ACQUISITION OF THYMIDINE KINASE ACTIVITY BY HERPES SIMPLEX INFECTED MOUSE FIBROBLAST CELLS*

Saul Kit and D. R. Dubbs

Division of Biochemical Virology
Baylor University College of Medicine, Houston, Texas

Received March 6, 1963

It has previously been shown that thymidine kinase activity is induced in cells infected with vaccinia and other pox viruses (Kit, Dubbs and Piekarski, 1962; Kit, Piekarski and Dubbs, 1963; McAuslan and Joklik, 1962). The present communication demonstrates that thymidine kinase activity is also induced in mouse fibroblast cells infected with herpes simplex virus (O'Connell strain) (Scherer, 1953). The cells employed in this study were derived from Earle's L-cells (Clone 929). The cell strains [L- (TK^-) and a clonal subline, L-M (TK^-)1D] are devoid of demonstrable thymidine kinase activity, fail to incorporate bromodeoxyuridine or thymidine-H^3 (TDr-H^3) into DNA, and are resistant to growth inhibition by bromodeoxyuridine, iododeoxyuridine, or relatively high concentrations of TDr (Dubbs and Kit, 1963; Kit, Dubbs, Piekarski, and Hsu, 1963). Hence, the acquisition of thymidine kinase activity following virus infection is unequivocally demonstrable by enzymatic, biochemical, and radioautographic methods (Kit and Dubbs, 1962; Kit, Dubbs and Hsu, 1963; Kit, Piekarski and Dubbs, 1963).

Table 1 illustrates the fact that TDr-H^3 was not incorporated into the DNA of uninfected L-M (TK^-) or L-M (TK^-) 1D cells when challenged with a two hour

* Aided by grants from the Leukemia Society, Inc., the National Cancer Institute (CA-06829-02, CA-06656-01), the National Science Foundation (GB-620), and the American Medical Association (ERF #71). The experiments were carried out with the expert technical assistance of David Cullop, Barbara Davis and Milton Anken.

TDr-H^3 pulse. However, it has also been shown that uninfected L-M (TK^-) 1D cells fail to incorporate TDr-H^3 into DNA after a 19 hour incubation. Cells incubated with encephalomyocarditis, an RNA virus, or with a heated herpes simplex preparation (56° for 30 minutes), likewise did not incorporate TDr-H^3 into DNA. However, the capacity to utilize TDr-H^3 for DNA synthesis was acquired by cells infected with active herpes simplex virus.

Table 1

EFFECT OF HERPES SIMPLEX VIRUS ON THE INCORPORATION OF THYMIDINE-H^3 (TDr-H^3) INTO THE DNA OF L-M (TK^-) 1D CELLS

Expt.	Input Multiplicity: PFU per Cell	Time PI Before Adding TDr-H^3 for 2 Hours	c/m per μg DNA		% Cells with Nuclei Labelled	
			Control	Herpes Infected	Control	Herpes Infected
a	1	3	0	13	0	13%
		5	0	29	0	23%
b	1	5	0	28	—	—
c	approx. 10 to 20	5	0	156	0	67%
		8	0	41	0	66% *
		13	—	2	—	13% **
d***	20	3	0	101	—	—

* Nuclei lightly labelled; ** Nuclei very lightly labelled; *** L-M (TK^-) cells

Approximately 6 to 8 x 10^6 cells, which had been grown for 2 days in suspension cultures in 25 x 150 mm test tubes, were centrifued and the medium was discarded. In experiments a and b, the cells were resuspended in 10 ml of medium containing herpes simplex virus. In experiment c, the cells were resuspended in 5 ml of medium containing herpes simplex virus. After an absorption period of 1 hour at 37°, additional growth medium was added to each test tube to give a final volume of 25 ml and the suspensions were further incubated for the indicated periods of time. TDr-H^3 (0.1 ml per tube, equivalent to 10 μcuries and 0.37 μg) was added for a period of 2 hours. In experiment d, 5 ml of a herpes simplex preparation was added to 20 ml of a cell suspension and the suspensions were incubated at 37° for 3 hours prior to the addition of TDr-H^3.

The amount of TDr-H^3 incorporated into DNA depended upon the input multiplicity of virus to cells and upon the time after infection that the cells were challenged with TDr-H^3. The specific activity of the DNA was much greater when the input multiplicity was about 10 to 20 PFU per cell (Table 1, experiments c and d) than when the input multiplicity was about 1 PFU per cell (Table 1, experiments a and b). At 5 to 7 hours PI, approximately two-thirds of the cells incorporated TDr-H^3 into DNA at the higher input multiplicity, as compared with about one-quarter of the cells at the lower input multiplicity. At eight hours or more PI, extensive cell destruction occurred, so that DNA labelling at 13 to 15 hours PI was relatively low (Table 1, experiment c).

