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Half-Lives of Ornithine Decarboxylase and *S*-Adenosylmethionine Decarboxylase Activities during the Cell Cycle of Chinese Hamster Ovary Cells

Pia S. H. Berntsson,[1] Kersti Alm, and Stina M. Oredsson
Department of Animal Physiology, Lund University, Helgonavägen 3 B, SE-223 62 Lund, Sweden

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Cells in mitosis were seeded immediately after being harvested by the mitotic shake off technique from a culture of exponentially growing Chinese hamster ovary cells. At 2, 5, 7, 10, and 12 h after seeding, cycloheximide was added. Cells were sampled at various times after cycloheximide addition and the ornithine decarboxylase (ODC) and *S*-adenosylmethionine decarboxylase (AdoMetDC) activities were determined. Flow cytometric analysis showed that cells sampled at 2, 5, 7, 10, and 12 h after seeding were found in mid G_1, at the G_1/S transition, in mid S phase, at the S/G_2 transition, and in late G_2, respectively. The half-lives of ODC and AdoMetDC activities varied during the cell cycle. The half-life of ODC activity showed a biphasic pattern with increases in connection to the G_1/S and S/G_2 transitions while the half-life of AdoMetDC activity increased only at the G_1/S transition.

Key Words: **polyamines; ornithine decarboxylase; S-adenosylmethionine decarboxylase; mitotic shake off; cell cycle; half-life.**

The polyamines—putrescine, spermidine and spermine—are essential for normal cell proliferation (1, 2). When polyamine biosynthesis is inhibited and polyamine pools are depleted, cell proliferation ceases. The specific roles of polyamines in cell proliferation is however still enigmatic. One approach to understand the role of the polyamines is to elucidate the regulation of the enzymes involved in polyamine biosynthesis. Much effort has gone into studying ornithine decarboxylase (ODC; EC 4.1.1.17) and S-adenosylmethionine decarboxylase (AdoMetDC; EC 4.1.1.50). ODC is the rate limiting enzyme for the synthesis of putrescine. AdoMetDC is rate limiting for the synthesis of spermidine and spermine when putrescine is present.

[1] To whom correspondence should be addressed. Fax: +46 46 222 45 39. E-mail: Pia.Berntsson@zoofys.lu.se.

The half-lives of ODC and AdoMetDC activities have been investigated in asynchronously proliferating cells *in vivo* and *in vitro* after administration of cycloheximide. *In vivo* experiments show a half-life of ODC activity ranking from 10 to 24 minutes (3, 4) and for AdoMetDC activity from 22 to 67 minutes (5, 6). In a majority of those experiments, liver and kidney have been the organs of investigation. Experiments with cells in culture show a variation in the half-life of ODC activity from 5 minutes to 8 h (7, 8) and of AdoMetDC activity from 48 minutes to 8 h (9). The variations may be related to cell type, different experimental protocols/manipulations and the condition during which the cells were grown. The half-lives of ODC and AdoMetDC activities during the cell cycle phases have never been determined. We have investigated the activities and mRNA levels of ODC and AdoMetDC during the cell cycle of Chinese hamster ovary (CHO) cells (10). In CHO cells, the specific activities of ODC and AdoMetDC increased biphasically during the cell cycle and the mRNA levels approximately doubled (10).

In the present paper, we have added further information pertaining to the regulation of ODC and AdoMetDC by determining the half-lives of the enzyme activities during the cell cycle of CHO cells.

MATERIALS AND METHODS

Materials. Growth medium components were purchased from Biochrom, Berlin, Germany. Tissue culture plastics were purchased from Nunc, Roskilde, Denmark and from Corning, New York. Propidium iodide (PI) and cycloheximide was purchased from Sigma, St. Louis, MO. S-[*carboxyl*-^{14}C]Adenosyl-L-methionine (55 mCi/mmol) was purchased from Amersham Sweden AB, Solna, Sweden. L-[1-^{14}C]Ornithine (52 mCi/mmol) was purchased from New England Nuclear Du Pont, Scandinavia AB, Stockholm, Sweden.

