Short Communication

Cell Cycle Regulation of Deoxyribonucleoside Kinase Activities in Cultured Mouse Fibroblasts*

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Deoxyribonucleoside triphosphate biosynthesis occurs periodically in eucaryotic cells and in close correlation to DNA synthesis. Therefore, resting cells have very low deoxyribonucleoside triphosphate pools that increase dramatically as cells enter the S phase of the cell cycle. It is generally assumed that enzymes and proteins required for DNA replication are synthesized primarily during the S phase. This includes enzyme responsible for both *de novo* synthesis and for salvage of deoxyribonucleotides,[1] such as ribonucleotide reductase, thymidylate synthetase and thymidine (Thd) kinase. Several studies have shown that deoxycytidine kinase (dCyd kinase, NTP: deoxycytidine-5′-phosphotransferase, EC 2:7:1.74) also belongs to this group of enzymes.[2,3,4] However, it has also been shown that deoxyadenosine (dAdo) and deoxyguanosine kinase activities changed very little in extracts of cells from different phases of the cell cycle.[3,4]

We have recently purified dCyd kinase from human leukemic spleen and have shown that the same enzyme (a dimer of 30 K subunits) phosphorylates deoxycytidine, deoxyadenosine and deoxyguanosine efficiently, probably with the involvement of two different forms of the enzyme.[5] It is therefore surprising that changes in dCyd kinase activity during the cell cycle were found not to be accompanied by changes in purine deoxyribonucleoside kinase activity. However, considerable variability between species and tissues in the properties of dCyd kinase and in deoxyribonucleoside salvage metabolism in general has been observed.

To clarify the regulation of the activity of the salvage pathway in a very well studied mammalian cell culture model system we have investigated the cell cycle regulation of dCyd-, Thd- and dAdo-kinase activities in serum-stimulated mouse 3T3 fibroblasts.

Experimental

Cell synchronization. 3T3 mouse fibroblast cells were seeded at high density (13×10^3 cells per cm^2) in Dulbecco's modified Eagles medium supplemented with 10 % heat-inactivated horse serum and incubated for three days, during which time the cell number increased only marginally. At day four the medium was changed and the serum content reduced to 5 %, and one day later fresh medium containing 10 % serum was added to the cells. At various times thereafter cells were harvested, and cell extracts were prepared by freezing and thawing the cells three times in buffer containing 50 mM Tris HCl (pH 7.6), 2 mM dithiotreitol, 20 % glycerol and the protease inhibitors: phenylmethylsulfonyl fluoride (0.5 mM), pepstatin (1 μM) and benzamide (5 mM).

Enzyme assays were performed as follows: Thymidine kinase activity was measured with 5 μM

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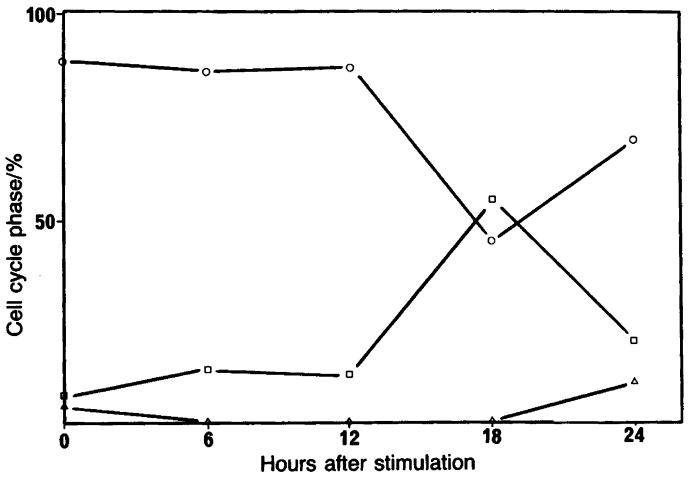

Fig. 1. The proportion of cells in different phases of the cell cycle at various times after addition of serum, as determined by rapid-flow cytometry. Cell numbers were counted manually. (○) G1, (□) S, (△) G2 plus M.