Radioautographic experiments have revealed that vaccinia infected L-M (TK^-) 1D cells contain discrete foci of DNA radioactivity in the cell cytoplasm. In a very small proportion of the cells, radioactivity barely above the background level, was also noted in the cell nuclei. On the other hand, herpes simplex infected cells exhibited an intense DNA labelling throughout the nucleus, but no cytoplasmic radioactivity. These findings are consistent with the concept that vaccinia and herpes simplex, respectively, are replicated in the cell cytoplasm and nucleus.

At present, it has not been established whether in herpes simplex infected cells: (1) thymidine kinase is formed in the cytoplasm and is transferred to the nucleus; (2) thymidine kinase is formed in the cytoplasm, catalyzes the synthesis of thymidylate-H^3 in the cytoplasm and, thereafter, the thymidylate-H^3 passes to the nucleus; or (3) thymidine kinase is formed in the nucleus and catalyzes the synthesis of thymidylate in the nucleus. Once available in the nucleus, thymidylate-H^3 may be utilized for herpes simplex DNA synthesis. Probably, part of the nuclear radioactivity also represents host-cell chromosomal DNA biosynthesis.

Table 2 shows that sonic extracts of L-M (TK^-) 1D cells lack thymidine kinase activity but that sonic extracts prepared from herpes simplex infected cells manifest appreciable enzymatic activity. At an input multiplicity of about 10 to 20 PFU per cell, thymidine kinase activity was demonstrable at 2 hours PI and increased rapidly over the next four hours (Table 2, experiment b). The enzyme was localized in the "soluble" fraction of the cells. Thus, the supernatant fraction obtained by centrifuging the cell sonicates at 144,000 g for one hour accounted for all of the enzymatic activity found in the crude cellular sonicates.

Table 2

INDUCTION OF THYMIDINE KINASE ACTIVITY IN L-M (TK^-) 1D CELLS AFTER HERPES SIMPLEX INFECTION

Expt.	Input Multiplicity: PFU per Cell	Hours PI Before Preparing Enzyme	μμMoles TMP Formed per μg DNA in 10 minutes at 38°	
			Control	Herpes Simplex Infected
a	1.3	6	0	7.3
b	approx. 10 to 20	2	0	2.4
		4	—	57.6
		6	—	153
c	12	4	0	77.4

Thymidine kinase activity was measured in sonic extracts of noninfected and virus infected cells as previously described. (Kit, Piekarski and Dubbs, 1963)

In control experiments, it was shown that sonicates prepared from cells which were incubated with heated herpes preparations (56° for 30 minutes) exhibited no measurable thymidine kinase activity. Neither was thymidine kinase activity found in the growth medium obtained from infected cells at 6 hours PI, nor in the herpes simplex viral preparations, per se.

The experiments cited here demonstrate clearly that herpes simplex, which contains only 4.5×10^6 molecular weight equivalents of DNA per particle (Ben-Porat and Kaplan, 1962), as well as vaccinia, which has 160×10^6 molecular weight equivalents of DNA per particle (Allison and Burke, 1962; Joklik, 1962), is capable of inducing thymidine kinase in infected cells. The possibility that infection of L-M (TK^-) 1D cells by other DNA viruses leads to thymidine kinase induction is currently under investigation.

REFERENCES

Allison, A. C. and Burke, D. C., J. Gen. Microbiol. 27, 181 (1962).

Ben-Porat, T. and Kaplan, A. S., Virology 16, 261 (1962).

Dubbs, D. R. and Kit, S., Exptl. Cell Research (in press).

Joklik, W. K., J. Mol. Biol. 5, 265 (1962).

Kit, S. and Dubbs, D. R., Virology 18, 274 (1962).

Kit, S., Dubbs, D. R. and Hsu, T. C., Virology 19, 13 (1963).

Kit, S., Dubbs, D. R. and Piekarski, L. J., Biochem. and Biophys. Research Comm. 8, 72 (1962).

Kit, S., Dubbs, D. R., Piekarski, L. J. and Hsu, T. C., Exptl. Cell Research (in press).

Kit, S., Piekarski, L. J. and Dubbs, D. R., J. Mol. Biol. 6, 22 (1963).

McAuslan, B. R. and Joklik, W. K., Biochem. and Biophys. Research Comm. 8, 486 (1962).

Scherer, W. F., Am. J. Path. 29, 113 (1953).