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SHORT COMMUNICATION

INFECTION OF BROMODEOXYURIDINE RESISTANT HELA CELLS WITH SV40 INFECTIOUS DNA

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After infection of animal cells with animal viruses, certain enzyme activities are induced or enhanced (Kit and Dubbs, 1969).

For instance after infection of permissive or nonpermissive host cells with SV 40 virus, dCMP deaminase, thymidine kinase, DNA polymerase and thymidylate kinase are induced (Kit, 1967). On polyoma virus infection, the induced thymidine kinase activity is not coded by viral genome and is of cell origin (Basilico et al., 1969). Recently, Kit et al. (1970c) also clarified the origin of human adenovirus type 5 induced thymidine kinase activity using bromodeoxyuridine (BUdR) resistant HeLa S_3 cells (HeLaBU 25) and found that it was also of cell origin.

This paper describes studies on thymidine kinase activity in HeLaBU 25 cells infected with SV 40 infectious DNA. Human cells are usually much less sensitive to infection with SV 40 virus than monkey cells but are susceptible to infection with SV 40 infectious DNA (Swetley et al., 1969; Kit et al., 1970a). Infectious DNA was prepared as described elsewhere (Kit et al., 1968) using PAS-phenol-extraction and nitrocellulose column chromatography. The BUdR resistant HeLa S_3 cell line, HeLaBU 25, was established and kindly supplied by Dr. Saul Kit of Baylor College of Medicine, Houston, Texas (Kit et al., 1966). The procedure for infection with infectious DNA was described by Kit et al. (1968). As already reported by Kit et al. (1968), SV 40 DNA is about 0.01–0.001 times as infectious as intact virus on CV-1 cells. There are technical limitations to obtaining a sufficient input multiplicity of infection. Under our experimental conditions, 3% of BUdR resistant HeLa cells (HeLaBU 25) infected with SV 40 DNA at an input multiplicity of 0.1 showed SV 40 specific intranuclear T-antigen (Table 1).

We used two methods to test thymidine kinase activity after infection of HeLaBU 25 cells with SV 40 infectious DNA. One method was to measure ^{3}H-thymidine (^{3}H-dT) incorporation into DNA in the presence or absence of hypoxanthine (10^{-4} M), aminopterin (10^{-5} M) and glycine (10^{-5} M). These drugs were used to enhance ^{3}H-dT incorporation by inhibiting thymidylate synthetase (Littlefield, 1965). Table 2 shows the results of this type of experiment. Apparently SV 40 infection did not induce thymidine kinase and ^{3}H-dT incorporation was somewhat less in SV 40 infected cells than in control cells. Table 3 shows the growth of SV 40 virus in HeLaBU 25 cells after infection with SV 40 DNA. Since SV 40 can grow in HeLaBU 25 cells as in other human cells (Kit et al., 1970a), if SV 40 induces thymidine kinase, more ^{3}H-dT should

be incorporated into the DNA of SV 40 infected cells than into that of noninfected cells.

The other method used was titration of the infectious virus in the presence and absence of BUdR (25 μg/ml). Since HeLaBU 25 cells can not grow in the presence of hypoxanthine, aminopterin and glycine, it is evident that they must be deficient in thymidine kinase activity. If SV 40 induces thymidine kinase in these cells, BUdR should be incorporated into DNA and progeny SV 40 which incorporate BUdR should be killed on exposure to visible light. The results of this type of experiment are shown in Table 4. Apparently there is no difference between the virus yields with and without BUdR.

In this type of experiment, there are several possible combinations of host cells and SV 40 virus or DNA. The system we employed

TABLE 1. *Induction of intranuclear T-antigen in HeLaBU25 cells infected with SV40 DNA*

Hours after infection	T-antigen positive/total	% of T-antigen positive cells
24	21/1500	1.4
48	45/1500	3.0
72	33/1000	3.3

The procedure for infection is described in the legend of table 4. The input multiplicity of infection was 0.1PFU/cell. At each time, T-antigen positive cells were measured in 2 or 3 cultures and non-infected cultures. For T-antigen assay, the indirect immunofluorescent technic was employed using tumor bearing hamster serum and anti-hamster-gammaglobulin rabbit serum.

TABLE 2. *Incorporation of ^{3}H-dT into DNA in the presence and absence of hypoxanthine (H), aminopterin (A) and glycine (G)*

Group No.	Infection with SV40 DNA[a]	Addition of HAG[b]	^{3}H-dT incorporated into DNA (Average cpm/culture[c])
1	+	−	3.9×10^4
2	+	+	6.2×10^4
3	−	−	5.0×10^4
4	−	+	8.7×10^4

a Input multiplicity of 0.1 PFU/cell.
b Hypoxanthine (10^{-4}M), Aminopterin (10^{-5}M), Glycine (10^{-5}M).
c Average of two cultures.

The final concentration of thymidine was 5μc/ml and 1μg/ml. Seventy-five hours after infection, cells were collected and excess ice-cold 5% TCA was added. The mixture was centrifuged and the pellet was washed three times with ice-cold 5% TCA. Then 1.0 ml of 0.5 N perchloric acid was added and the mixture was heated at 70C for 30 min. The mixture was centrifuged and the radioactivity in the supernatant was measured in a Beckman liquid scincillation counter.

TABLE 3. *Growth of SV40 virus in HeLa BU25 after infection of these cells with SV40 infectious DNA*

Hours after Infection	pfu/culture
1.5	0
45	2.0×10^1
92	3.2×10^3

Confluent monolayers of HeLaBU25 cells (2 oz. vessels) were infected with SV40 infectious DNA using DEAE-dextran (1mg/ml) at an input multiplicity of 0.2 PFU/cell. At the times indicated in the table, cultures were frozen. Before titration of virus, they were thawed and sonicated at 10kc for 3 min. Infectious virus in the samples was assayed using green monkey kidney cell monolayers.

TABLE 4. *Virus yield in HeLaBU25 cells in the presence and absence of BUdR (25μg/ml)*

BUdR (25μg/ml)	PFU/culture
+	1.2×10^4
−	2.3×10^4

Cultures of HeLaBU25 cells in 2 oz. bottles were infected with SV40 infectious DNA using DEAE-dextran at an input mutiplicity of 0.1PFU/cell. Cells were harvested 70 hr after infection. Cultures were frozen and thawed once and sonicated at 10kc for five minutes. Plaque assay was done on primary green monkey kidney cell cultures. One hour after infection, no infectious virus was found by plaque assay.

seems the best since the thymidine kinase-less green monkey kidney cell line is not available and human cells are much less sensitive to SV 40 virus. Hatanaka and Dulbecco (1967) reported on SV 40 specific thymidine kinase. Their results may be explained in two ways. One is that genetic information for thymidine kinase is present in the viral genome, and the other is that the second gene locus for thymidine kinase in the host cell chromosome is derepressed. Kit et al (1970b) reported that induction of thymidine kinase occurs in cells infected with SV 40 virus strain which was rescued from BUdR resistant SV 40-transformed mouse cells (mKS-BU 100). This phenomenon can also be explained in two ways. One is that viral coded thymidine kinase is not produced in SV 40 transformed mouse cells and the other explanation is that SV 40 induced thymidine kinase activity is of host cell origin.

Our results can also be explained in two ways. First, the SV 40 genome does not code thymidine kinase. Second, the failure to induce thymidine kinase activity may be due to the very low efficiency of infection by infectious DNA (Kit et al., 1970a).

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