but these changes were less significant than after inhibition of transcription of xRNA (Figs. 1b and 2a).

Just as after the action of inhibitors on the immediate mitosis, disturbance of transcription of total RNA and, to a lesser degree, suppression of protein synthesis in the first half of interphase caused predominantly the development of C-like mitoses in the late wave of proliferation. Other forms of pathology of mitosis (chromatid and chromosome bridges, deletion of chromosomes during movement, etc.) were encountered much less frequently. The appearance of a large number of colchicine-like mitoses (with hyperspiralization, swelling, and adhesion of chromosomes) after treatment with actinomycin D and puromycin was perhaps connected with

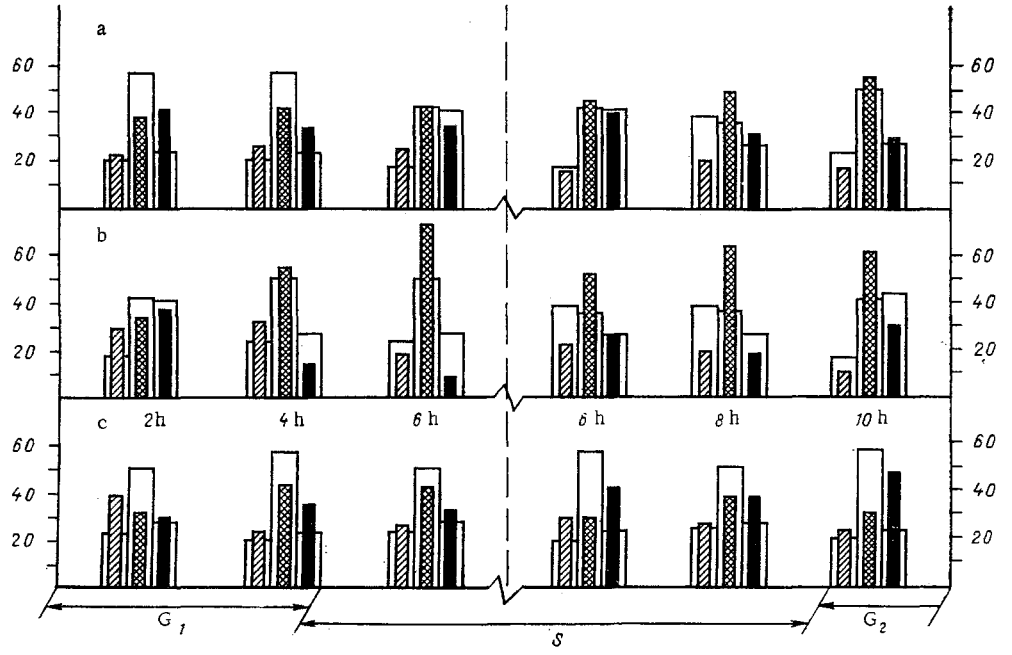

Fig. 2. Changes in ratio between phases of mitosis in late wave of divisions after exposure to inhibitors in different periods of interphase (results of one experiment). Left half of graph shows action of inhibitors in first half of interphase, right half – second half of interphase. a) Puromycin (10 μg/ml); b) actinomycin D (1 μg/ml); c) actinomycin D (0.1 μg/ml). 1) Number of prophases (oblique shading); 2) number of metaphases (cross-hatching); 3) number of ana- and telophases (black columns); 4) corresponding control values (unshaded columns). Ordinate, number of phases of mitosis (in % of corresponding MI).

inhibition of certain proteins responsible for spiralization of the chromosomes in prophase. Disturbance of the mitotic regime as a result of inhibition of synthesis at the beginning of the cycle was due, it can tentatively be suggested, to disorganization of the normal sequence of replication of the genome, manifested not only in the first mitosis after exposure, but also in late cell division.

Whereas after suppression of synthesis of xRNA, rRNA, and proteins in the first half of interphase the second mitosis after synchronization was characterized by predominance of C-mitoses, after disturbance of the same processes in the second half of interphase (especially at the end of the S-period or in the G_2-period) delay of mitosis in metaphase (predominance of metaphases with scattering of the chromosomes) and disturbance of the formation of the mitotic apparatus were typical (Fig. 3). These changes were most marked after blocking of transcription of xRNA, they were also considerable after treatment with puromycin, but they were absent after the addition of small doses of actinomycin D to the cells. As regards rRNA, delay of cell division in metaphase was evidently not associated at all with it. Meanwhile rRNA plays the most important role in the reconstruction of daughter nuclei, for after treatment with actinomycin D in a dose of 0.1 μg/ml at any period of interphase typical delay of mitosis in ana- and telophase was observed in the late wave of division.

