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Transfer of Thymidine Kinase to Thymidine Kinaseless L Cells by Infection with Ultraviolet-Irradiated Herpes Simplex Virus

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L cells lacking thymidine kinase (TK) activity (Ltk$^-$ cells) have been stably transformed to a TK-positive phenotype by infection with ultraviolet-irradiated herpes simplex virus (HSV-UV). The highest frequency of the Ltk$^-$ to Ltk$^+$ transformation observed in these experiments was approximately 10^{-3}, whereas no measurable transformation was observed (less than 10^{-8}) in the absence of HSV-UV infection. Cell lines of HSV-transformed Ltk$^+$ cell lines contain 7 to 24 times as much TK activity as do the parental Ltk$^-$ cells, and they have been maintained in culture for a period exceeding 8 months. The kinetics of thermal inactivation of the TK activity derived from an Ltk$^+$ HSV-transformed cell line and the TK activity from Ltk$^-$ cells lytically infected with infectious HSV are similar. Both of these TK activities are much more thermolabile than the TK activity present in wild-type L cells. A mutant strain of HSV which does not induce TK activity during lytic infection does not cause the Ltk$^-$ to Ltk$^+$ transformation. These data suggest that either an HSV TK gene has been transferred to Ltk$^-$ cells or that an HSV gene product has caused the expression of a previously repressed cellular enzyme.

There has been much interest recently concerning the development of methods by which external genetic information could be stably introduced into an eukariotic cell line. As a first step, we proposed to look for a carrier mechanism which would enable the desired information to enter the cell and be established in an inheritable manner. An obvious choice was a mammalian virus. Our requirements for such a suitable virus were (i) that there be some basis for belief that the virus is capable of a nonlytic relationship with cells, (ii) that the virus be a nuclear deoxyribonucleic acid (DNA) virus, and (iii) that it contain a gene(s) that could be selected for in a tissue culture system.

In view of these criteria, herpes simplex virus (HSV) was chosen. HSV is able to establish persistent infections in man such as herpes labiallis. Evidence has been obtained (18) indicating that Epstein-Barr (EB) virus, a herpesvirus, is vertically transmitted in cultured cell lines derived from Burkitt tumors and is able to transform human leukocytes in vitro in regard to their growth characteristics (8, 21). Zur Hausen and Schulte-Holthausen (24) have shown that the tumor tissue from Burkitt tumors contains DNA homologous to EB virus DNA. Marek's disease of chickens (4) and lymphomas in rabbits (H. Hinze, Bacteriol. Proc., p. 157, 1969) have been shown to be associated with viral agents having an HSV morphology. There is, therefore, much evidence to indicate that HSV and other viruses with similar morphology are able to establish nonlytic relations with animal cells.

HSV is a DNA-containing virus (1), and its DNA replicates in the nucleus of infected cells (20). A nuclear DNA virus was chosen for study because it seemed that it would offer maximum opportunity for possible genetic interchange between the genomes of the virus and the cell.

Kit et al. (10) have reported that by cultivating LM cells (Ltk$^+$ cells) in the presence of successively increasing concentration of bromodeoxyuridine (BUDR), an altered cell strain was produced which has very low amounts of thymidine kinase (TK) activity (Ltk$^-$ cells). HSV is able to induce TK activity in Ltk$^-$ cells during the course of a lytic infection (9). This HSV-induced TK

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activity has been shown to be immunologically different from the Ltk$^+$ TK activity (12).

The existence of a virus which is potentially a carrier of a TK gene and a cell line which is deficient in this activity present the opportunity to test the possibility that HSV-infected Ltk$^-$ cells are able to acquire permanently the virus TK activity.

Since HSV has a strong cytopathic effect in Ltk$^-$ cells, ultraviolet-irradiated HSV (HSV-UV) were used for infection. UV irradiation of transducing particles prior to infection increases the frequency of stable transduction in bacterial systems (7). It has been shown that vaccinia that had been inactivated by UV irradiation is able to initiate TK synthesis in Ltk$^-$ cells, indicating that a UV-inactivated virus may still contain a functional TK gene and that the gene can be expressed by a UV-killed virus (17).

Littlefield (13) has shown that TK-containing cells (Ltk$^+$ cells) can be selected from an Ltk$^-$ population by including aminopterin, thymidine, hypoxanthene, and glycine in the tissue culture medium (HAT medium). By using a similar system, we have infected Ltk$^-$ cells with HSV-UV and then selected for cells which have acquired TK activity (Ltk$^+$ cells).

