NON-FUNCTIONAL THYMIDINE KINASE CISTRON IN BROMODEOXYURIDINE RESISTANT STRAINS OF HERPES SIMPLEX VIRUS*

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Thymidine (TdR) and deoxyuridine (UdR) kinase activities are induced following infection of mouse fibroblast cells with vaccinia (Kit, Dubbs and Piekarski, 1962; Kit, Piekarski and Dubbs, 1963a) or herpes simplex viruses (Kit and Dubbs, 1963a). The data to be presented show that enzyme induction by herpes simplex can be prevented by puromycin and actinomycin D. Moreover, mutant herpes simplex strains highly resistant to 5-bromodeoxyuridine (BUdR) have been obtained (Kit and Dubbs, 1963b). The mutant herpes simplex strains are deficient in TdR and UdR kinase inducing activities.

The BUdR-resistant LM (TK^-) strain of mouse fibroblasts (Dubbs and Kit, in the press; Kit, Dubbs, Piekarski and Hsu, 1963) and the O'Connell strain of herpes simplex (Scherer, 1953) were employed in these studies. The LM (TK^-) cells lack TdR and UdR kinase activities and fail to incorporate TdR-H^3 or BUdR into DNA.

Figure 1 illustrates the fact that TdR kinase activity was absent from uninfected LM (TK^-) cells but that enzyme activity could be detected at 2.5 hours

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post inoculation (PI) of herpes simplex virus. By 5.5 hours PI, high levels of TdR kinase were induced.

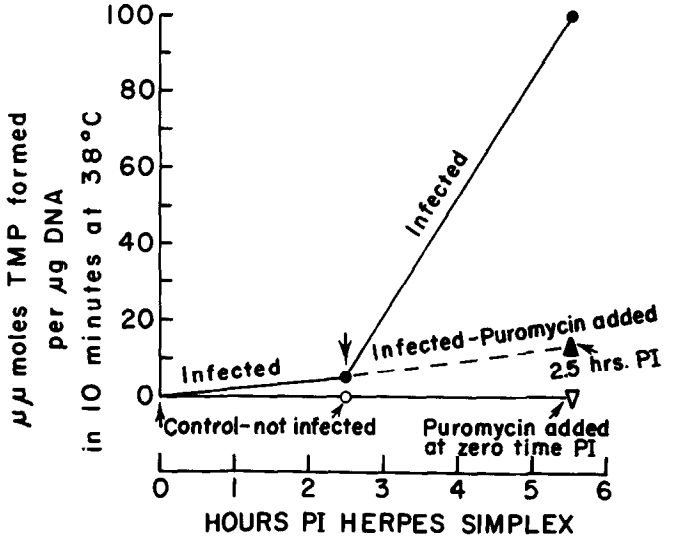

Figure 1: Effect of puromycin on the induction of thymidine kinase by herpes simplex infected LM (TK^-) cells. Puromycin concentration: 5×10^{-5}M. Input multiplicity: 4.5 PFU/cell. Each tube contained 10^7 cells in a total volume of 25 ml of medium.

Puromycin (5×10^{-5}M), when added to the medium at the time of virus infection, prevented TdR kinase induction. Moreover, the addition of puromycin at 2.5 hours PI almost completely prevented the increase of TdR kinase activity normally observed between 2.5 and 5.5 hours PI.

Enzyme preparations from noninfected cells also failed to catalyze the phosphorylation of UdR. However, UdR kinase activity was induced following virus infection (Table 1). (Possibly, the same enzyme catalyzes the phosphorylation of TdR and UdR.)

At a concentration of 0.5μg/ml, actinomycin D inhibited TdR and UdR kinase induction by about 97% (Table 1). At higher concentrations of actinomycin D, the kinase activities were virtually undetectable. The puromycin (Allen and Zamecnik, 1962) and actinomycin D (Goldberg and Rabinowitz, 1962; Levinthal,

Table 1

Effect of actinomycin D on the induction of thymidine and deoxyuridine kinase activities in 2-day-old LM (TK^-) cells at 5 hours post inoculation of herpes simplex virus.