Cell culture. CHO cells were sub-cultured twice a week in RPMI 1640 medium supplemented with 10% fetal calf serum (FCS) and antibiotics. The cultures were incubated at 37°C in a water-saturated atmosphere of 5% CO_2 in air.

Mitotic shake off. The procedure has earlier been described thoroughly (10). In short, 30×10^6 cells were seeded in 100 ml medium in

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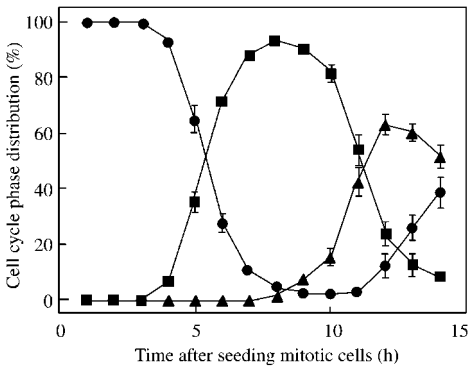

FIG. 1. Distribution of cells in G_1, S, and G_2 phases at various times after seeding mitotic Chinese hamster ovary cells. Cells in mitosis were harvested from an asynchronously growing cell culture by the mitotic shake off technique. The mitotic cells were reseeded in tissue culture dishes at time 0. At indicated times after seeding, cells were harvested for flow cytometry analysis of the cell cycle phase distributions. At time 0, at least 98% of the cells were in mitosis as judged in the fluorescence microscope after staining with propidium iodide. (●) % cells in the G_1 phase; (■) % cells in the S phase; (▲) % cells in the G_2 phase. The symbols are the mean from 13 experiments and error bars represent ± S.E.M.

a 850 cm^2 tissue culture roller bottle which was gassed with 5% CO_2 in air before it was placed on a roller bottle apparatus (Roller Culture Apparatus, Wheaton, Millville, NJ) at a speed of 1 rpm in a 37°C incubator. At day 2 after seeding, the bottle was moved to a specially designed roller bottle apparatus in the 37°C incubator and the speed was set at 3 rpm. Every ten minutes, the speed was increased to 100 rpm for 30 s and the medium containing detached cells was poured into a 60 cm^2 tissue culture dish. The roller bottle was then washed with 40 ml prewarmed (37°C) RPMI 1640 medium containing 0.1% FCS and 5 mM HEPES. Thereafter, 15 ml prewarmed RPMI 1640 medium containing 10% FCS and 5 mM HEPES was added and the roller bottle was gassed with 5% CO_2 in air, before being placed on the roller apparatus at a speed of 3 rpm. The collection procedure was repeated for each sample. Before starting the collection of samples, the 10 initial detached cell populations were discarded.

Flow cytometric (FCM) analysis. Cells were harvested by trypsinization at times 1–14 h after seeding mitotic cells. The cells were pelleted at 1000× g for 10 min at 4°C and the medium was decanted. The cells were resuspended in a buffer (40 mM Na^- citrate pH 7.6; 250 mM sucrose; 0.7 M dimethylsulfoxide) and stored at −20°C until analysis. Before FCM analysis, the cells were pelleted and resuspended in the DNA intercalating fluorescent dye propidium iodide (PI) (PBS pH 7.4; 50 μg/ml PI; 100 μg/ml RNase; 0.6% NP-40) (11). Immediately prior to FCM analysis, the cell suspension was suctioned three times through a cannula (0.7 mm diameter) and filtered through a 50 μm nylon mesh. Analysis of DNA content was done with an Ortho Cytoron Absolute, equipped with a 15 mW argon-ion laser (Ortho Diagnostic Systems, Raritan, NJ). The laser line at 488 nm was used for excitation. Ten thousand nuclei per sample were analyzed. The impulses were digitized and sorted by a 256 multichannel analyzer and stored as list data files according to the FCM standard 1.0 by an acquisition software, running on a PC, onto a data file server Micro Vax. Nuclear doublets and triplets were excluded by electronic threshold settings (11).

