DEREPRESSION OF ORNITHINE-δ-TRANSAMINASE SYNCHRONIZED WITH THE LIFE CYCLE OF HELA CELLS CULTIVATED IN SUSPENSION

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Summary. Hela cells in suspension culture appear to multiply in a naturally synchronized manner with a cell cycle of about 22 hours. Ornithine-δ-transaminase activity develops in parallel with this stepwise increase in cell number with peaks at 20, 45 and 65 hours. These enzymatic pulsations are damped out by proline.

Ornithine-δ-transaminase (OTA) is a reversible enzyme amphi-directionally controlled by the end products of two amino acid pathways which share it as a common step (Volpe, Strecker, 1968; Volpe, Sawamura, Strecker, 1969). Its properties have been described using both partially purified (Peraino, Pitot, 1963; Strecker, 1965) and crystallized (Matsuzawa, Katsunuma, Katanuma, 1968) preparations. The development of OTA in Chang's liver cells cultivated in vitro has been followed for 96 hours (Strecker, Eliasson, 1966). Data are presented now which seem to suggest that the curve of enzyme development may be related to the cell cycle. It was found that during 3-4 days of growth in suspension Hela cells divided in a stepwise manner in an apparent natural synchronization. Under these conditions OTA appeared to develop in synchrony with the cell cycle, whereas in unsynchronized Chang's cells, under the same conditions, only a single peak of enzyme activity was obtained during the 4-day growth.

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Methods. Chang's liver (Chang, 1954) and Hela (Gey et al., 1952) cells were cultivated in suspension at 37°C in Joklik-modified Eagle minimum essential medium which contains 10% calf serum (Hela) or horse serum (Chang's) previously heated for two hours at 50°C. In the experiments with media lacking one essential amino acid (Arg or His) the serum was purified on Sephadex-G-50 (Piez et al., 1960), and the absence of free amino acids checked by Silica gel thin layer chromatography using butanol: acetic acid: water (55:15:30) as the solvent. Proline was added to the medium at a 6 mM concentration. At different periods of time, as shown in figures, the cells obtained from 200 ml of suspension were harvested, washed with 0.25 M sucrose at pH 7.1, resuspended in 5.0 ml of this solution, frozen for 12 hours at -20°C, then thawed and sonicated for one minute. OTA was assayed according to Strecker (1965), and its activity expressed in terms of μmoles of P5C (Δ^1-pyrroline-5-carboxylate) produced in 1 hour at 37°C in 0.05 M potassium phosphate buffer at pH 7.2. The method of Lowry (Lowry et al., 1951) was used for protein determination. DNA was determined according to Giles and Myers (1965). Cell division was calculated by counting double cells in the phase-contrast microscope, and was expressed as a percentage of the total cell number.

Results. As reported previously (Strecker, Eliasson, 1966), OTA in Chang's cells reached a peak of activity approximately 48 hours after inoculation of cells into fresh medium. This period coincided with the maximum accumulation of double cells in the suspension (Fig. 1). Under identical growth conditions to those used for Chang's liver cells, Hela cells appeared to grow in a stepwise manner with three regular cycles, each with a periodicity of a little less than a day (Fig. 2B). During the first cycle the percentage of double cells decreased from 80 to 35; during the second cycle this value increased to 60%; during the third cycle the number of double cells returned to the zero-time high level. DNA in the culture increased concurrently also in a stepwise fashion. Corresponding with these synchronized patterns, in three days of growth of a Hela culture

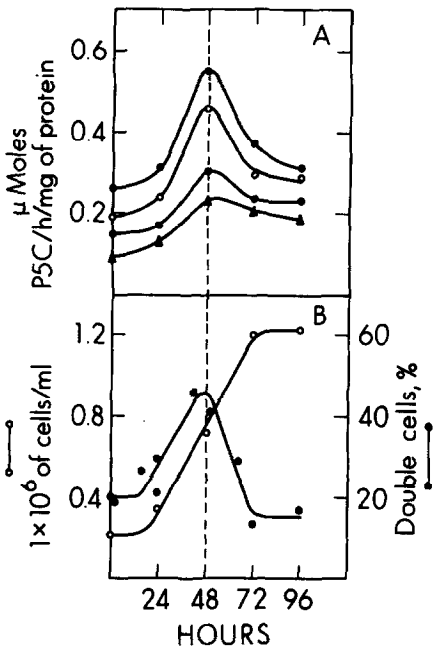

Fig. 1. Ornithine-δ-transaminase activity and cell division in Chang's cells during a 96 hour culture growth in suspension. A - The profile of the derepression curves does not depend on the method of preparation of the enzyme extraxt: Δ——Δ the cells were washed with Hanks salt solution, suspended in 0.05 M Tris-HCl buffer at pH 7.8, then frozen for 12 hours and thawed; o——o the cells were treated as before, in addition to being sonicated; ●——● the cells were washed and suspended in 0.25 M sucrose at pH 7.1, frozen for 12 hours and thawed; ●——● the same treatment with sucrose, plus sonication. B - Cell division (●——●) and culture growth (o——o).

