JOURNAL OF VIROLOGY, Jan. 1974, p. 36-41
Copyright © 1974 American Society for Microbiology
Vol. 13, No. 1
Printed in U.S.A.

Analysis of Minimal Functions of Simian Virus 40

III. Evidence for "Host Cell Repair" of Oncogenicity and Infectivity of UV-Irradiated Simian Virus 40

N. H. SEEMAYER AND V. DEFENDI
The Wistar Institute, Philadelphia, Pennsylvania 19104

Received for publication 17 July 1973

The in vitro transforming capacity of simian virus 40 (SV40) for Syrian hamster cells is highly resistant to inactivation by UV light in comparison to infectivity. In the same cell system, we demonstrated a "host cell repair mechanism" sensitive to caffeine which is, to a large extent, responsible for the high resistance to UV inactivation of the transforming capacity of SV40. The survival of infectivity of UV-irradiated SV40 in CV-1 cells was also sensitive to caffeine, again indicating host cell repair. On the other hand, depression of normal cell DNA synthesis by hydroxyurea during the first 24 h postinfection only modestly reduced, and to a similar extent, the transforming capacity of UV-irradiated and nonirradiated SV40.

In a previous paper, we reported that the transforming capacity in vitro of simian virus 40 (SV40) is increased after weak UV irradiation (5,000 to 10,000 erg/mm^2) and that this capacity is highly resistant to strong UV irradiation (10,000 to 60,000 erg/mm^2) in comparison to a reduction of infectivity of 4 to 5 logs (25).

In this paper, we present evidence that caffeine (known to inhibit DNA host cell repair of UV-irradiated bacteria and mammalian cells [16, 19]) reduces the enhancement effect and the high resistance of transforming capacity of UV-irradiated "complete" and "defective" SV40. The survival of replication of UV-irradiated SV40 also is decreased in the presence of caffeine. We therefore propose that host cell repair plays a central role in transformation as well as in replication of UV-irradiated SV40.

MATERIALS AND METHODS

Virus. Preparation of the "complete" and "defective" SV40 pool (Rh 911 [9]) by the method of Uchida et al. (30) has been described previously (25).

The virus titer was estimated in CV-1 cells (13) and calculated by the method of Reed and Muench (20). The titer of the "complete" pool was $10^{8.4}$ mean tissue culture infective doses ($TCID_{50}$)/ml; that of the "defective" pool was $10^{6.0}$ $TCID_{50}$/ml. UV irradiation of SV40 has been previously described (25).

Cell cultures. Preparation and cultivation of primary Syrian hamster kidney cultures has been reported (26). CV-1 cells were grown in Eagle minimal essential medium supplemented with 8% fetal calf serum; maintenance medium contained 2% fetal calf serum.

Transformation assay. The transformation assay of Syrian hamster kidney cells has been described in detail (24). Briefly, logarithmically growing Syrian hamster kidney cells were infected with SV40 at a multiplicity of infection (MOI) of approximately 1,000 mean infective doses (ID_{50}) or 10 ID_{50} for the "complete" or "defective" SV40 pool, respectively. The transformed colonies were scored under the microscope at 16 to 18 days postinfection as described (24).

Chemicals. Caffeine (Eastman Kodak Co., Rochester, N.Y., 14650) was added to medium to a final concentration of 1, 2, and 5 mM. The medium containing caffeine was added to cell cultures after 1 h of virus adsorption at 37 C and maintained for 24 h. Thereafter the medium with and without caffeine was replaced with fresh medium containing no caffeine.

Various concentrations of caffeine were tested to determine the effect on cultured Syrian hamster kidney cells. We observed no obvious morphological alteration or decrease of growth capacity of the cells using concentrations of 1, 2, and 5 mM. At a concentration of 10 mM caffeine, however, the cells began to detach and growth was impaired. For this reason, 5 mM caffeine was determined to be the maximum tolerable concentration.

Hydroxyurea (HU) (A grade no. 400046, lot no. 803031, Calbiochem, San Diego, Calif.) was added to medium to a final concentration of 1 mM. After 1 h of virus adsorption at 37 C, HU medium was added to cell cultures for different periods of time as indicated under Results. Thereafter the medium was replaced with fresh medium without HU.

RESULTS

Effect of caffeine on transformation. The transforming capacity of UV-irradiated SV40 in logarithmically growing Syrian hamster kidney cells followed the pattern previously reported (25). An enhancement of transformation capacity occurred when the virus sample was irradiated with UV for 2 min, and transformation frequency remained high after stronger UV irradiation (Fig. 1). In the presence of 5 mM caffeine for 24 h after virus adsorption, different values for transformation frequency of UV-irradiated SV40 were obtained. Thus, in the presence of caffeine, transformation frequency was remarkably reduced even at the lowest tested dosage of UV irradiation (2,500 erg/mm^2) when there is essentially no reduction in transformation efficiency in the absence of caffeine. This reduction was evident in all virus samples irradiated and varied directly with the amount of UV irradiation.

