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ORNITHINE DECARBOXYLASE INDUCTION AND NUCLEOLAR RNA SYNTHESIS IN FRIEND LEUKEMIA CELLS

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Summary: The induction of ornithine decarboxylase and the stimulation of nucleolar RNA synthesis following dilution of stationary phase Friend Leukemia Cells into fresh medium were studied. Ornithine decarboxylase activity and the rate of nucleolar RNA synthesis reached maximum values within 4 hours after dilution, with ornithine decarboxylase levels increasing 10-20 fold and nucleolar RNA synthesis increasing by about 60% during this period. 0.5 mM putrescine effectively inhibited the rise in ornithine decarboxylase following cell transfer, but did not prevent increases in the rate of nucleolar RNA synthesis.

In cultured mammalian cells, amino acid starvation decreases the rate of nucleotide incorporation into nucleolar RNA (1-3). This effect is seen both with actual amino acid deprivation and with functional deprivation caused by the presence of an amino acid analogue, such as histidinol. In FL cells,[1] incorporation of [^{3}H]uridine into nucleolar RNA is inhibited by 75% two hours after the addition of histidinol to the culture (3). For the purposes of this communication, we shall assume that incorporation of [^{3}H]uridine into nucleolar RNA is a valid index of the rate of synthesis of that RNA. Our justification for this assumption is given in reference 3.

The mechanism by which amino acid starvation decreases the rate of nucleolar RNA synthesis is not understood. Grummt and Grummt proposed that amino acid starvation, by decreasing intracellular levels of ATP and GTP, produces a pleiotypic response that includes a decrease in the rate of nucleolar RNA

[1]Abbreviations: FL, Friend Leukemia; ODC, Ornithine Decarboxylase.

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synthesis (2). However, we failed to observe decreases in ATP and GTP levels in FL cells following amino acid starvation (3).

An alternative mechanism is suggested by recent studies on the enzyme ornithine decarboxylase (L-ornithine carboxy-lyase, EC 4·1·1·17) and its role in nucleolar RNA synthesis. This enzyme, which catalyzes the decarboxylation of ornithine to putrescine, has a half-life of between 10 and 20 minutes (4). Therefore, the intracellular level of ODC is able to respond rapidly to changes in its rate of synthesis or degradation.

Several lines of evidence led Manen and Russell to suggest that ODC may function as an initiation factor for RNA polymerase 1 (5): 1) RNA polymerase 1 and ODC activities are tightly correlated during stimulation of cells with various hormones and also during treatment with inhibitors, 2) the half lives for disappearance of ODC and RNA polymerase 1 activities in cycloheximide treated cells are identical, and 3) ODC displays a strong and specific affinity for RNA polymerase 1.

Studies with many different types of mammalian systems indicate that a many-fold increase in ODC activity occurs very early after cells are stimulated to proliferate (6,7). When FL cell cultures are stimulated to proliferate by dilution of stationary phase cultures with fresh medium, they increase their rate of uridine incorporation into RNA about two fold during the first few hours (8). In this paper, we report that, as expected, this stimulation increases nucleolar RNA synthesis rates and ODC levels in FL cells. However, we also show that addition of putrescine at the time of dilution into fresh medium blocks the increase in ODC levels but does not prevent the increase in nucleolar RNA synthesis.

Experimental Procedures

Cells: Friend Leukemia cells [cell line 745(15), clone 18, obtained from Dr. David Kabat] were grown in Eagle's medium supplemented with 10% fetal calf serum as previously described (9). Cells were diluted into fresh medium at a density of 5-7 x 10^5 cells/ml 48 hours prior to use for experiments. Cells to be used for experiments were centrifuged 3 minutes at room temperature at 1/2 speed in an International Clinical centrifuge (Model CL). They were resuspended in fresh medium at a density of 1 x 10^6 cells/ml.

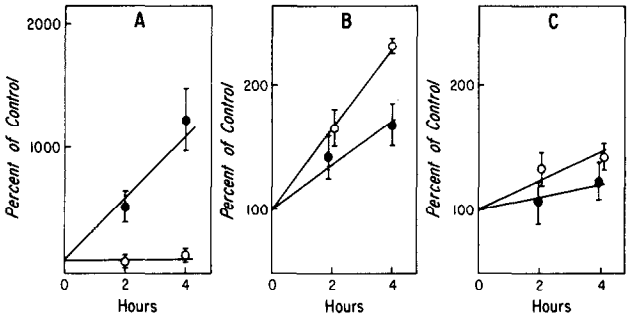

Figure 1. ODC levels, nucleolar and nucleoplasmic RNA synthesis rates at various times after resuspension in fresh medium in the presence and absence of 0.5 mM putrescine. A. ODC specific activity. B. Nucleolar RNA synthesis rate. C. Nucleoplasmic RNA synthesis rate. Open circles, with putrescine; closed circles, without putrescine. The error bars indicate the range of values obtained in two independent experiments; the lines are drawn through the average values. All values are given as percent of the zero time control.

ODC assays: Cell extracts were prepared and ODC activity in extracts was measured by the method of Prouty (10). Protein concentration of crude cell extracts was determined by the method of Bradford (11).

Nucleolar and nucleoplasmic RNA synthesis rates: Nucleolar and nucleoplasmic RNA synthesis rates were determined by measuring incorporation of [^{3}H]uridine into nucleolar and nucleoplasmic fractions as previously described (3).

Results and Discussion

When stationary phase FL cells are suspended in fresh medium at a cell density (1×10^6 per ml) that permits growth to resume, ODC levels increase by approximately 12 fold within 4 hours whereas the nucleolar RNA synthesis rate increases by about 65% during the same period. Nucleoplasmic RNA synthesis is not significantly affected. These results, which are shown in Figure 1, agree with the results of earlier studies (3,7). When 0.5 mM putrescine is added to the cells at the time of resuspension in fresh medium, the increase in ODC activity was completely abolished, whereas the increase in nucleolar RNA synthesis was, if anything, greater than that observed in the absence of putrescine.

We have not established whether the mechanism by which putrescine blocks induction of ODC in FL cells involves regulation at the translational level

(12) or whether it involves induction of an ODC antizyme (13). Whatever the mechanism, our observation that ODC induction can be dissociated from stimulation of nucleolar RNA synthesis does not support the proposal of Manen and Russell that ODC is an initiation factor for RNA polymerase 1 (5). However, since we measured ODC activity, rather than the level of ODC protein, our results do not rule out the possibility that putrescine may cause inhibition of the enzymatic activity of ODC without impairing the ability of this protein to stimulate nucleolar RNA synthesis. Studies with monospecific antibodies to ODC (14) could resolve this question.

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