EFFECT OF INHIBITION OF SYNTHESIS IN THE S-PERIOD ON THE SUBSEQUENT COURSE OF MITOSIS IN A SYNCHRONIZED CHINESE HAMSTER CELL CULTURE

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Transcription of total nuclear RNA and rRNA was inhibited by actinomycin D and protein synthesis inhibited by puromycin in the first and second halves of the S-period of the mitotic cycle in a synchronized culture of Chinese hamster cells. In the first case a reduction in mitotic activity was observed in the first and second waves of cell proliferation after synchronization regardless of the time of administration of antibiotics. At the same time an increase was observed in the number of pathological mitoses such as C-mitoses and scattering of the chromosomes in metaphase, and suppression of rRNA synthesis led to delay in emergence of the cells from mitosis (an increase in the fraction of ana- and telophases), associated most frequently with pathological mitoses such as chromatid and chromosomal bridges. Inhibition of protein synthesis in the first half of the S-period usually did not affect the mitotic regime of the cell culture in the first (nearest) wave of divisons but considerably inhibited cell division in the distant proliferation wave. It can tentatively be suggested that the cells enter the S-period with a complete set of proteins for the initiation and maintenance of genome replication, at least in the first stages of this process.

KEY WORDS: mitosis; synchronized cell culture; S-period; actinomycin D; puromycin.

The role of RNA and proteins synthesized in the course of the S-period and, in particular, whether they are necessary only for maintenance and completion of replication of the genome or whether there is a closer correlation between them and the course of mitosis, still remain matters for debate [1]. In the writer's view analysis of different forms of pathological mitoses after modification of the mitotic activity of cells through the action of inhibitors of RNA and protein synthesis in the S-period would shed some light on this problem.

EXPERIMENTAL METHOD

Synthesis of total nuclear RNA (xRNA) and nucleolar RNA (rRNA) was inhibited by actinomycin D (AMD, in doses of 1 and 0.1 μg/ml respectively) and protein synthesis was inhibited by puromycin (10 μg/ml) in a synchronized culture of Chinese hamster cells (strain B11dU FAF-28, clone 237) in the S-period. The cells were synchronized by mitotic selection after preliminary treatment with colcemid [3, 8]. The inhibitors were added to the cells either 3 h (first half of the S-period) or 6 h (second half of the S-period) after emergence of the cells from the colcemid block. After incubation for 3 h with the inhibitors the cells were carefully washed in Hanks' solution, after which incubation was continued in fresh medium at 37°C until the first (13 h) or second (25 h) wave of mitoses after the colcemid block, when the cells were fixed (alcohol : acetic acid, 3 : 1). In each 1000 cells of the preparations the mitotic index (MI), phases of mitosis, index of pathological mitoses (PM), and the different forms of pathology of mitosis were counted. All the results were subjected to statistical analysis.

EXPERIMENTAL RESULTS

Both inhibition of synthesis of all types of high-molecular-weight RNA and selective suppression of transcription of rRNA only at any stage of the synthetic period caused a decrease in MI of the cells in the first and second proliferation waves after synchronization Fig. 1). By contrast with the low dose, the high dose of AMD always led to a sharp increase in the relative proportion of PM.

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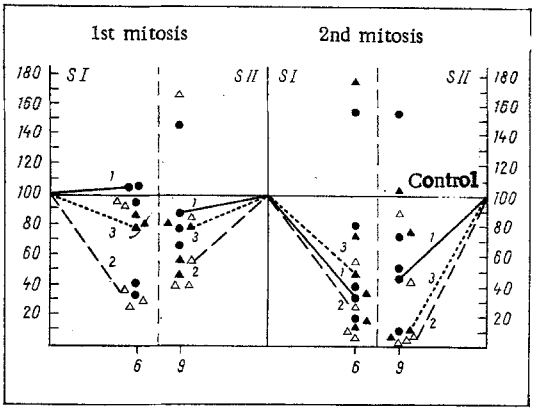

Fig. 1. Changes in MI during first and second waves of cell proliferation after treatment with inhibitors of RNA and protein synthesis. 1) Puromycin (10 μg/ml); 2) AMD (1 μg/ml); 3) AMD (0.1 μg/ml). Abscissa, time of ending of incubation of cells with antibiotics in first (SI) and second (SII) halves of S-period (in h); ordinate, MI (in % of control).