in suspension, three distinct derepressive phases of ornithine-δ-transaminase appeared (Figure 2A). In the following experiments more attention was paid to the production of the enzyme during the first phase of growth of Hela cells. In the absence of an essential amino acid in the medium, during the first 18 hours of growth, the amount of protein per cell underwent a striking decrease (Strecker, Eliasson, 1965; Volpe, 1969). In media lacking Arg or His, in contrast with this total protein decrease, the persistent presence of an OTA peak of activity became even more significant (Fig. 3).

It was reported previously that at 48 hours the maximum OTA activity in Chang's cells was suppressed by proline which is the main end product of the pathway in which

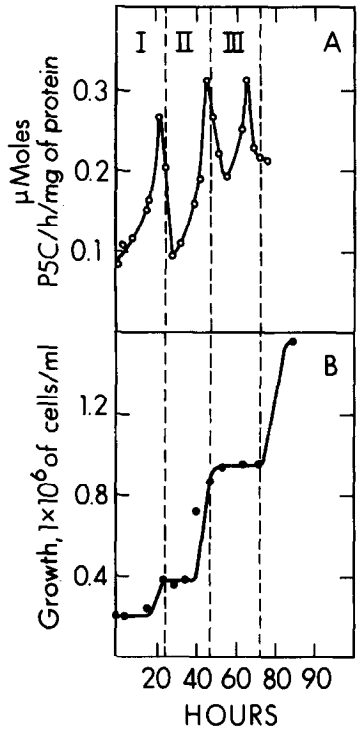

Fig. 2. Ornithine-δ-transaminase activity in Hela cells cultivated for 80 hours in suspension. A - OTA peaks of activity in three cycles of the cell growth. B - Synchronized growth of Hela cells.

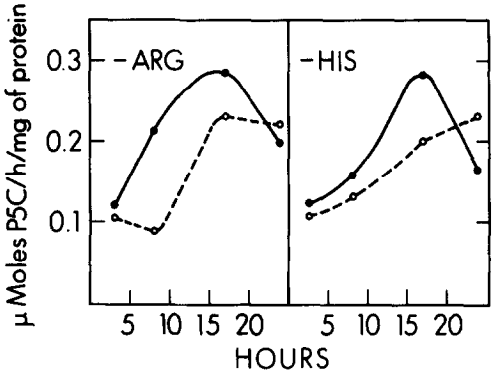

Fig. 3. Ornithine-δ-transaminase activity during the first cycle of growth in Hela cells cultivated in absence of arginine or histidine: ●——● control curve; o——o OTA activity in the presence of 6 mM proline.

ornithine-δ-transaminase represents the second step, and that other non-essential amino acids did not produce any specific effect on this enzyme (Volpe, Strecker, 1968). Just

as the single peak of OTA activity in Chang's cells was suppressed by proline, all the three peaks of ornithine-δ-transaminase activity seen in Hela were lowered by this amino acid (Table I, Fig. 3). The data indicate that the suppressive effect of proline increased with time, so that in the first cycle the peak of OTA activity was decreased by proline by about 15%, whereas the third cycle was decreased by this amino acid by about 40%.

TABLE I. Proline effect on the ornithine-δ-transaminase activity in Hela cells cultivated in suspension. For each cycle the total OTA activity was defined as the area beneath the peak. The ratio (+ PRO/- PRO) refers to the peaks in the presence of proline and in its absence.

Cycles	Hours of growth	+ PRO/- PRO
I	0 - 24	0.86
II	25 - 48	0.79
III	49 - 72	0.63

Discussion. The reason for the observed natural synchronization of Hela cells under the conditions of growth used in this laboratory (Sawamura, 1967; Volpe, 1968) is unknown and is subject of further investigation.

The correspondence between the maximum OTA production at 48 hours and the highest level of division in growing Chang's cells hints at a correlation between the mechanism of enzyme control and a parameter of the cell cycle such as division. The correspondence between the three peaks of OTA activity and the three phases of cell growth in a Hela culture in a more direct way suggests this connection. At the same time, the finding that ornithine-δ-transaminase activity is lowered by proline in Hela as well as in Chang's cells suggests a common mechanism of its control in these two neoplastic lines.

The correlation of ornithine-δ-transaminase production with the cell cycle is confirmed by the experiments in which Hela lines were synchronized artificially with

thymidine (Volpe, Strecker, 1969).

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