MATERIALS AND METHODS

Virus. HSV type 1 was obtained from David Yohn in 1966. Except where noted otherwise in this paper, stock virus was prepared in primary rabbit kidney (PRK) monolayers as follows. Cell layers that had been infected 20 to 24 hr previously were frozen and thawed twice in growth medium. This lysate was centrifuged at 500 $\times$ *g* for 10 min, cultured for sterility, and stored at -70 C. Virus stocks prepared in this way had 10^5 to 10^7 plaque-forming units (PFU)/ml and 10^9 to 5×10^{10} particles/ml.

HSV (B2006), a strain of HSV, was obtained from Saul Kit and Del Dubbs and has been described by them (5). This is a mutant HSV that does not induce TK activity in Ltk$^-$ cells during a lytic infection.

The cell line on which a particular virus stock had been grown and the number of consecutive passages in that cell line are indicated by an abbreviation for the cell line followed by the passage number. For example, HSV-PRK p = 7 refers to herpes simplex virus that has been serially passaged in PRK cells seven times.

Virus preparations were not sonically treated before or after UV irradiation because sonic treatment did not increase the titer of infectious virus preparations.

UV irradiation of virus. A GE Germicidal Lamp G8T5 was used to irradiate suspensions of stock virus. Viral suspensions in growth medium were irradiated at a distance of 13 inches (33.3 cm) from the lamp. Irradiation at this distance produced a UV intensity of 23 ergs per mm^2 per sec. Samples (2 ml) of stock virus suspensions were irradiated in 6-cm plastic petri dishes. During irradiation the petri dishes were rotated at 100 rev/min on a rotary platform shaker and were exposed to atmospheric oxygen at room temperature.

Media. Growth medium contained Eagle's medium supplemented with the nonessential amino acids and 5% calf serum (6). TK plus selective medium was growth medium with the following additions: methotrexate (6.0×10^{-7} M), thymidine (1.6×10^{-5} M), adenosine and guanosine (each at 5×10^{-5} M), and glycine (10^{-4} M).

Cell lines. Ltk$^-$ cells were obtained from Kit et al. (10). These cells were cloned once and were serially cultivated in growth medium containing 20 μg of BUDR per ml. The phenotypic characterization of this cell line is that (i) it is resistant to growth inhibition by BUDR at 20 μg/ml, (ii) it incorporates very little tritiated thymidine into its DNA, and (iii) high speed (100,000 $\times$ *g* $\times$ 1 hr) supernatant fluid extracts of Ltk$^-$ cells contain very low levels of TK activity.

A9 cells were obtained from Littlefield (13). This cell line was used as the L cell type that contained TK activity (Ltk$^+$ cells). These cells were grown in growth medium without 8-azaguanine, except in the experiments involving phosphoribosyl transferase activity.

KB cells were obtained from the cell service unit at Roswell Park Memorial Institute. They were grown in monolayer cultures in growth medium.

Cell lines derived from Ltk$^-$ cells infected with HSV-UV and grown in TK plus selective medium are referred to in this paper as Ltk$^+$-transformed cells. Specific Ltk$^+$-transformed cell lines will be identified as Ltk$^+$ HSV-. The number following the hyphen after HSV identifies the particular clone.

PRK cells were obtained from 10-day-old rabbits, and tissue cultures were prepared from them as described by Merchant et al. (19).

Human embryonic lung cells were obtained from Jun Minowada at Roswell Park Memorial Institute. This cell line was grown in Eagle's medium containing 10% fetal calf serum.

Virus plaque titrations. Plaque titrations were performed as described by Roizman and Roane (22), except that monolayers of PRK cells were used.

Virus particle counts. Virus particle counts were performed as described by electron microscopy (23) and standardized by using latex particles.

Colony counting. In the experiments in which colony counts were made, four to six replicate petri dishes were counted for each determination. The Ltk$^+$ HSV-transformed colonies varied greatly in size. Colonies of less than 50 cells were not counted. Data are expressed as the average number of Ltk$^+$-transformed colonies per dish.

Tritiated thymidine uptake. This was performed as described by Breslow and Goldsby (3). The medium used for all cell types was TK plus selective medium without methotrexate.

