CHANGES IN L-ORNITHINE DECARBOXYLASE ACTIVITY DURING THE CELL CYCLE

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Received April 14, 1976

SUMMARY

The activity of L-ornithine decarboxylase (L-ornithine carboxy-lyase; EC 4.1.1.17), the enzyme that catalyzes the initial and rate-limiting step in polyamine biosynthesis, has been studied in Chinese hamster ovary fibroblasts synchronized by selective detachment of mitotic cells. At various times after plating the distribution of cells among the G1, S and G2+M phases of the cell cycle was calculated from DNA distributions obtained by high-speed flow cytometric analysis. At these same times determination of the cellular L-ornithine decarboxylase activity showed that polyamine (putrescine) synthesis was initiated in mid-G1, that the rate of synthesis was maximal prior to DNA synthesis, and that it decreased during the S phase. A second increase in enzyme activity occurred before mitosis.

INTRODUCTION

The polyamines putrescine, spermidine and spermine have been implicated in growth processes (1,2). A rapid increase in the activity of L-ornithine decarboxylase (L-ornithine carboxy-lyase; EC 4.1.1.17), the enzyme that catalyzes putrescine synthesis and which is the rate-limiting enzyme in polyamine synthesis, has been observed in cells subjected to a number of different growth stimuli, e.g., in cultured hepatoma cells (3,4) and in baby hamster kidney cells (5) after the addition of serum or insulin, in mouse

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kidney cells (6) after infection with polyoma virus, in various target organs after hormone treatment (7-9), and in liver after partial hepatectomy (10,11). This increase in the activity of L-ornithine decarboxylase is frequently maximal within 6 hours after stimulation. However, in extended time studies of cells stimulated to synthesize DNA and divide, an additional peak of enzyme activity has been observed to occur late in the G1 or early in the S phase of the cell cycle (4,6,10-12).

To date, most studies on polyamine biosynthesis have been concerned with those changes which occur in the activity of L-ornithine decarboxylase within the first few hours after stimulation. In a recent publication we presented data which suggest that this early increase in enzyme activity is unrelated to normal cell cycle events, whereas the increase in putrescine synthesis, which occurs in late-G1, or early-S, is closely related to the cell's preparation for DNA synthesis and division (13). Further evidence to support this hypothesis is presented in the present report.

We have used a method which produces a synchronous, homogeneous population of cells by selective detachment of mitotic cells from monolayer cultures of exponentially growing (asynchronous) Chinese hamster ovary (CHO) cells. This technique was originally devised for HeLa cells (14) but it was subsequently modified for CHO cells (15). It allowed us to study polyamine synthesis in relation to a cell cycle that was not perturbed by synchronizing agents or affected by changes in the concentration of growth-promoting humoral or serum factors. Furthermore, the cells grew with very low dispersion for more than one cell cycle so that polyamine synthesis could be studied not only during the G1 phase but also during the S, G2 and M phases of the cell cycle.

MATERIALS AND METHODS

A subclone of the CHO cell line, adapted to monolayer growth, was used in these experiments. The cells were grown free of pleuropneumonia-like organisms in monolayer culture using α medium supplemented with 10% fetal calf serum (Flow Laboratories), penicillin (100 IU/ml) and streptomycin (100 mcg/ml) (GIBCO). To obtain synchronous populations of cells from asynchronous cultures we used the mitotic selection technique described by Terasima

and Tolmach (14) and by Petersen et al. (15). The mitotic cells were plated into plastic Falcon flasks and cultures were harvested at 2-hour intervals by trypsinization. Samples were removed for flow cytometric analysis and for L-ornithine decarboxylase assay.