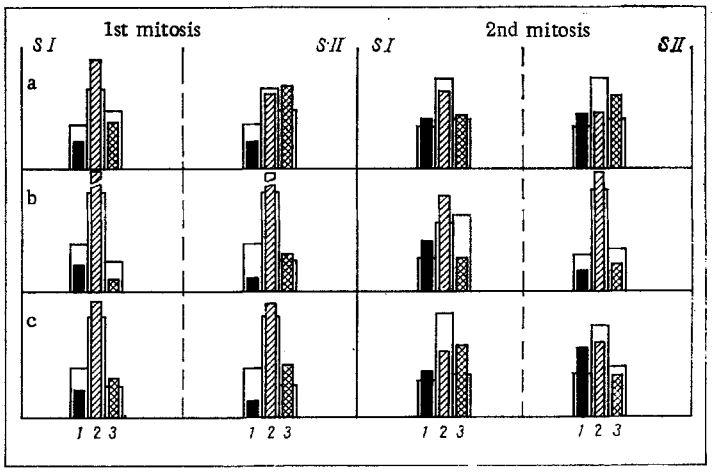

Fig. 2. Changes in ratio between phases of mitosis during first and second waves of cell proliferation after addition of inhibitors. Narrow columns represent experiment; wide unshaded columns appropriate control; 1) prophase, 2) metaphase, 3) anaphase and telophase. a) Puromycin (10 μg/ml); b) AMD (1 μg/ml); c) AMD (0.1 μg/ml). All data calculated as percentages of corresponding MI.

Inhibition of protein synthesis after addition of puromycin in the first half of the S-period in most cases had very little effect on the mitotic regime of the cell culture in the first, nearest, wave of divisions, whereas it suppressed cell division in the second, distant wave. Under the influence of this inhibitor on the cells a decrease in MI was always found in the final stages of the S-period at whatever time of active cell proliferation it was studied, but suppression of mitosis induced by puromycin was always weaker than that observed under the influence of the high dose of AMD (Fig. 1).

Besides inhibiting mitotic activity the antibiotics also caused changes in the duration of the individual phases of mitosis. For instance, in the nearest wave of mitosis the cells were characteristically held up in metaphase as a result of the action of all the inhibitors, whereas inhibition of xRNA synthesis induced this effect in the distant wave of proliferation also (Fig. 2). Depression of synthesis in the cell (by the different doses of AMD and by puromycin) was manifested functionally sometimes in the distant mitosis as an increase in the prophase index (Fig. 2). This phenomenon was evidently more attributable to difficulties preventing the cells from passing into the next stages of division than to stimulation of proliferative activity. Suppression of rRNA transcription throughout the S-period also led to delay in the emergence of the cells from mitosis (higher proportions of ana- and telophases in the stages of active proliferation), and this effect was most frequently associated with PM such as chromatid and chromosomal bridges.