Thymidine kinase assay. The TK assay was as described by Kit et al. (11) with the following modifications. Pellets of cells were suspended in a sonically treated buffer (five times their volume) containing 0.01$_1$ M maleate tris(hydroxymethyl)aminomethane (Tris; *p*H 6.5), 0.15 M KCl, 0.02 M $MgCl_2$, 0.001 M mercaptoethanol, 2×10^{-4} M adenosine triphosphate (ATP), 10^{-5} M thymidine and were sonically treated.

The standard assay mixture contained in a total of 120 μliters the following final concentration of solutes: 0.1 M maleate Tris (*p*H 6.5), 0.01 M ATP, 0.02 M $MgCl_2 \cdot 6H_2O$, 8.3×10^{-5} M ^{3}H-thymidine (specific activity approximately 1 mCi/mmole), and between 100 and 700 μg of protein. Under these conditions, enzyme activity was proportional to the amount of cell extract added and to the time of incubation, up to 30 min at 36.5 C.

The amount of phosphylated thymidine produced was measured as described by Breitman (2). Protein was determined by the method of Lowry et al. (15).

Thermal denaturation experiments. Cell extracts were prepared in a manner similar to that described for the TK assay. Each sample, containing 4.4 mg of protein in a total volume of 2 ml, was incubated at 52 C in a water bath. Samples of 0.2 ml were removed at indicated time intervals, and portions were assayed for TK activity.

Preparation of HSV antiserum. New Zealand rabbits were immunized by three successive injections of purified HSV-KB p = 10. The first injection was given intravenously and contained 5×10^{10} virus particles. Two subsequent intramuscular injections of 10^{10} HSV particles followed the first injection at monthly intervals. The HSV used for immunization was purified by two cycles of rate zonal sedimentation in 5 to 40% Ficol (Pharmacia Ltd.) gradients.

RESULTS

Reversion frequencies of Ltk$^-$ cells to Ltk$^+$ cells. In these experiments, spontaneous reversion of Ltk$^-$ cells to the Ltk$^+$ phenotype is less than 1 in 10^8. In the course of 20 experiments (measured by the formation of colonies by Ltk$^-$ cells in TK plus selective medium), 10^6 cells were seeded in each of a total of 100 petri dishes containing selective medium. No colony formation was observed. Similar results have been reported by Littlefield (14).

Transformation of Ltk$^-$ to Ltk$^+$ via infection with HSV-UV. Formation of colonies in TK plus selective medium was observed when Ltk$^-$ cells were infected with HSV-UV. These experiments were carried out as follows. Sparse monolayers of Ltk$^-$ cells, growing in 6-cm diameter plastic petri dishes, were inoculated with 0.2 ml of HSV-UV. After an adsorption period of 1 hr, the inoculum was aspirated, and 5 ml of growth medium containing 0.2% pooled human gamma globulin (Hyland) was added to each dish. The infected cells were then incubated for 24 hr to allow for gene establishment and expression. The growth medium then was removed and replaced with an equal volume of TK plus selective medium. TK plus selective medium caused between 50 and 80% of the cells to die and detach from the surface of the petri dishes. At 5 days after infection, the suspension of dead cells and medium was aspirated and replaced with fresh TK plus selective medium. The dishes were then incubated for a total of 16 to 20 days at 37 C in a humidified incubator with a CO_2-air atmosphere at 10 and 90%, respectively. Colonies that had formed were then either stained with crystal violet-formaldehyde at 0.1 and 3.7%, respectively, or used for clone isolation. Under these circumstances colony formation was regularly observed. Colony size and morphology varied widely. Approximately one-fourth to one-third of the colonies grew to a size of 2 mm or larger in diameter. A continuous spectrum of smaller colonies also formed. Many of the small colonies did not increase in size, even if incubation was continued for more than 19 days. Often the cells of these small nongrowing clones showed cytological evidence of thymidine starvation and probably represented cells in which TK activity was lost after a few divisions.

No colony formation was observed when cells were infected with unirradiated HSV. In this case a massive cytopathic response, typical of herpes infection and not characteristic of the effect of the TK plus selective medium, was observed.

Infection of Ltk$^-$ cells with UV-irradiated vaccinia did not result in the formation of Ltk$^+$ colonies.

Cells containing phosphoribosyl transferase (PRT) activity can be selected for in a tissue culture system by using HAT medium as described by Littlefield (13). A9 cells are deficient in PRT activity. Infection with HSV-UV did not enable A9 cells to form colonies in HAT medium.

Factors affecting the frequency of Ltk$^-$ to Ltk$^+$ transformation. The following factors have been found to alter the frequency of the HSV-irradiated Ltk$^-$ to Ltk$^+$ transformation: UV dose, virus dose, time of incubation in growth medium after infection, and cell density.

