POLYAMINES AND NEOPLASTIC GROWTH:STABILIZATION OF ORNITHINE DECARBOXYLASE DURING TRANSFORMATION

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SUMMARY: Transformation of chick embryo fibroblasts by Rous sarcoma virus leads to the stabilization of ornithine decarboxylase. A two-fold increase in the half-life of the enzyme is noted after infection with the wild type Schmidt-Ruppin,but not after infection with the temperature-sensitive mutant T5, under restrictive conditions. The half-life of the enzyme doubles soon after shifting the temperature of a RSV-T5 infected culture from 42° to 37° and after adding fresh medium to cultures of normal chick embryo fibroblasts at relative high densities.

Ornithine decarboxylase (ODC, L-ornithine-carboxylase EC. 4.1.1.17) is the rate-limiting enzyme in the biosynthetic pathway of the naturally occurring polyamines (1-4) .One remarkable property of this enzyme is its extremely short half-life, which is 10 to 20 minutes (5).

Recent studies indicated that ODC activity increases in tissues undergoing rapid growth and is also elevated in tumor cells. It has been suggested that activation of ODC is an early event that occurs during the expression of malignancy (6). This suggestion is based on the increase in ODC activity 5 hours after application of tumor-promoting agents to mouse epidermis (7) and by the use of RSV-T5, a temperature-sensitive mutant of Rous sarcoma virus (8). This mutant multiplies at 42° , but transforms only after shifting the temperature to 37° . Approximately 1 hour after the temperature shift, the activity of ODC increases (8) whereas a morphological change of the cells is noted much later.

Elevated activity of ODC may be explained either by increased synthesis

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of this enzyme or by a decreased rate of degradation. In some tumor cells, such as hepatoma (9) or glioma (10) cells, activation of ODC can be explained by increased levels of cellular cAMP.

In this communication we wish to present evidence for the stabilization of ODC during the transformation of chick embryo fibroblasts by RSV-T5 — the temperature-sensitive mutant — or by RSV-SR (Schmidt-Ruppin) — a wild-type of this oncogenic virus. This increase in the half-life of ODC may explain the apparent increase in ODC activity during neoplastic growth and may resemble a similar stabilization which occurs during rapid growth.

The Schmidt Ruppin (SR) strain of RSV and the temperature-sensitive mutant T5 were grown on cultures of chick embryo fibroblasts as described elsewhere (11): 3.0×10^6 cells were seeded on 5 cm plastic dishes in Eagle's minimal essential medium supplemented with 15 percent tryptose phosphate broth, 5 percent inactivated calf serum, tyrosine (72 mg/liter) and glutamine (348 mg/liter). The activity of ODC was determined as previously described (11): two dishes were taken for each point, rinsed twice with 3 ml of ice-cold phosphate-saline buffer, then drained and stored at -70° . Seven-tenths of the assay buffer (0.1 mM EDTA, 0.04 mM pyridoxal phosphate, 3 mM dithiothreitol in 10 mM sodium phosphate buffer) was added to the plates, and the cells scraped off with a rubber policeman. Cells were then frozen and thawed twice.

Enzyme activity was determined in the supernatant fluid obtained after centrifuging at 4500 g for 10 minutes in tubes equipped with a rubber stopper supporting a polyethylene center well. The reaction mixture contained 0.2 ml of extract and 20 µl of DL[1-^{14}C]ornithine (0.08 µc, 15 µc/µmole, Radiochemical Centre, Amersham) and analyzed as described (11).

The half-life of the enzyme was determined by adding cycloheximide (at a final concentration of 20 µg/ml) to the various cultures. At desired times the reaction was stopped by removing the medium and the inhibitor. Plates were washed with ice-cold buffered saline as above and stored at -70° .