Analysis of ODC and AdoMetDC activities. At 2, 5, 7, 10 and 12 h after seeding mitotic cells, cycloheximide (50 μg/ml) was added to the cultures. Cells were harvested by trypsinization at time 0 (the time of cycloheximide addition) and at various times up to 90 minutes after cycloheximide addition. After counting in a hemocytometer, the cells were pelleted at 1000× g for 10 min at 4°C and the medium was decanted. The cells were resuspended in 200 μl ODC buffer (0.1 M Tris-HCl pH 7.5; 0.1 mM EDTA; 2.5 mM DTT) and stored at −80°C until analysis. The cell homogenates were thawed on ice. To determine ODC activity, the cell homogenates were used for measuring the release of $^{14}CO_2$ from carboxy-labeled ornithine in the presence of saturating levels of pyridoxal 5′-phosphate and L-ornithine (12). To determine AdoMetDC activity, the cell homogenates were used for measuring the release of $^{14}CO_2$ from carboxy-labeled AdoMet in the presence of unlabeled AdoMet and putrescine (13).

RESULTS

Synchronously growing CHO cells were obtained by selection of mitotic cells from exponentially growing cultures by the mitotic shake off technique (10, 14). The progression through the cell cycle of the seeded cells was monitored by DNA FCM. For 3 h after seeding, only G_1 cells were detected (Fig. 1). Thereafter, the G_1 phase fraction decreased as the S phase fraction increased. The majority of the cells were at the G_1/S transition at 4–5 h after seeding. The number of cells in S phase reached a maximum at 7–9 h after seeding. Thereafter the S phase fraction decreased as the G_2 phase fraction increased. The majority of the cells were at the S/G_2 transition at 10–11 h after seeding. The G_2

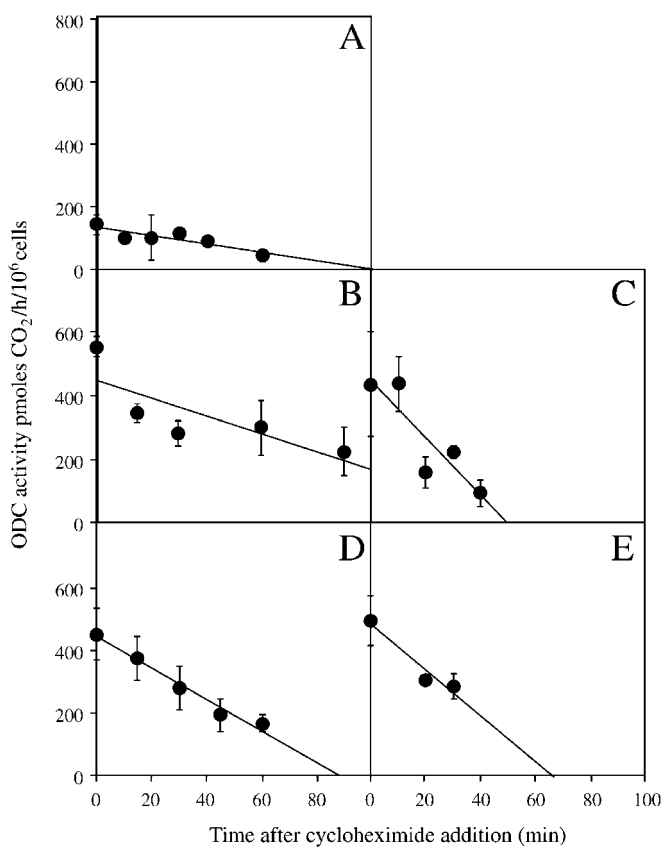

FIG. 2. The rate of decrease of the ODC activity after addition of cycloheximide at 2 (A), 5 (B), 7 (C), 10 (D), or 12 (E) h after seeding CHO cells in mitosis. The symbols are the mean of n = 3–6 from 9 experiments and error bars represent ± S.E.M.

