EFFECT OF INHIBITORS OF RNA AND PROTEIN SYNTHESIS ON THE COURSE OF MITOSIS IN A SYNCHRONIZED CULTURE OF CHINESE HAMSTER CELLS

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The period of synthesis of different types of RNA and proteins related to the first mitosis after synchronization in interphase was investigated by means of inhibitors in a synchronized culture of Chinese hamster cells. Analysis of the mitotic index and forms of pathology of mitosis showed that proteins functionally connected with cell division are mainly synthesized in the second half of interphase. The most essential factor for the action of puromycin during this period is induction of C mitoses by suppression of synthesis of the protein responsible for spiralization of the chromosomes in prophase. Suppression of rRNA transcription at any stage affected cytotomy and reconstruction of the daughter nuclei, and was accompanied by delay in the emergency of the cells from mitosis. A high dose of actinomycin D, as an inhibitor of synthesis of total nuclear RNA, by contrast with puromycin, was most effective in the first half of interphase. It is suggested that at least three types of RNA, differing in their functional connections with the next mitosis, are synthesized during this period: an RNA for replication of the genome, an RNA for tubulin synthesis and, finally, an RNA which is a structural component of the division spindle.

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

In previous investigations on Chinese hamster cell cultures [1, 6] the dynamics of synthesis of proteins and total cellular RNA was studied throughout interphase and an attempt was made to determine the period of synthesis of proteins and RNA connected with mitosis [3]. Considering the limitations of morphological analysis of the distribution of labeled precursors above the dividing cells, in the present investigation an indirect approach was used to determine the period of synthesis of the different types of RNA connected with mitosis, with the aid of actinomycin D, a specific inhibitor of RNA synthesis. To differentiate between the direct action of actinomycin D on mitosis (for example, inhibition of synthesis of the RNA of the mitotic apparatus) from its indirect action (disturbance of the synthesis of tubulins and enzymes associated with inhibition of RNA synthesis), puromycin was used in parallel experiments to suppress protein synthesis.

EXPERIMENTAL METHOD

In the experiments of series I, on an asynchronous population of a culture of Chinese hamster cells (strain B11dii FAF-28, clone 237) the optimal doses of actinomycin D necessary for selective inhibition of nucleolar RNA (rRNA) and for total nuclear RNA (xRNA) were determined from the degree of inhibition of incorporation of [^{3}H]uridine (5 μCi/ml, specific activity 16.1 Ci/mmole, exposure with cells 10 min) 1, 2, and 4 h after treatment with actinomycin D (0.05, 0.1, and 1.0 μg/ml; from Reanal). In the experiments of series II the effect of puromycin (10 μg/ml; from Serva) and of selected optimal concentrations of actinomycin D at different periods of interphase on the course of mitosis was compared in a synchronized culture of Chinese hamster cells. Synchronization was carried out by mitotic selection after preliminary treatment of the cells with colcemid [5, 14]. Collected metaphase cells were dispersed in the proportion of 100,000 cells to 1 ml in penicillin flasks containing 2 ml of culture medium. The mean duration of the mitotic cycle for cells of this particular strain was 13-14 h: t_{G_1} = 3-4 h, t_S = 7-8 h, and t_{G_2} = 1-1.5 h. The inhibitors were added starting from the time when the cells emerged from colcemid block, after 2 h (beginning of the G_1 period), after 4 h (G_1 period and beginning of the S period), and after 6 h (G_1 period and first half of the S period), and also during

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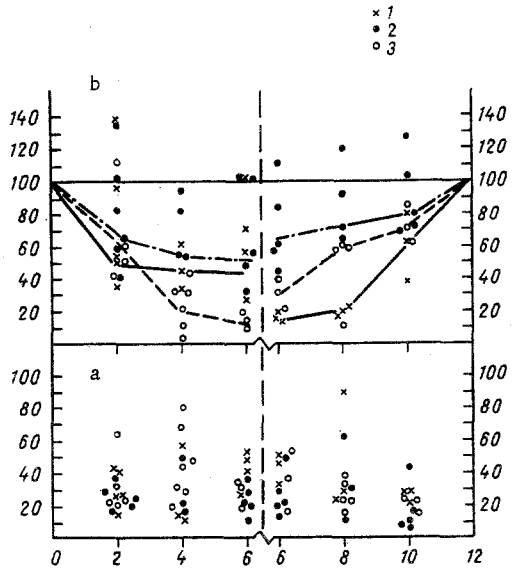