The frequency of colony formation versus the dose of UV received by HSV is shown in Fig. 1. Optimal colony formation was observed when HSV was irradiated for 9 to 12 min. Note that virus that had been irradiated until no infectivity could be detected was still effective in causing the Ltk$^-$ to Ltk$^+$ transformation. The number of colonies formed varied linearly with relative virus dose in the experiment shown in Fig. 2. In several experiments, however, this relation was not linear, comparing undiluted virus versus the lowest virus dilution. This effect is observed in Fig. 3. Dilution of the undiluted virus (plotted as a relative dose of 25) by a factor of 5 did not reduce the number of colonies produced by a factor of 5. The subsequent dilution of these virus preparations by a factor of 5 did reduce the number of colonies by approximately one-fifth. The use of high multiplicities alone, however, cannot account

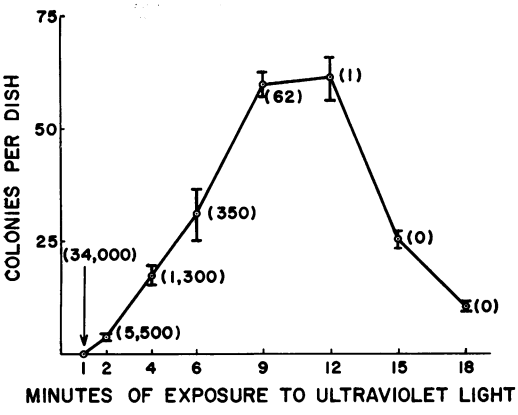

FIG. 1. *An HSV-PRK p = 7 preparation, containing 6.9 × 10⁹ particles and 6.3 × 10⁶ plaque-forming units (PFU) per 0.2 ml before irradiation, was exposed to UV irradiation for the intervals indicated on the abscissa. The number of PFU per 0.2 ml surviving a given dose of UV is given by the number in parentheses by each point. A 0.2-ml amount of this preparation was added to petri dishes, each of which contained 3.8 × 10⁵ Ltk⁻ cells. Other details are as described in Materials and Methods. Vertical lines through the points of this and the other figures represent the magnitudes of the standard deviations of the graphed values.*

for this nonlinearity, since the multiplicity used in the experiment shown in Fig. 3 is lower than in Fig. 2. This effect was independent of the amount of UV irradiation given to the virus.

The HSV-UV infected cells were incubated in growth medium for variable periods prior to the addition of TK plus selective medium (Table 1). The optimum period of incubation in growth medium was found to be 24 hr.

The number of colonies formed depended also on the number of cells present in the petri dish at the time of infection. Optimal cell density was between 2.5 × 10⁵ and 6.3 × 10⁵ cells per petri dish. Suppression of colony formation was observed when higher cell densities were used (Fig. 4).

Treatment of HSV-UV with HSV immune rabbit serum prevented colony formation (Table 2). Heating of HSV-UV at 50 C inactivated the transforming activity of the virus (Table 3). Incubation of HSV-UV with deoxyribonuclease had no significant effect (Table 3).

To demonstrate the formation of Ltk⁺ HSV transformation, it was necessary to use high multiplicities of virus particles per cell. Other experiments indicated that the efficiency of HSV particle uptake by Ltk⁻ cells is very low, so that the number of virus particles adsorbed is much lower than the number of virus particles added. For example, after being exposed to a monolayer of Ltk⁻ cells for 1 hr, HSV-UV inocula were removed and used to infect a second set of Ltk⁻ monolayers. Ltk⁺ HSV-transformed colonies (70 to 90%) formed on the second set. The proportion of HSV-UV particles adsorbed by Ltk⁻ monolayers under the same conditions used in the transformation experiments was determined by counting virus particles in solution before and after being exposed to Ltk⁻ cells. In one experiment, no decrease in particle count was observed.