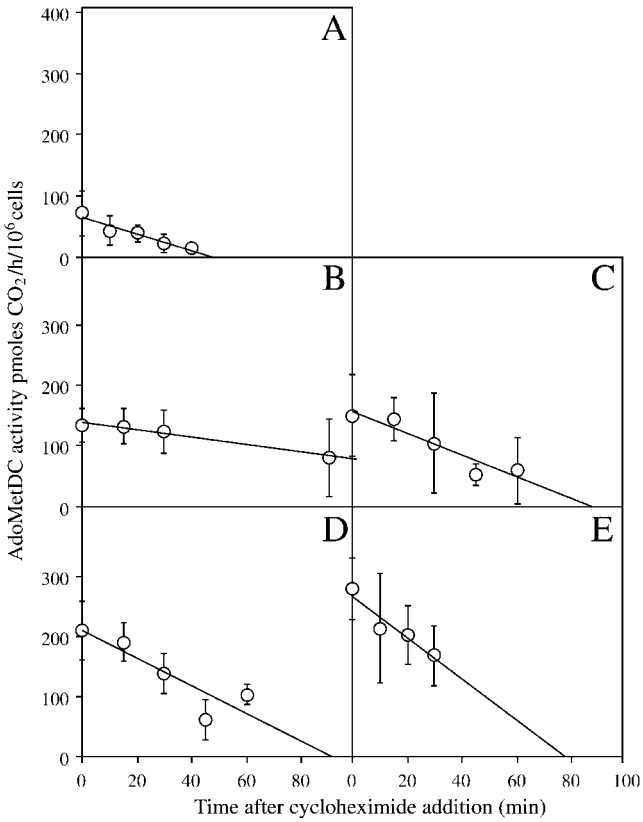

FIG. 3. The rate of decrease of the AdoMetDC activity after addition of cycloheximide at 2 (A), 5 (B), 7 (C), 10 (D), or 12 (E) h after seeding CHO cells in mitosis. The symbols are the mean of n = 3–8 from 20 experiments and error bars represent ± S.E.M.

phase fraction reached a maximum at 12–13 h after seeding. On the basis of this experiment, we chose to look at the half-lives of ODC and AdoMetDC activities at 2, 5, 7, 10 and 12 h after seeding when the cells were found in mid G_1, at the G_1/S transition, in mid S phase, at the S/G_2 transition and in late G_2, respectively. In addition, those time points appeared to be relevant to study in relation to the changes in the activities of ODC and AdoMetDC that we have previously found (10).

The rates of decrease in the ODC and AdoMetDC activities after addition of cycloheximide (an inhibitor of protein synthesis) at various times after seeding mitotic CHO cells are shown in Figs. 2 and 3, respectively. At some times points, the S.E.M. is rather large, however, that is a consequence of the complex experimental system. Since we wish to study totally unperturbed cells, each analysis is performed on a single population of detached cells. The data in Figs. 2 and 3 comprise 179 individual mitotic shake offs from 20 different experiments. Usually when studying the half-life of a protein, a starting population of cells is divided into the individual sampling populations. That is not possible in this case since each mitotic shake off only yields 2–4 $\times$ 10^5 cells and fewer cells than that cannot be used for the analysis of ODC and AdoMetDC activities. Figure 4 is a compilation of the half-lives of ODC and AdoMetDC activities calculated by linear regression analysis of the data in Figs. 2 and 3. The most notable change in the half-life of the AdoMetDC activity occurred at the G_1/S transition. The half-life of the AdoMetDC activity increased from 24 minutes in mid G_1 to 111 minutes at the G_1/S transition i.e. approximately a 4.6-fold increase. In mid S phase, the half-life was notably lower (43 minutes) compared to that found at the G_1/S transition and it remained at that level throughout the cell cycle. The half-life of the ODC activity increased from 50 minutes in mid G_1 to 80 minutes at the G_1/S transition i.e. a 1.6-fold increase. The half-life of ODC activity then decreased remarkably and was 25 minutes in mid S phase. At the S/G_2 transition, the half-life of the ODC activity showed a second increase and the half-life was approximately 1.8-fold longer than that found in mid S phase. In late G_2 phase, the half-life of the ODC activity was somewhat decreased compared to that found at the S/G_2 transition. In conclusion, the half-life of the ODC activity showed a biphasic pattern with increases in connection to the G_1/S and S/G_2 transitions while the half-life of the AdoMetDC activity increased only at the G_1/S transition.