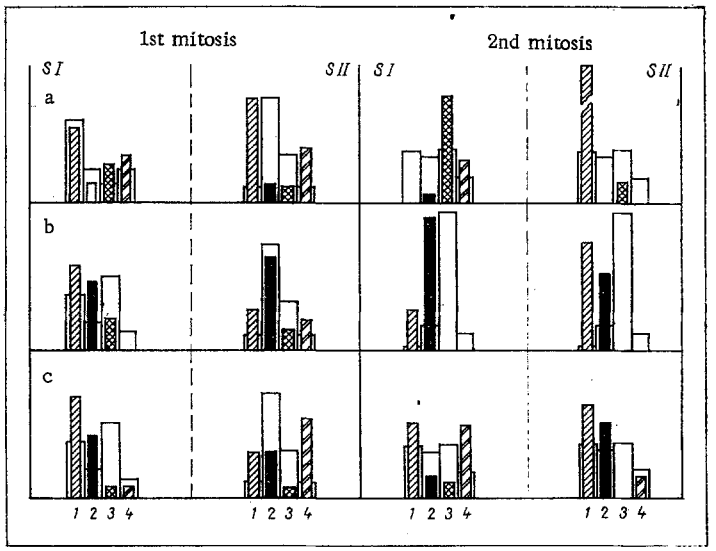

Fig. 3. Changes in ratio between different forms of PM during first and second waves of cell proliferation after addition of inhibitors. Narrow columns represent different forms of PM after addition of inhibitors, wide unshaded columns appropriate control. 1) C-mitoses; 2) scattering of chromosomes in metaphase; 3) chromatid and chromosomal bridges; 4) deletions of chromosomes during movement. All data calculated as percentages of corresponding total number of PM. Remainder of legend as in Figs. 1 and 2.

The most characteristic feature of inhibition of RNA and protein synthesis during genome replication was predominance of C-mitoses in the subsequent proliferation waves. This was evidently connected with suppression of the synthesis of a certain type of protein responsible for normal spiralization of the chromosomes. Another type of PM, namely scattering of chromosomes in metaphase, was connected with destruction of the division spindle of the cell, and was most marked in the nearest mitosis after contact of the cells with AMD in a dose of 1 μg/ml in the first half of the S-period, whereas in the distant proliferation wave it was most marked after incubation of the cells with the same dose of antibiotic throughout the complete phase of genome replication (Fig. 3).

To judge from the character of the changes in MI and the PM spectrum (deletions of chromosomes, bridges) under the influence of puromycin, proteins synthesized in the beginning of the S-period were functionally inert in relation to the nearest mitosis, and also evidently in relation to genome replication (MI in this case remained at almost the control level). This conclusion is probably not completely valid for proteins and enzymes which are synthesized in the final stages of the S-period (a very small decrease in MI in the first mitosis). These results confirm the views of other investigators [5, 6] on the existence of a reserve of proteins in the cell for the initiation and maintenance of genome replication, at least in the first stages of this process, and which accumulate in the cell in the G_1-period of the cycle. According to these views, inhibition of protein synthesis does not lead to the immediate cessation of replication of DNA templates – DNA synthesis continues at the normal rate, and does not stop until some time later, when the reserves of enzymes and structural proteins for replication are exhausted. As regards histones, a certain proportion of this fraction of basic nuclear proteins is evidently synthesized before the beginning of the S-period [4, 7], and the synthesis of the remainder manages to be made good after removal of the inhibitory effect of puromycin and before the entry of the cells into mitosis.

The appearance of PM such as scattering of chromosomes in metaphase in the nearest mitosis under the influence of the high dose of AMD (incubation of the cells with AMD in the first half of the S-period), and also in the 2nd mitosis (inhibition of transcription of xRNA throughout the greater part of the S-period) points to close functional connections between certain types of xRNA synthesized during these periods and subsequent cell divisions (transcription of RNA of the mitotic apparatus?).

Induction of PM of chromosomal type (C-mitoses, deletions of chromosomes, bridges) coupled with a decrease in MI is characteristic of the action both of puromycin (2nd mitosis) and of AMD (especially the low concentration of AMD in the first and second proliferation waves). In all probability this is due to disorganization of the normal order of genome replication both as a result of the direct action of AMD of the DNA template and through disturbance of the synthesis of the necessary enzymes. For instance, other workers have shown [2] that inhibition of protein synthesis throughout the synthetic period interferes with entry of the cells into the S-period of the next mitotic cycle, and this is inevitably manifested as a decrease in the mitotic activity of the cells during their second proliferation wave.

It can be concluded from these results that cells enter the synthetic period with a reserve set of proteins for the initiation and maintenance of genome replication, at least in the first stages of this process. Regulation of proliferation at the level of protein synthesis thus takes place mainly in the G_1-period (protein synthesis for DNA) and in the G_2-period (protein synthesis for the division spindle). As regards proteins of the S-period (especially those synthesized in the initial stages of this period), it can be postulated that they are relatively independent and functionally inert relative to the last cell division (the 1st mitosis). This conclusion is not valid for examination of functional connections between xRNA synthesis in interphase and the course of the next mitosis, for at the RNA level the regulation of cell proliferation extends also to the synthetic period: Certain types of RNA transcribed in this phase of the cycle are functionally essential for normal cell division.

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