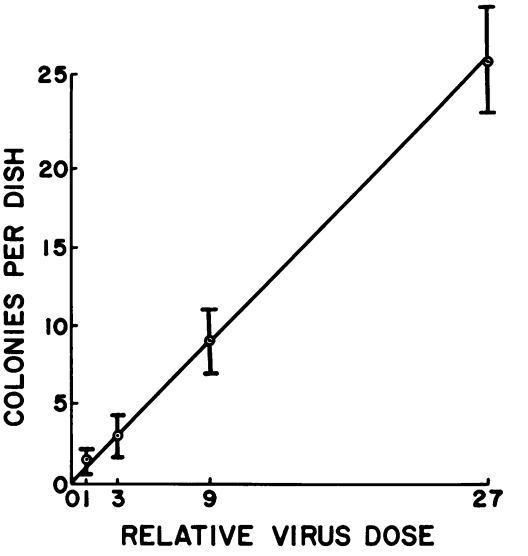

FIG. 2. *Petri dishes containing 1.4 × 10⁶ Ltk⁻ cells/dish were infected with 0.2 ml of HSV-Ltk⁻ p = 1 that had been UV irradiated for 9 min. The particle count of the undiluted preparation was 3.1 × 10⁹ particles/0.2 ml. A relative virus dose of 27 corresponds to the undiluted virus suspension.*

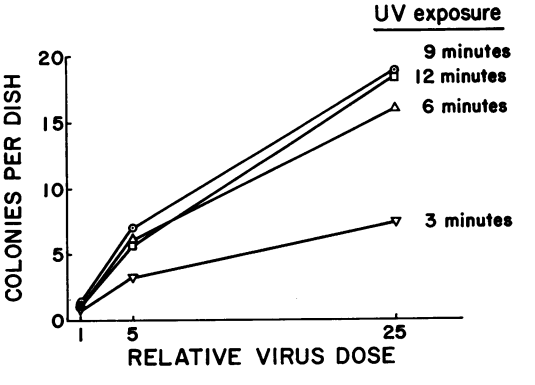

FIG. 3. *Petri dishes containing 1.7 × 10⁶ Ltk⁻ cells/dish were infected with 0.2 ml of HSV-PRK p = 3 that had been irradiated for the intervals indicated. The particle count of the undiluted virus suspension was 1.2 × 10⁹/0.2 ml. Relative dose of 25 represents the undiluted inoculum.*

In a second experiment, a 15% decrease was observed. It is therefore concluded that in these experiments only a relatively small fraction of the total HSV particles of an inoculum are taken up by the Ltk⁻ cells after 1 hr of adsorption. For this reason, the number of Ltk⁻ to Ltk⁺-transforming events per infecting particle has not been computed.

TABLE 1. *Effect on colony formation of incubation of infected cells in growth medium prior to the addition of selective medium*

Period of incubation in growth medium (hr)[a]	Avg no. of colonies	SD[b]
0[c]	1.7	2.1
12	7.2	1.3
24	46.8	9.7
36	—[d]	
48	—[d]	

[a] Ltk⁻ cells (8.8×10^5 per dish) were exposed to Ltk⁻ HSV p = 1 (ca. 8.8×10^8 particles) that had been irradiated with UV light for 8 min. Virus was allowed to adsorb to the cells for 1 hr at room temperature. After adsorption 5 ml of growth medium containing 0.2% pooled human gamma globulin was added to each petri dish, and the dishes were incubated at 36.5 C for variable intervals as indicated. This interval of incubation was terminated by adding 0.05 ml of a solution containing methotrexate (6.0×10^{-5} M), thymidine (1.6×10^{-3} M), adenosine and guanosine (each at 5×10^{-3} M), and glycine (10^{-2} M). Cells were then incubated for 18 days, counting from the time of infection. Colonies were fixed, stained, and counted as previously described.

[b] Standard deviation.

[c] At the time of infection, each petri dish contained approximately 2.3×10^6 Ltk⁻ cells.

[d] Cell multiplication during incubation in growth medium for periods exceeding 24 hr produced relatively dense monolayers. Under these conditions colonies were small and difficult to count.

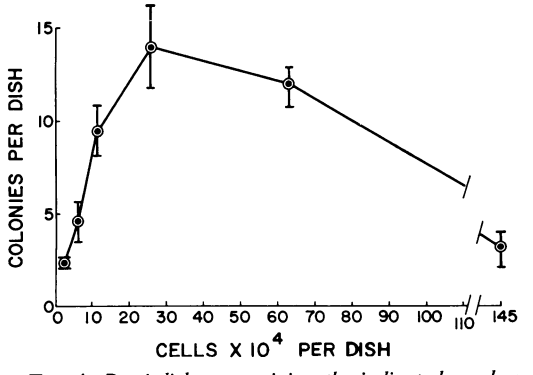

FIG. 4. *Petri dishes containing the indicated number of Ltk⁻ cells were inoculated with 0.2 ml of a suspension of HSV-PRK p = 7 that had been irradiated for 9 min. The virus preparation contained 6.9 × 10⁹ particles (6.3 × 10⁶ plaque-forming units before irradiation) per 0.2 ml.*