Fig. 1. Changes in MI and number of pathological mitoses in next wave of mitoses following action of inhibitors at different periods of interphase in a synchronized culture of Chinese hamster cells. Left side of graph shows action of inhibitors in first half of interphase; right side, in second half 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, 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 second half of interphase – after 6 h (second half of the S period and G_2 period), after 4 h (end of the S period and G_2 period), and after 2 h (mainly the G_2 period). After incubation with the inhibitors the slide with the cells was carefully washed in Hanks's solution and transferred to fresh nutrient medium, in which incubation of the cells continued at 37°C until the first wave of mitoses after synchronization, when the material was fixed (alcohol : acetic acid – 3 : 1). Only in a few cases, when the period of inhibition continued up to the next mitosis, were the cells fixed immediately after incubation with the antibiotics. The mitotic index (MI), the phases of mitosis, the number of pathological mitoses, and also the different types of pathology of mitosis were calculated in each preparation per 1000 cells. All the results were subjected to statistical analysis.

EXPERIMENTAL RESULTS

Analysis of the incoporation of [^{3}H]uridine into the nucleus and nucleolus led to the choice of actinomycin D in a concentration of 1 μg/ml for the cells of this strain in order to inhibit the synthesis of total xRNA, and a dose of 0.1 μg/ml for the selective inhibition of rRNA transcription.

Experiments on a synchronized cell culture showed that treatment with a high dose of actinomycin D at different periods of the cycle was always followed by a decrease in MI in the wave of mitoses immediately after synchronization, i.e., throughout interphase different types of RNA functionally connected with the subsequent entry of the cell into division were synthesized. Under these circumstances the inhibition of transcription of total xRNA in the first half of interphase depressed MI more strongly than in the second half (Fig. 1). This phenomenon correlated with an increase in the number of pathological mitoses induced by this dose of actinomycin D in the initial periods of the cycle (G_1 period to the beginning of the S period; see Fig. 1). De-

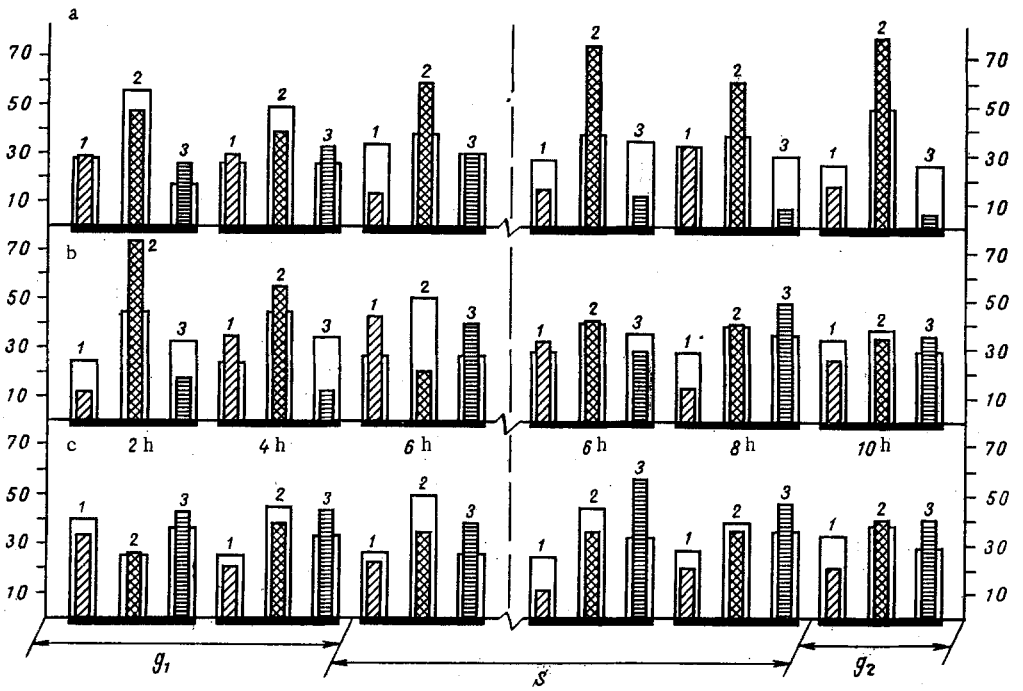

Fig. 2. Changes in ratio between phases of mitosis in next wave of divisions after action of inhibitors at different periods of interphase (results of one experiment given). Left side of graph shows action of antibiotics in first half of interphase; right side, in second half of interphase: a) puromycin (10 μg/ml); b) actinomycin D (1 μg/ml); c) actinomycin D (0.1 μg/ml). 1) Number of prophases; 2) number of metaphases; 3) number of ana- and telophases; unshaded columns) control. Abscissa, time after emergence of cells from mitosis (in h); ordinate, number of phases of mitosis (% of corresponding MI).