The progression of cells through the cell cycle was monitored by measuring their DNA content at various times after plating. For this purpose a high-speed flow cytometric technique was used. The cells were fixed in formalin (10% formalin in phosphate-buffered saline) and Feulgen-stained with the fluorescent dye acriflavine according to the method described by Gill and Jotz (16). This treatment leaves each cell with a stain content proportional to its DNA content. The stained cells were analyzed on the Livermore bicolor flow cytometer (17-19). In this device the cells flow at uniform velocities through an intense laser beam at rates up to 1,000 cells/second. Each cell receives constant illumination for the same period of time so that resulting fluorescence is proportional to the amount of dye in the cell and hence a measure of the cellular DNA content. The result of analyzing a large number of cells (10^6) from each sample is a DNA distribution. The time sequence of DNA distributions from the synchronous cell populations was analyzed according to the procedure of Gray (20) to reveal estimates for the fraction of cells in each phase of the cell cycle as a function of time (Figure 1A).

Putrescine synthesis was studied by measuring the L-ornithine decarboxylase activity according to the method described by Jänne and Williams-Ashman (21) in extracts of cell samples obtained from the same cultures that had been analyzed for their cellular DNA content. The enzyme assay, as applied in our laboratory, has been described in detail by Heby et al. (22). Cell samples were sonicated in 200 µl of ice-cold 100 mM glycylglycine buffer (pH 7.2) containing 0.1 mM Na_2EDTA and 5 mM dithiothreitol. The 45,000 x g (90 min) supernatant fraction was used for the enzyme assay.

RESULTS

Synchronously growing CHO cells were obtained by selection of mitotic cells from exponentially growing cultures by shake treatment (14,15). Cultures prepared by the shake treatment consisted of at least 90% mitotic cells, and following plating, the cells grew with very low dispersion for more than one cell cycle. The cell cycle dispersion, which describes the standard deviation in cell cycle transit time over one complete cycle (23), was only 1.7 hours out of 14.5 hours for the total cycle time.

Figure 1A shows the time sequence of the DNA distributions as obtained by computer simulation. After 2 hours of growth, nearly all the cells had divided and the cell population almost exclusively consisted of G1 phase cells. From 2 through 6 hours, essentially all cells were progressing through the G1 phase of the cell cycle. No cells were engaged in DNA synthesis for about 6 hours following plating. After this time, however, virtually the entire population

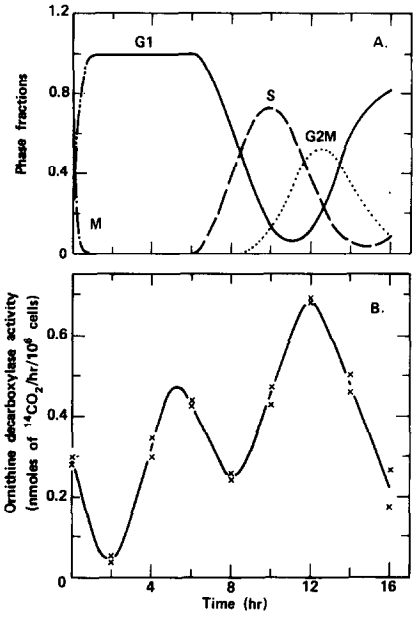

Figure 1A. Fraction of cells in each phase of the cell cycle as a function of time after plating of mitotic cells. The DNA content distribution of cells was determined by flow cytometry at 2-hour intervals following plating of the mitotic cells. These experimental distributions were analyzed by a computer model which permits the simulation of a sequence of DNA distributions. The model parameters include average phase transit times for the G1, S, and G2M phases and associated dispersions, information about the rate of DNA synthesis and an estimate of the variability of the measured values of cellular DNA content. These 8 parameters are varied until a match is obtained between the sequences of experimental and simulated DNA distributions. Once these parameters are estimated, they can be used to generate values for the fraction of cells in the G1, S, and G2M phases at any time during the course of the experiment such as the fractions shown here. The values for the fraction of cells in the G1 and M phases in the first hour were not taken from the model but were extrapolated from the experimentally determined result that, at time 0, the phase fractions for G1 and M were 0.27 and 0.63, respectively.