TABLE 2. *Neutralization of colony-forming activity of HSV-UV by herpes antiserum*

Serum treatment[a]		Avg no. of colony	SD[b]
Type	Dilution		
Immune	1/4	0.0	
Preimmune	1/4	7.4	2.6
Immune	1/40	0.5	0.55
Preimmune	1/40	11.3	2.2
Immune	1/320	8.9	3.1
Preimmune	1/320	6.0	4.0
Balanced salt solution		6.3	2.7

[a] HSV-PRK p = 1 (ca. 2×10^9 particles/0.2 ml) was irradiated with UV light for 9 min. Irradiated virus (1.2 ml) was combined with 0.4 ml of serum or serum dilutions and incubated at 37 C for 1 hr in plastic test tubes. The sera used were heat-inactivated at 56 C for 1 hr. Samples (0.2 ml) of virus-serum mixtures were added to petri dishes containing 1.4×10^6 cells per dish. Addition of growth medium and thymidine kinase plus selective medium was carried out as described in Materials and Methods. Colonies were stained and counted 17 days after infection.

[b] Standard deviation.

TABLE 3. *Effects of heat and deoxyribonuclease digestion on colony-forming activity of HSV-UV*[a]

Treatment[b]	Avg no. of colonies	SD[c]
0 C	6.3	2.7
56 C for 5 min	No colonies formed	
56 C for 30 min	No colonies formed	
Deoxyribonuclease digestion	8.0	3.2

[a] HSV-PRK p = 1 (ca. 2×10^9 particles/0.2 ml) was irradiated with UV light for 9 min.

[b] HSV-UV was either heated as indicated in the table or incubated at 37 C for 15 min in the presence of 400 μg of bovine pancreatic deoxyribonuclease per ml. Samples (0.2 ml) of these preparations were inoculated on 1.4×10^6 Ltk⁻ cells. Other procedures are as described in Materials and Methods.

[c] Standard deviation.

Effect of BUDR on colony formation. The omission of thymidine from TK plus selective medium suppressed colony formation (*see* Table 4). The substitution of thymidine with BUDR or iododeoxuridine in TK plus selective medium completely suppressed colony formation.

Properties of the Ltk⁺-transformed clones. A

series of 10 Ltk$^+$ HSV-transformed cell lines, derived from single clones, have been subcultured in TK plus selective medium twice a week for the past 8 months. The ability of these cell lines to incorporate tritiated thymidine into cellular DNA and the level of TK activity in supernatant fluid extracts of these cells are shown in Table 5. Relative to the Ltk$^-$ cell line, the Ltk$^+$ HSV-transformed cell lines were between 460 and 1,900 times more active in taking up tritiated thymidine into cellular DNA and contained 7 to 24 times as much TK activity.

Identity of the origin of the TK activity in Ltk$^+$ transformed cells. A mutant strain of HSV, HSV(B2006), does not induce TK activity in Ltk$^-$ cells during lytic infection. Infection of Ltk$^-$ cells with HSV-UV (B2006) did not cause the formation of colonies in TK plus selective medium (Table 6). This suggests that the infecting HSV must contain a functional TK gene to be able to cause the production of Ltk$^+$ cells.

HSV strains, which had been grown on Ltk$^-$ cells and then inactivated with UV, were able to cause the production of Ltk$^+$-transformed cells (Table 6). This suggests that the production of Ltk$^+$-transformed cells by HSV-UV is not due to the encapsidization of a fragment of the host cell DNA in the HSV virion. HSV which had been passed a greater number of times on Ltk$^-$ cells was not used because this strain of HSV replicates poorly on Ltk$^-$ cells.

Studies on the kinetics of thermal inactivation of TK activity were carried out with cell extracts derived from (i) Ltk$^+$ cells (A9 cells), (ii) Ltk$^-$ cells lytically infected with HSV, and (iii) Ltk$^+$ HSV-transformed cells (Fig. 5). The TK activity of the Ltk$^+$ HSV-transformed cells was similar

TABLE 4. *Effects of thymidine, bromodeoxyuridine (BUDR), and iododeoxyuridine (IUDR) on colony formation of HSV-UV infected Ltk$^-$ cells*[a]

Ingredient added to medium[b]	Avg no. of colonies	SD[c]
No addition	2.6	1.1
Thymidine	12	5.4
BUDR (20 μg/ml)	No colonies formed	
IUDR (20 μg/ml)	No colonies formed	

[a] HSV-Ltk$^-$ p = 1 containing 3.1×10^9 particles/0.2 ml was irradiated with UV light for 6 min. This virus (0.2 ml) was inoculated into petri dishes containing 3×10^6 Ltk$^-$ cells. Other procedures are as described in Materials and Methods.