These experiments to study the action of actinomycin D and puromycin of cells in the second half of interphase, and also earlier experiments with blockade of protein synthesis [1] and autoradiographic studies [2] show conclusively that proteins and xRNA synthesized at the end of the S-period–G_2-period of the mitotic cycle constitute a "reserve pool" of dividing cells, some of which will be utilized in the immediate mitosis, but most of which are intended for construction of the mitotic apparatus for the next cell division. The mechanism of this process requires further detailed analysis. It has been suggested [4, 5] that proteins (tubulins) and RNA of the mitotic apparatus, on the completion of the immediate mitosis, are redistributed between the daughter cells (the tubulins and monomers and oligomers) and are preserved as a "reserve pool." In the course of the next interphase some of the "building material" of the spindle undergoes natural degradation, and for that reason in the final stages of the cycle (and as regards RNA of the spindle, even earlier) – by the end of the G_1-period and beginning of the S-period – the mechanism of RNA and protein synthesis is reactivated

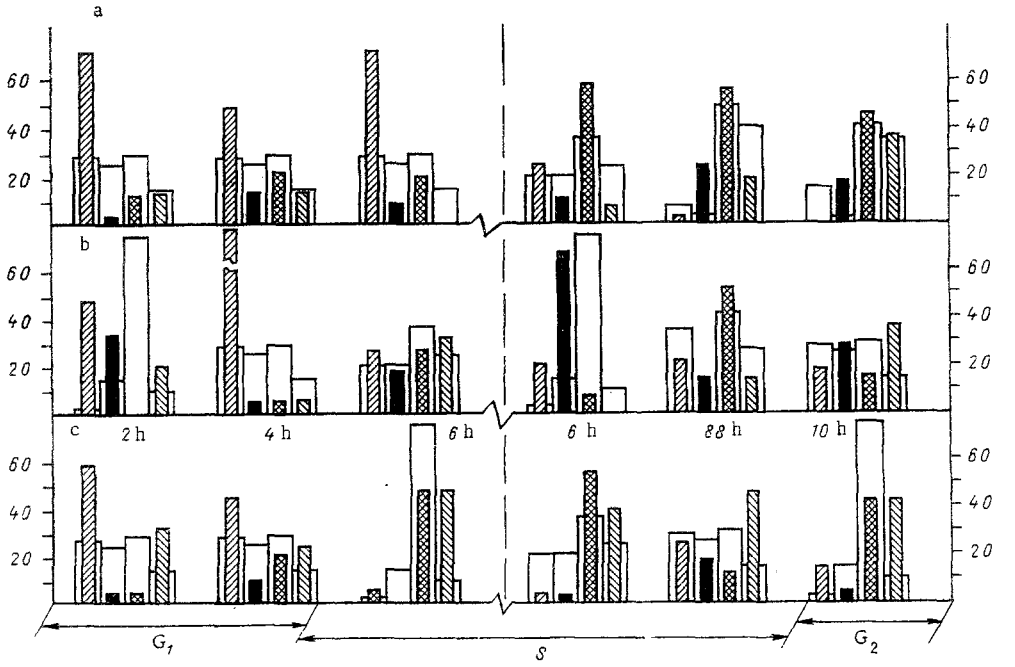

Fig. 3. Changes in ratio between pathological mitoses in late wave of division after treatment with inhibitors in different periods of interphase (results of one experiment). a) Puromycin (10 μg/ml); b) actinomycin D (1 μg/ml); c) actinomycin D (0.1 μg/ml). 1) C-mitoses; 2) scattered metaphases; 3) deletion of chromosomes; 4) bridges; 5) corresponding control values provided that the particular type of pathological mitosis is found in the control. Ordinate, number of different forms of pathological mitoses (in % of corresponding level of pathological mitoses).

and their pool in the cell is replenished to the physiological level. In other words, the division spindle of each mitosis consists partly of "fresh" proteins and RNA, synthesized before mitosis, and partly of "preserved" components, left over from the previous cycle.

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