Virus	Actinomycin D concentration μg/ml	Thymidine kinase	Deoxyuridine kinase
		μμmoles nucleotide formed per μg DNA in 10 minutes at 38°	
Noninfected	0	0	0
Parental herpes simplex	0	356	355
Parental herpes simplex	0.5	9	8
Parental herpes simplex	1.0	1	1
Parental herpes simplex	2.0	0	0
Parental herpes simplex	6.0	0	0

The input multiplicity of herpes simplex virus was 1.2 PFU/cell. The virus adsorption period was 30 minutes at 38°C. Actinomycin D was added to the cells at 30 minutes PI.

Keynan and Higa, 1962) experiments indicate, respectively, that protein and DNA-dependent-RNA synthesis are required for kinase induction in the herpes simplex infected LM (TK^-) cells.

The parental herpes simplex virus has been plaqued in LM (TK^-) cells and clonal sublines have been isolated and passaged over 8 times in LM (TK^-) cells. Table 2 shows that following infection with parental clonal strain 104, the LM (TK^-) cells display TdR and UdR kinase activities and actively incorporate TdR-H^3 into DNA.

By propagating and plaquing herpes simplex virus in LM (TK^-) cells in medium containing 25μg/ml BUdR, we have isolated several mutant herpes simplex strains resistant to BUdR. Details concerning the isolation of the herpes simplex mutants will be presented elsewhere.

As shown in Table 2, infection with 4 of the herpes simplex mutants (2006, 2011, 2012, and 2018) failed to induce incorporation of TdR-H3 into DNA or to

Table 2

Thymidine and deoxyuridine kinase activities and thymidine -H^3 uptake into DNA by 2-day-old LM (TK^-) cells following infection by parental or mutant strains of herpes simplex

Herpes simplex strains	Input multiplicity: PFU/cell		Cts/min per μg DNA*	μμmoles nucleotide formed in 10 min at 38°C per μg DNA**	
	TdR-H^3 Expt.	Enzyme Expt.		Thymidine kinase	Deoxyuridine kinase
Noninfected	-	-	0	0	0
Parental Clone 104	1.0	1.0	309	61.0	106.0
Mutant 2006	1.8	3.2	0	1.0	0
Mutant 2011	0.9	0.6	0	0.8	1.0
Mutant 2012	2.8	1.5	0	1.7	0
Mutant 2018	1.1	2.0	0	1.7	0.3
Mutants 2011 plus 2006	1.1 1.2	-	0	-	-
Mutants 2011 plus 2012	1.1 0.6	-	0	-	-
Mutants 2011 plus 2018	1.1 0.8	-	0	-	-
Mutants 2012 plus 2006	0.6 1.2	-	0	-	-
Mutants 2012 plus 2018	0.6 0.8	-	0	-	-
Mutants 2018 plus 2006	0.8 1.2	-	0	-	-

*2-6 hours PI. **5 hours PI. 0.1 ml of TdR-H^3 (10 μcuries) was added to each tube containing 25 ml.

induce TdR and UdR kinase activities in LM (TK^-) cells. Moreover, there was no detectable incorporation of TdR-H^3 into DNA when the LM (TK^-) cells were mixedly infected with pairs of herpes simplex mutants.

These experiments and similar experiments performed with cells infected with parental and mutant strains of vaccinia virus (Dubbs and Kit, unpublished experiments; Kit and Dubbs, 1963b; Kit, Dubbs and Piekarski, 1963; Kit, Piekarski and Dubbs, 1963ab) provide strong support for the concept that information for the synthesis of TdR (UdR) kinase resides in the herpes simplex and vaccinia genomes. The kinases may facilitate the synthesis of thymidylate and thymidine triphosphate, despite the presence in the cells of powerful deoxyuridylic and thymidylic acid phosphatases.

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