[b] Growth medium containing methotrexate (6.0×10^{-7} M), adenosine and guanosine (each at 5.0×10^{-5} M), and glycine (10^{-4} M).

[c] Standard deviation.

TABLE 5. *Rate of incorporation of tritiated thymidine into DNA and thymidine kinase activity of Ltk$^+$ HSV-transformed cell lines*

Cell lines	Incorporation of ^{3}H-thymidine into DNA[b] (counts per min per cell division per hr)	Thymidine kinase activity[c]
Ltk$^+$ (A9)	1.42	0.81
Ltk$^-$	0.003	0.03
Ltk$^+$ HSV-3[a]	2.4	0.46
Ltk$^+$ HSV-11	3.2	0.44
Ltk$^+$ HSV-20	2.7	0.85
Ltk$^+$ HSV-21	2.8	0.72
Ltk$^+$ HSV-22	2.0	0.63
Ltk$^+$ HSV-23	1.4	0.65
Ltk$^+$ HSV-28		0.60
Ltk$^+$ HSV-33	1.6	0.33
Ltk$^+$ HSV-39	5.7	0.59
Ltk$^+$ HSV-40	2.9	0.26

[a] Ltk$^+$ HSV-transformed cell lines were isolated from clones derived from Ltk$^-$ cells that had been infected with HSV-UV as described in Materials and Methods. The number after the HSV is an arbitrary clone designation.

[b] Monolayer cultures of the indicated cell lines were exposed to growth medium containing adenosine and guanosine (each at 5×10^{-5} M), glycine (10^{-4} M), and ^{3}H-thymidine (10^{-7} M), (specific activity of 10^4 μCi/μmole) for 1 hr. The rate of cell division was determined by counting replicate monolayer cultures 8 hr before and 21 hr after the cells were exposed to the tritiated thymidine. The experimental error was approximately 15%.

[c] Picomoles of thymidine monophosphate per μg of protein in the uncentrifuged cell sonically treated material per 20 min of incubation.

TABLE 6. *Ltk$^-$ to Ltk$^+$ transformation by using a thymidine kinase-deficient HSV*

Virus type	MOI[a]	Frequency of Ltk$^+$ HSV transformation[b]
HSV-HEL p = 3	18,000	8×10^{-4}
HSV(B2006)[c]-PRK p = 3	16,400	None
HSV-Ltk$^-$ p = 1	14,700	1.9×10^{-3}

[a] Multiplicity of infection (particles of HSV-UV added per cell). All virus preparations were irradiated for 9 min. The cell count per dish was 8.5×10^4 cells.

[b] This data was computed as follows: Ltk$^+$ HSV-transformed colonies per Ltk$^-$ cells per dish at time of infection.

[c] HSV(B2006) is an HSV mutant that does not induce thymidine kinase activity during lytic infection.

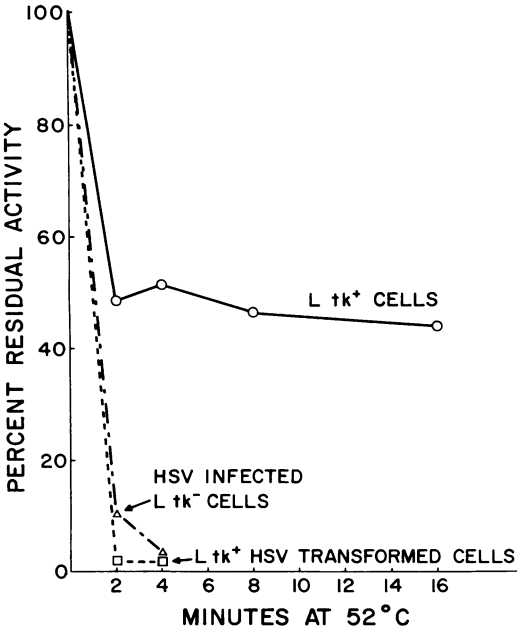

FIG. 5. *Extracts were prepared from Ltk+ cells, (A9 cells) HSV-infected Ltk- cells (8 hr postinfection), and Ltk+ HSV-20 clone of transformed cells. Before heating, these extracts were all diluted to a protein concentration of 2.2 mg/ml. The extracts were quickly brought to 52 C, and samples were removed and quickly cooled at the times indicated in the figure.*

(in respect to the kinetics of thermal inactivation) to the activity present in cells lytically infected with HSV and was much more thermolabile than the TK activity found in wild-type Ltk+ cells.