pression of rRNA synthesis affected MI only to a slight degree following contact between cells and antibiotic at any stage of the cycle. However, the number of pathological mitoses was somewhat greater than at the beginning of the cycle when a low concentration of actinomycin D acted in the second half of interphase (except the G_2 period; see Fig. 1).

Puromycin, like actinomycin D, strongly inhibited the entry of the cells into the next mitosis at whatever time it acted in the course of the cycle. However, unlike actinomycin D, the sharpest decrease in MI and simultaneous increase in the number of pathological mitoses were observed chiefly when protein synthesis was suppressed in the second half of interphase (Fig. 1). Inhibition of protein synthesis in the G_1 period under these circumstances usually led to delay in the emergence of the cells from mitosis, but as suppression spread to the S period, metaphase delay, which was usually particularly well marked when the cells were in contact with the inhibitor at the end of the S period and in the G_2 period, began to appear (Fig. 2a). Meanwhile, as the cells advanced from the G_1 into the G_2 period, puromycin was found to induce a much higher level of c-like mitoses than in the control. In the G_1 period, the c mitoses were joined by a different form of pathology of cell division, namely chromatid and chromosomal bridges. Possibly the bridges, as a result of linkage of the chromosomes in anaphase, were responsible for anaphase delay after the action of puromycin in the G_1 period. As the cells advanced from the S period to the G_2 period (especially in the latter), inhibition of protein synthesis led not only to c mitoses, but also to the appearance of yet another form of pathology – to scattering of the chromosomes in metaphase (Fig. 3a), although the number of the latter was significantly less than in late mitosis arising under similar circumstances. The weaker effect of puromycin at the beginning of interphase was evidently due to its indirect action on mitosis – through a disturbance of the synthesis of structural proteins and enzymes for the initiation and maintenance of DNA replication [9, 10, 13] – for after the action of puromycin in the G_1 period and beginning of the S period, the chromosomal anomalies which mainly appeared were bridges and c mitoses. It can tentatively be suggested that the strengthening of the effect of puromycin in the second half of interphase on cell division is due to the fact that it is at the end of the S period and in the G_2 period that the proteins directly connected with mitosis (tubulins) and also the enzymes and structural components of the nuclear membranes and nucleolus are synthesized [2, 4, 8, 12]. The most important factor for