Figure 1B. L-Ornithine decarboxylase activity during the cell cycle of CHO fibroblasts. The mitotic cells were plated at time 0 and cell cultures were analyzed for their enzyme activities at 2-hour intervals. The data points are from 2 parallel experiments, where growth medium and incubation conditions were exactly the same. The entire experiment was repeated 3 times; the temporal relationship to the cell cycle was similar whereas the peak heights varied somewhat between the experiments.

commenced the production of DNA; 8 hours after plating 40% of the cells had entered the S phase and by 10 hours 70% of the cells were in the S phase, 15% were in the G1 phase and 15% had entered the G2+M phase. At 12 hours after plating the majority of the cells were progressing through the G2 and M phases

of the cell cycle. Approximately 15 hours after plating, the majority of the cells had completed one cycle and 75% of the cells possessed the DNA content of G1 cells.

Figure 1B shows that L-ornithine decarboxylase activity increases markedly in mid-G1 and maximal activity occurs prior to DNA synthesis. A significant decrease in enzyme activity takes place in the S phase, but as the cells start to enter the G2 and M phases the enzyme activity increases again. Before cell division L-ornithine decarboxylase activity reaches a new maximum at about the same level as the maximum occurring at the G1/S border.

The pattern of change of L-ornithine decarboxylase activity was similar in 5 experiments, as was the temporal relationship to the cell cycle. However, the peak heights varied somewhat between the experiments, probably because of differences among various batches of sera and among incubation conditions. Therefore, in Figure 1B, the data points are from 2 parallel experiments where growth medium and incubation conditions were identical.

DISCUSSION

In view of our findings, it seems as if only the peaks of L-ornithine decarboxylase activity which occur in late-G1 and in G2+M are related to the cell cycle of continuously dividing cells. The absence of an increase in L-ornithine decarboxylase activity in early-G1 suggests that the presence of such early activity, as that reported for other cell systems (3-11), may be due to physical changes in the cell in response to particular synchronization techniques. It is also possible that its early expression is dependent on the cell type and on the condition of the cell, i.e. whether it is resting or proliferating.

A role for the polyamines in the cell's traverse of the cycle, particularly in the initiation and/or continuation of DNA synthesis is indicated by our current and past observations, as well as by those of others: 1) spermidine is required for the initiation stage of ϕX 174 phage DNA replication (24); 2) spermidine strongly stimulates the activity of DNA-dependent DNA polymerase B

of rat brain (25); 3) putrescine synthesis increases prior to the initiation of DNA synthesis in WI-38 fibroblasts (13); 4) the polyamines begin to accumulate during early S phase in AKR leukemic cells (26); 5) fewer rat brain tumor cells are found in the S phase of the cell cycle when spermidine and spermine synthesis is inhibited by methylglyoxal-bis(guanylhydrazone) (27); 6) methylglyoxal-bis(guanylhydrazone) reduces phytohemagglutinin-stimulated (28) and concanavalin A stimulated (29) DNA synthesis in lymphocytes; and 7) putrescine, which stimulates the proliferation of human fibroblasts, accomplishes its effect by shortening the cell cycle mainly by reduction of the length of time required for DNA replication (30). Furthermore, a possible role for the polyamines in cell division is suggested by the premitotic increase in L-ornithine decarboxylase activity (putrescine synthesis), in view of the fact that Rao and Johnson (31) found that putrescine and spermine specifically promoted premature chromosome condensation.

ACKNOWLEDGEMENTS

This work was performed under auspices of the Energy Research and Development Administration with the support of NCI Grants CA-13525 and CA-15515, U.S. Public Health Service Grant 5R0114533 and gifts from the Phi Beta Psi Sorority, the Joe Gheen Medical Foundation, and the Association for Brain Tumor Research. L.J.M. is the recipient of NCI Research Career Development Award CA-00112.

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