DISCUSSION

The Ltk- cells used in these experiments had no measurable reversion to Ltk+ cells (less than 10^{-8}). When Ltk- cells were infected with HSV-UV, a transformation frequency to the Ltk+ genotype of 10^{-5} to 10^{-3} was observed. The number of colonies formed was proportional to the relative virus dose used and depended also on the amount of UV received by the virus. Colony formation was neutralized by HSV antiserum, was thermolabile, and was not affected by deoxyribonuclease digestion. These data indicate that the appearance of Ltk+-transformed cells was caused by the infection of Ltk- cells with HSV-UV.

The failure of a TK-minus mutant of HSV (one that does not induce TK activity during lytic infection) to cause the transformation of Ltk- to Ltk+ cells provides genetic evidence to suggest that the new TK activity in the Ltk+ HSV-transformed cells is either derived from a structural gene in the HSV genome or a previously repressed product of the cells was derepressed by an HSV gene product. The kinetics of thermal denaturation of the TK activity of the Ltk+-transformed cells is similar to that of the TK activity induced by HSV infection and different from the wild-type TK enzyme activity. These observations suggest that the TK activity present in Ltk+ HSV-transformed cells is associated with a different protein than the TK activity of wild-type L cells.

Experiments now in progress indicate that the major TK activities of Ltk+ and Ltk+ HSV-transformed cells migrate at different rates in polyacrylamide gel electrophoresis. These results will be reported separately.

The clones that arose in TK plus selective medium differed from the parental Ltk- cells by the following criteria. (i) They did not grow in BUDR-containing media; (ii) they grew continuously in TK plus selective medium; (iii) they contained 7 to 24 times more TK activity than did Ltk- cells, and (iv) they incorporated 960 to 1,900 times more tritiated thymidine into cellular DNA than did Ltk- cells.

The addition of TK plus selective medium prior to 24 hr after HSV-UV infection strongly inhibits colony formation. The requirements for a period of incubation in growth medium after infection suggests that gene expression takes approximately 24 hr, i.e., there is a lag between infection and the occurrence of TK activity in colony-forming cells. In support of this, the inclusion of BUDR in the growth medium added to the cells after infection (and then replaced with TK plus selective medium 24 hr later) does not significantly inhibit colony formation. These results are compatible with the following hypothetical sequence of events resulting in the formation of Ltk+ transformed cells. (i) DNA synthesis is required for the "establishment" of the TK gene activity in the potentially Ltk+ HSV-transformed cells and (ii) expression of the TK activity in this cell.

The significant decrease in colony number when cell density is greater than 6.3×10^5 cells per dish may be due to contact inhibition of the newly transformed cell or the formation of diffusible inhibitory macromolecules (e.g., interferon). A similar process may be operative when HSV-UV infected cells are incubated in growth medium for more than 24 hr prior to the addition of the TK plus selective medium. In this circumstance, a dense monolayer may form due to cell growth during this period.

To our knowledge, this is the first instance in which a gene of a known function has been stably acquired by an eukariotic cell line due to virus infection. This may be a first step in establishing a system in which viruses are used as carrier

mechanisms to enable desired information to be transferred to a mammalian cell and to be established in an inheritable fashion.

In bacterial systems UV irradiation increases the frequency of transduction (7), presumably by increasing the probability of recombination. Casto (Bacteriol. Proc., p. 196, 1970) and Lytle et al. (16) have reported that the exposure of cultured cells to UV light increases the frequency of transformation by DNA-containing viruses. A similar effect may be operating in the Ltk^- cell to Ltk^+ cell transformation caused by HSV-UV.

Preliminary experiments have indicated that Ltk^+-transformed cell lines have increased resistance to UV irradiation as compared to the parental strain. This would be consistent with the possibility that HSV-UV can confer UV repair capability. It may therefore be possible to insert this information into cell lines lacking UV repair mechanisms, e.g., cell lines derived from patients with xeroderma pigmentosa.

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