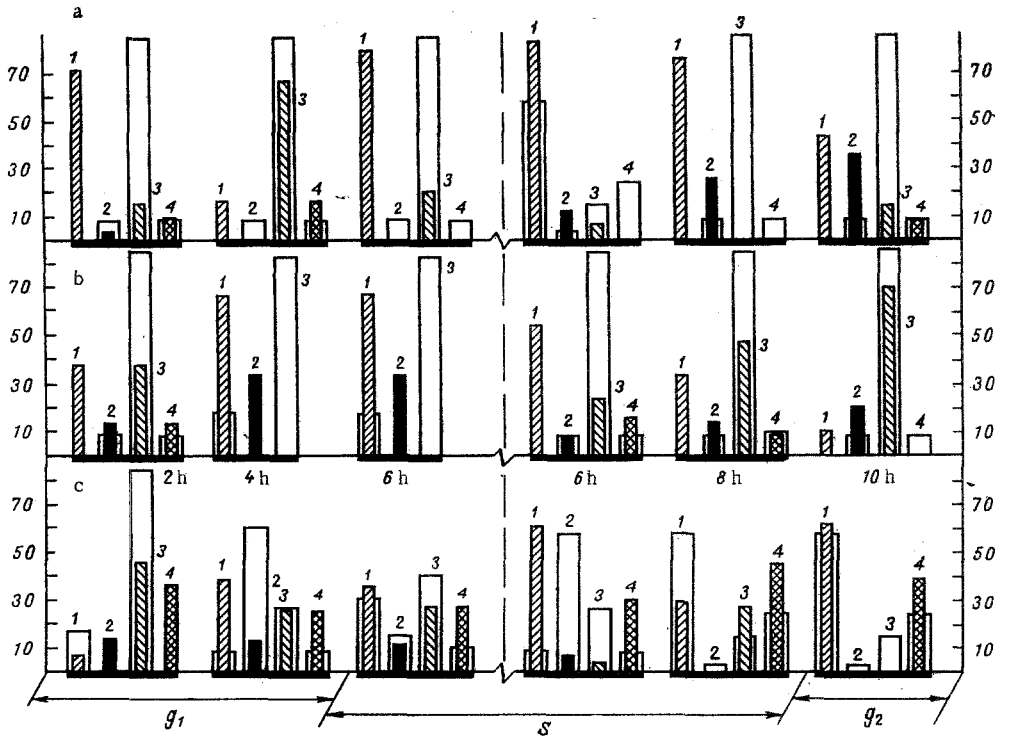

Fig. 3. Changes in ratio between pathological mitoses in next wave of divisions after treatment with inhibitors at different periods of interphase (results of one experiment). a) Puromycin (10 μg/ml); b) actinomycin D (1 μg/ml); c) actinomycin D (0.1 μg/ml). 1) c mitoses; 2) scattered metaphases; 3) chromosomal deletions; 4) bridges. Unshaded columns – control. Abscissa, time after emergence of cells from colcemid block (in h); ordinate, number of different forms of pathological mitoses (in % of corresponding level of pathological mitoses).

the action of puromycin on interphase of the next mitosis is probably suppression of synthesis of the protein (proteins?) responsible for spiralization of the chromosomes in prophase. Its inhibition is expressed as predominance of c-like mitoses with hyperspiralization and with fusion of the chromosomes in the next wave of divisions.

In the first half of the cycle, actinomycin D in a high dose, as an inhibitor of the entry of cells into mitosis and as an inducer of pathological mitoses was more effective than puromycin. Moreover, whereas puromycin led to metaphase delay after administration in the S to G_2 periods, in the case of suppression of transcription of xRNA the analogous effect occurred chronologically sooner: it was observed already in the G_1 period, whereas in the S period under these conditions the cells were predominantly in prophase (Fig. 2b). Administration of actinomycin D at the end of the S period and in the G_2 period was accompanied by delay of the emergence of the cells from mitosis (a high telophase index).

After contact between the cells and actinomycin D in a high dose, besides changes in the course of the individual phases of mitosis, an increase in the number of pathological mitoses was observed: c mitoses (throughout interphase), bridges (G_1 and S period), scattering of the chromosomes in metaphase (the first half of the cycle) (Fig. 3b).

Weakening of the action of actinomycin D compared with puromycin in the second half of interphase is difficult to explain at present. This phenomenon (and the telophase delay induced by actinomycin D in this period) may perhaps be connected with a disturbance by actinomycin D of RNA transcription for the synthesis of various secondary structural proteins and enzymes determining the processes of chromosome distribution, and also with a disturbance of the synthesis of RNP of the perichromosomal region [11].

Inhibition of transcription of rRNA in any phase had only a slight effect on the subsequent entry of the cells into mitosis, except in the early G_1 period: the level of depression of mitotic activity induced by a low dose of actinomycin D in the course of 2 h of the G_1 period remained almost unchanged during further incubation of the cells with the antibiotic (Fig. 1). The absence of rRNA synthesis at any stage of the cycle was most

frequently accompanied by delay in the emergence of the cells from mitosis (a high anaphase and telophase index) (Fig. 2c). Parallel with the increased representation of these stages of mitosis the number of bridges also was increased, especially in the S period. The ill-defined metaphase delay following the use of the smaller dose of actinomycin D was most frequently accompanied by accumulation of c-like mitoses (Fig. 3c).

Analysis of changes in the mitotic regime after exposure to inhibitors of RNA and protein synthesis suggests that in the first half of interphase at least three types of RNA connected with the next mitosis are synthesized: an RNA necessary for replication of the genome; an mRNA which begins to accumulate at the beginning of interphase and is responsible for tubulin synthesis at the end of the cycle and, finally, a structural RNA (with high molecular weight and not inhibited by low doses of actinomycin D), which is probably a component of the mitotic spindle [7].

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