[^{3}H]Thd (specific activity ca. 1 mCi mmol^{-1}), 2 mM ATP, 2 mM $MgCl_2$, 5 mM sodium fluoride, 5 mM dithiotreitol and 25 mM Tris HCl (pH 8.0). Deoxycytidine kinase activity was measured with 10 μM [^{3}H]dCyd (specific activity as for Thd), 10 mM ATP, 10 mM $MgCl_2$, 1 mM deoxyuridine, 15 mM sodium fluoride, 10 mM dithiotreitol, 1 mM cytidine and 50 mM Tris HCl (pH 7.6). Deoxyadenosine kinase activity was measured with 33 μM [^{3}H]dAdo (specific activity ca. 0.2 mCi mmol^{-1}), 10 mM ATP, 10 mM $MgCl_2$, 15 mM sodium fluoride, 50 μM of the adenosine deaminase inhibitor *erythro*-9-(2-hydroxy-3-nonyl)adenine (EHNA, a gift from Burroughs-Welcome, Research Triangle Park, NC, USA), 0.1 M KCl, 10 mM dithiotreitol and 50 mM Tris HCl (pH 7.5). In all cases the reactions were incubated at 37 °C and run for 0, 10 and 30 min. Fifty μl of the reaction mixture was spotted on Whatman DE 81 paper discs, which were washed three times for 30 min in 10 mM ammonium formate and then in water for 3 min. The 0 min value was subtracted from the values for the subsequent times, and the amount of dCMP formed under these conditions was calculated for extracts corresponding to 10^6 cells.

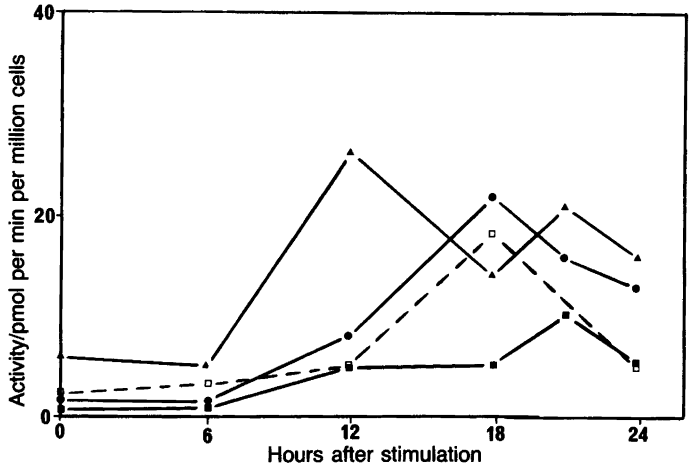

Fig. 2. Levels of thymidine (●), deoxycytidine (□, ■) and deoxyadenosine (▲) kinase activity in extracts prepared from 10^6 synchronized 3T3 mouse fibroblast cells. The activity of deoxycytidine kinase in extracts from a separate experiment is also shown (open symbols, dashed line). The cell cycle distribution was as shown in Fig. 1. The values represent the means of at least three determinations, with less than 20% deviation in the individual values from the mean value.

Results and discussion

Addition of serum to density-inhibited mouse 3T3 fibroblasts resulted in approximately 50 % of the cells entering the S phase in a synchronous manner 16 h later (Fig. 1). This was accompanied by a 10-fold increase in dCyd kinase activity in the cell extracts (Fig. 2). In a separate experiment, Thd-, dCyd- and dAdo-kinase activities were all determined in the cell extracts obtained at different times after serum addition (Fig. 2). A higher concentration of purine nucleoside (33 μM) than of pyrimidine substrates was used and an adenosine deaminase inhibitor (EHNA) was added to increase the efficiency of dAdo phosphorylation.

The cell cycle dependent increase in activity of the three deoxyribonucleoside kinases was different in that Thd-kinase increased 20-fold, dCyd-kinase 10-fold and dAdo-kinase 4-fold (calculated per cell) as cells entered the S phase (Figs. 1 and 2). The overall activities of Thd- and dAdo-kinases were twice as high as the dCyd-kinase activity in S phase cells, while in resting cells dAdo-kinase activity was highest. dAdo phosphorylation is most likely attributable to several enzymes, one of which is the dCyd-kinase enzyme; this enzyme is responsible for the increase in S phase cells. However, adenosine kinase can also phosphorylate dAdo and this may explain the relatively high activity observed in resting cells. The failure of earlier studies[3,4] to observe a cell cycle dependent increase in dAdo phosphorylation may be due to rapid deamination of dAdo.

The fact that the ratio between the various deoxyribonucleoside kinase levels varies during the cell cycle of mammalian cells is of importance when using different radioactive nucleosides to study cell growth and DNA precursor metabolism, as well in attempts to interfere with viral and cellular replication using deoxyribonucleoside analogues.

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References

1. Prescott, D. M. *Reproduction of Eucaryotic Cells,* Academic Press, New York 1976.
2. Wan, C. W. and Mak, T. W. *Cancer Res. 38* (1978) 2768.
3. Howard, D. K., Hay, J., Melvin, W. T. and Durham, J. P. *Exp. Cell Res. 86* (1974) 31.
4. Brent, T. P. *Cell Tissue Kinet. 4* (1971) 297.
5. Bohman, C. and Eriksson, S. *Biochemistry. Submitted for publication.*

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