THYMIDINE KINASE ACTIVITY IN SYNCHRONIZED HELA CELL CULTURES[1].

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Received July 20, 1965

Previous studies have described the synchronization of DNA synthesis and nuclear replication of HeLa cells through the induction and timed reversal of a thymidineless state (Rueckert and Mueller, 1960; Mueller, et al., 1962; Stubblefield and Mueller, 1962). This study reports periodic alterations in the level of thymidine kinase activity that occurs during a single replication cycle of a synchronized culture.

Methods and Results

Replicate monolayer cultures were grown for 24 hours in modified Eagle's medium[3]. before 10^{-6}M amethopterin was added to induce the thymidineless state. Sixteen hours later thymidine (1.0 μg/ml) was added which reversed the block and initiated a wave of DNA synthesis followed by a synchronous wave of cell division. Control cultures received 1.0 μg/ml thymidine at the time amethopterin was added.

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1. This work was supported by grant CY-1897 from the United States Public Health Service, by funds from the Alexander and Margaret Stewart Trust, and by grant E-286 from the American Cancer Society.
2. Predoctoral Fellow of the National Science Foundation, 1957 to 1961.
3. Eagle's medium supplemented with 10 per cent whole bovine serum, 2 X 10^{-5}M inositol, 5 X 10^{-5}M adenosine, and 10^{-4}M each of glycine and serine.

Cells from trypsinized cultures were counted with a Coulter automatic blood cell counter.

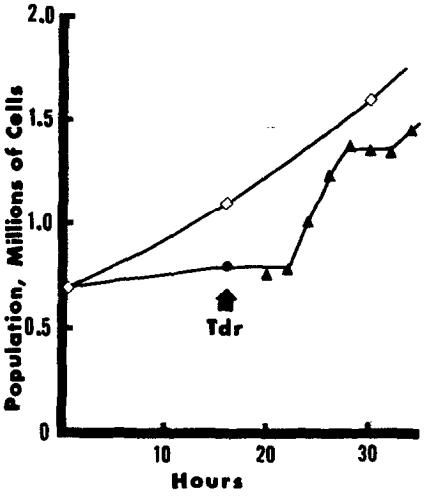

Fig. 1 Population kinetics of HeLa cells treated with amethopterin (10^{-6}M); (◇) supplemented with thymidine (1.0 μg/ml) (control), (●) thymidine omitted for 16 hours, and (▲) thymidine added at 16 hours.

Figure 1 compares the increase in cell number of control and synchronized cultures; the results are similar to those reported earlier. While the cells supplemented with thymidine increase logarithmically, the amethopterin-blocked population did not divide. Thymidine addition at 16 hours produced a synchronous wave of cell division 8 hours later.

The ability of HeLa cell extracts to phosphorylate thymidine was measured using the system described by Bollum and Potter (1959). Trypsinized cell suspensions (1 X 10^6 cells) were sonicated, frozen, and thawed in 0.2 M "Tris" buffer at pH 8.0. The supernatant fluid (0.05ml) was incubated with 0.5ml of substrate, consisting of $MgCl_2$ (5 X 10^{-3}M), 3-phosphoglyceric acid (6 X 10^{-3}M), ATP (5 X 10^{-3}M) and thymidine-2-C^{14} (3 X 10^{-5}M, 1.0 C/mole), dissolved in water. The 0.10ml

reaction mixtures were incubated 30 min at 37°C. The enzyme reaction was stopped by cooling and adding 100% (W/v) trichloroacetic acid to a final concentration of 5%. Aliquots of the supernate were chromatographed on Whatman No. 1 filter paper using an isopropanol-acetic acid-water (17:1:7) solvent system. The thymidine and phosphorylated derivatives were eluted and assayed for radioactivity. One enzyme unit was defined as the capacity to phosphorylate 10^{-7} moles of thymidine per hour, in accord with the definition of Lehman, et al. (1958).

The enzyme preparations contained considerable thymidylate phosphatase activity, but the 3-phosphoglyceric acid in the assay system strongly inhibited this enzyme.

Figure 2 shows the amounts of thymidine kinase in cultures blocked with amethopterin. The level of the enzyme rose strikingly in the thymidineless cells relative to control cultures supplemented with thymidine. When the blocked cultures received thymidine at 16 hours,

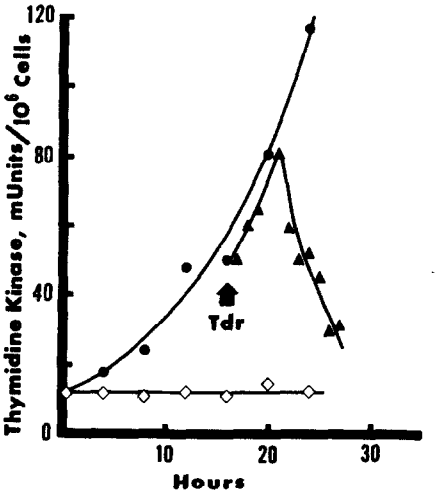

Fig. 2 Thymidine kinase activity in the HeLa cells treated as in Fig. 1. Cultures treated with amethopterin and thymidine (◇) maintained a constant level of enzyme as they grew logarithmically. In the absence of thymidine (●) the enzyme level rose sharply. In cultures given thymidine at 16 hours (▲) enzyme activity continued to increase until the cells began to divide 8 hours later.

the enzyme level was almost five times that of the controls. After the thymidine-reversal, the enzyme level continued to rise until DNA synthesis was complete and then decreased rapidly as the cells entered mitosis.

Discussion

The data suggest that the production of thymidine kinase is initiated in the cell cycle as the nucleus of the cell acquires competence for DNA synthesis. This occurs in the absence of thymidine and before DNA synthesis begins. Once the induction process has been initiated, the synthesis of the enzyme continues until division of the cell.

Littlefield (1965) reported that treatment of L-cells with 5-fluorodeoxyuridine failed to produce the increase in thymidine kinase activity reported here with HeLa cells. These results suggest that the synthesis of thymidine kinase is repressed by the phosphorylated derivatives of both thymidine and 5-fluorodeoxyuridine.

Summary

Thymidine kinase activity increases in HeLa cells which have been treated with amethopterin to produce a thymidineless state. The addition of thymidine after 16 hours results in a synchronous wave of DNA synthesis during which the enzyme activity continues to increase. After division of the cells this activity drops to the level of logarithmically growing, control cultures.

References

Bollum, F. J. and Potter, V. R., Can. Res. 19, 561 (1959).
Lehman, I. R., Bessman, M. J., Simms, E. S. and Kornberg, A., J. Biol. Chem. 233, 163 (1958).
Littlefield, J. W., Biochim. Biophys. Acta 95, 14 (1965).
Mueller, G. C., Kajiwara, K., Stubblefield, E. and Rueckert, R. R., Can. Res. 22, 1084 (1962).
Rueckert, R. R. and Mueller, G. C., Can. Res. 20, 1584 (1960).
Stubblefield, E. and Mueller, G. C., Can. Res. 22, 1091 (1962).