DISCUSSION

ODC and AdoMetDC are two enzymes subjected to a complex regulation at various levels. Both enzymes are regulated at transcriptional, translational, and post-translational levels, the latter regulation containing unique components for both enzymes (15, 16). The polyamines are involved in this regulation at various

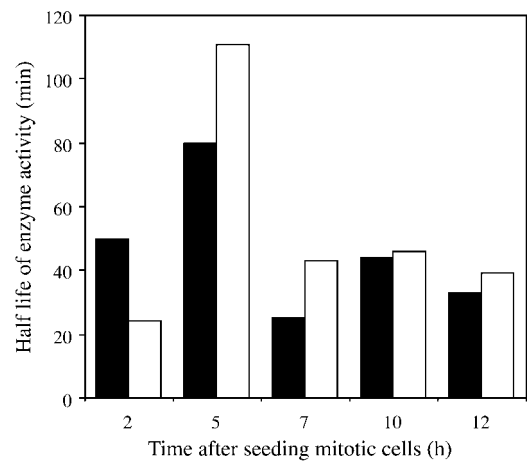

FIG. 4. The half-lives of ODC (■) and AdoMetDC (□) activities 2, 5, 7, 10, and 12 h after seeding CHO cells in mitosis. The data are obtained from Figs. 2 and 3. At 2, 5, 7, 10, and 12 h after seeding of cells in mitosis the cells were found in mid G_1, at the G_1/S transition, in mid S phase, at the S/G_2 transition, and in late G_2, respectively.

levels. Polyamine biosynthesis, as well as its regulation, has been studied in a number of *in vivo* and *in vitro* experimental systems. A majority of those studies have been performed on asynchronous cell systems. In a few studies using cells in culture, polyamine biosynthesis has been investigated during the cell cycle (17–19). We have earlier shown that the ODC activity was low in mitotic cells and that the enzyme was activated late in G_1 and reached a plateau in early S phase. A second burst in activity was observed during late S phase and maximal ODC activity was found at the S/G_2 transition. AdoMetDC activity increased in late G_1 and a first maximum was observed during the G_1/S transition. A second burst in activity was found in mid S phase. Maximal AdoMetDC activity was found in G_2 (10). In the present investigation, we have studied the half-lives of ODC and AdoMetDC activities during mid G_1, at the G_1/S transition, in mid S, at the S/G_2 transition and in late G_2, to get an insight into how post-translational mechanisms might contribute to the observed changes in enzyme activity.

As mentioned above, the ODC activity increased in conjunction with the G_1/S transition, a time point when there was an increase in the half-life of the enzyme activity. From this, the conclusion can be made that at least part of the regulation of the enzyme activity lies in a decreased rate of enzyme degradation, i.e. a post-translational control. We have found that the relative ODC mRNA level was approximately unchanged during G_1 to mid S phase (10), excluding that transcription may have a role in the change in ODC activity found during late G_1 to early S phase. In addition, an increased rate of translation may explain the increase in ODC activity at that time point. The increase in the AdoMetDC activity seen at G_1/S transition was also coincident with an increase in the half-life of the enzyme activity, thus, pointing to a regulation at the post-translational level. Transcription may have a part in the activation of AdoMetDC as AdoMetDC mRNA doubled in conjunction with the G_1/S transition (10). In the present paper we have found that the half-life of AdoMetDC activity was constant during mid S, the S/G_2 transition and late G_2. Thus, the maximal AdoMetDC activity found in G_2, was not caused by a change in the rate of degradation.

Taken together with our earlier results (10), the results of the present paper indicate that a change in the half-life of AdoMetDC does not participate in the activation of the enzyme in S phase. However, the increase in the half-life of ODC found at 10 h after seeding of mitotic cells points to a role of post-translational mechanisms in the second burst of ODC activity. Transcriptional mechanisms may also be involved since the level of ODC mRNA appeared to double during that time period (10).

To the best of our knowledge, this is the first paper studying the changes of the half-lives of any enzyme activities during the cell cycle. Our results show that there are distinct changes in the half-lives of ODC and AdoMetDC activities during the cell cycle. This can partly contribute to observed changes in enzyme activities. Taken together, our results show that ODC and AdoMetDC are regulated at transcriptional, translational, and post-translational levels during the cell cycle. Further studies are needed to unravel the full story of the regulation of polyamine biosynthesis at various levels during the cell cycle.

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