Control experiments suggested that half-life of ODC is not constant and

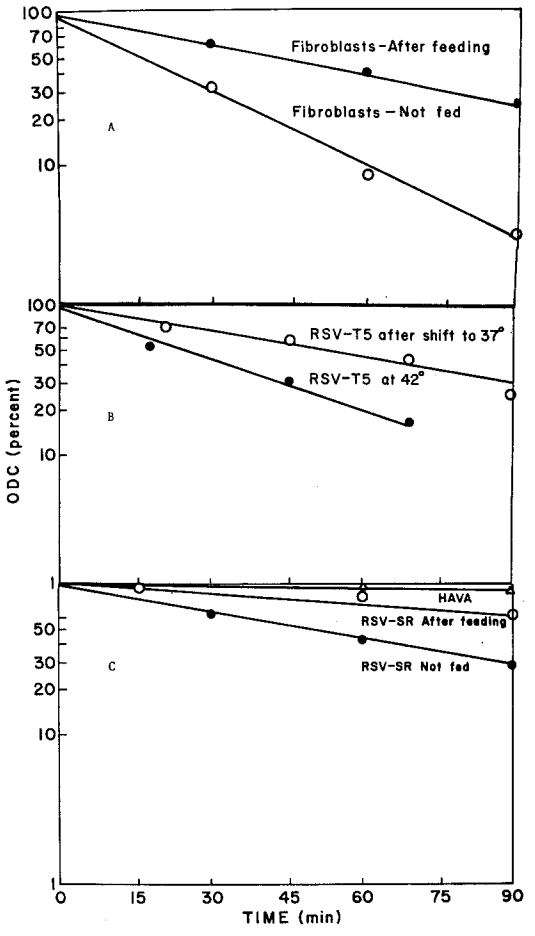

Fig. 1.(A) Stabilization of ornithine decarboxylase by feeding chick embryo fibroblasts. The activity of ornithine decarboxylase was determined in extracts obtained from 6-day-old chick embryo fibroblasts, last fed on day 5 (O) or 5 hours after feeding (●). Cycloheximide (20 µg/ml) was added to the cultures at zero time and extracts prepared after various time intervals. (B) Effect of infection with RSV-T5 on ornithine decarboxylase activity. Enzyme activity was determined in the extracts of 6-day-old chick embryo fibroblasts infected with the temperature-sensitive mutant RSV-T5 at 42° (●), or 5 hours after shifting the temperature to 37° (O). Cells were last fed on day 5. Cycloheximide (20 µg/ml) was added to the cultures at zero time. (C) Ornithine decarboxylase activity in RSV-SR-transformed cells. Enzyme activity was determined in the extracts of 6-day-old chick embryo

depends on the age of the culture and time of feeding. It may be seen (Fig. 1A) that ODC of chick embryo fibroblasts was stabilized considerably 5 hours after adding fresh medium to a 6-day-old culture. The half-life of the starved cells was 15 minutes compared to 40 minutes after feeding (Table 1).

The effect of transformation on the stability of ODC was next tested in chick embryo fibroblasts infected with the temperature-sensitive mutant RSV-T5. It is evident from Fig. 1B that the ODC extracted from cells infected at 42° (restrictive temperature) was labile and resembled that of the uninfected control (cf. Fig. 1A). On the other hand, when a RSV-T5-infected culture was shifted on the sixth day from 42° to 37° , a remarkable stabilization of ODC was noted 5 hours after the temperature shift (Fig. 1B). In this case, fresh

Table 1. Stabilization of ornithine decarboxylase

Preparation	Half-life (minutes)
Chick embryo fibroblasts - normal, starved	15
normal, 5 hours after feeding	40
infected with RSV-T5 at 42°	17
infected with RSV-T5, 5 hours after temp. shift	39
infected with RSV-SR at 42° , starved	50
infected with RSV-SR at 42° , 5 hours after feeding	120

fibroblasts infected with RSV-SR at 42° and last fed on day 5 (●), 5 hours after adding fresh medium to the transformed cells (o), 5 hours after adding HAVA (100 µg/ml) and fresh medium to the transformed cells (▲). Cycloheximide (20 µg/ml) was added to the cultures at zero time.

medium was not added to the cells prior to the temperature shift, yet, the half-life of ODC was changed from 17 to 39 minutes (Table 1).

Chick embryo fibroblasts infected with RSV-SR at 42° exhibited stable ODC when analyzed on the sixth day of culture (Fig. 1C). The addition of fresh medium to the culture increased the stability of the enzyme (Fig. 1C) and the half-life increased from 50 to 120 minutes.

We have recently shown (12) that HAVA (DL-α-hydrazino-δ-aminovaleric acid), an ornithine analogue, causes the accumulation of ODC in RSV-transformed chick embryo fibroblast. It may be seen that this analog brings about a dramatic stabilization of ODC when added to RSV-SR transformed cells along with fresh medium. It has already been suggested that HAVA interferes with the degradation of ODC by proteolytic enzymes (12-13).

Experiments described in this paper, thus demonstrate the analogy between neoplastic growth and rapid proliferation, caused by the addition of nutrients to cells in tissue culture. This stabilization is apparently due to a decrease in the activity of proteolytic enzymes, which normally inactivate ODC (14-16). Numerous recent studies, indeed, indicated the importance of proteases in the transformation process (17).

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