Particularly striking was the fact that the relative enhancement observed with the 2-min UV SV40 is virtually abolished by caffeine, but the residual transformation frequency still reaches a higher value than for the 30-s or 1-min UV-irradiated samples (Fig. 1). Similar results were obtained for the "defective" virus. UV irradiation of "defective" SV40 for 8 min reduced transformation frequency by approximately 50% in the presence of caffeine (Fig. 2). Caffeine concentrations of 1 and 2 mM showed a comparable effect on transformation capacity of UV-irradiated SV40. In contrast, caffeine did not alter the transformation frequency of nonirradiated virus.

The relation between transformation frequency in the presence or absence of caffeine and infectivity of UV-inactivated SV40 is demonstrated in Fig. 3. It is apparent that, even in the presence of caffeine, transformation frequency is much more resistant than infectivity. These results indicate that there is a caffeine-sensitive mechanism in Syrian hamster kidney cells, presumably a host cell repair mechanism, which can modify the transforming capability of UV-irradiated SV40.

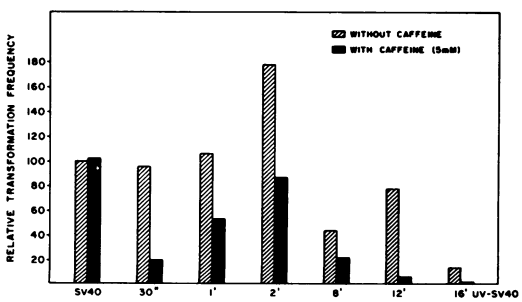

FIG. 1. *Relative transforming capacity of UV-irradiated and nonirradiated "complete" SV40, estimated in Syrian hamster kidney cells in vitro in presence of caffeine (5 mM) added immediately after virus adsorption for 24 h postinfection. (Infectious titer of untreated SV40, $10^{8.4}$ $TCID_{50}$/ml; MOI, 1,000 ID_{50}; transformation frequency, 100/10^4 cells, which in the graph is represented as 100%).*

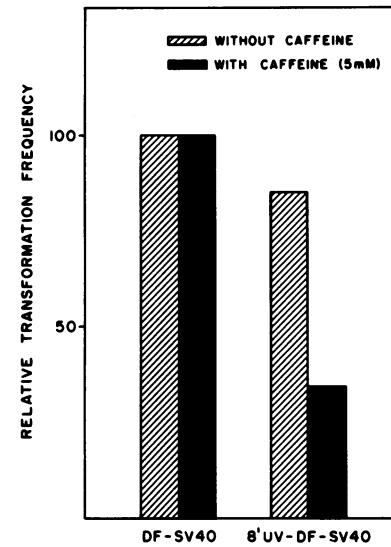

FIG. 2. *Relative transforming capacity of 8-min UV-irradiated and nonirradiated "defective" SV40, determined in Syrian hamster kidney cells in vitro in presence and absence of caffeine (5 mM) added immediately after virus adsorption for 24 h postinfection. (Infectious titer of nontreated "defective" SV40, $10^{6.0}$ $TCID_{50}$/ml; MOI, 10 ID_{50}; transformation frequency, 20/10^6 cells, which in the graph is represented as 100%.)*

Effect of caffeine on SV40 replication. Transformation requires only partial expression of the viral genome (10). For replication, however, all viral functions need to be expressed. Therefore, it was of interest to determine whether or not there is a caffeine-sensitive host function which affects the replication of UV-irradiated SV40. SV40 was UV irradiated for different periods of time and titrated in CV-1 cells in the presence and absence of caffeine (5 mM, for 24 h, postadsorption) (Fig. 4). The titer of nonirradiated SV40 was not influenced by this concentration of caffeine. Infectivity of SV40 after UV irradiation decreased at a rate similar to that previously described (25). But, in the presence of caffeine, infectivity of UV-irradiated SV40 decreased at a faster rate. The difference between survival curves in the presence and absence of caffeine increased as a function of UV irradiation time. Even in the

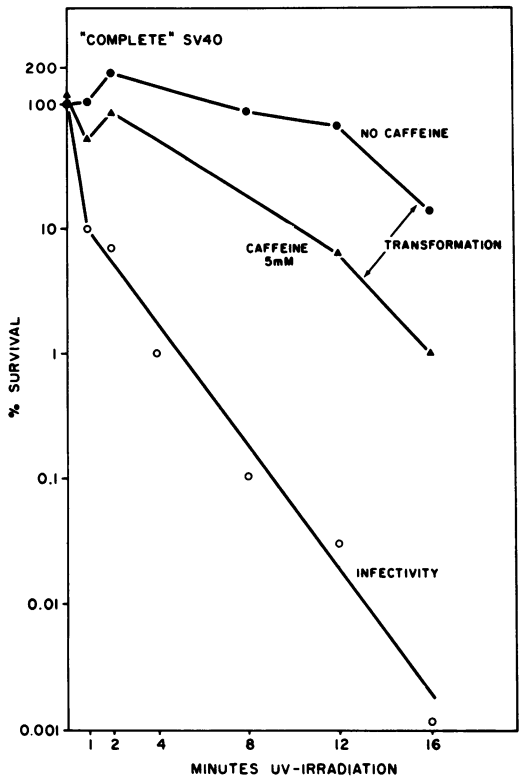

FIG. 3. *Survival of transforming capacity of UV-irradiated "complete" SV40, determined in Syrian hamster kidney cells in vitro in presence and absence of caffeine (5 mM, 24 h postinfection) and survival of infectivity in CV-1 cells.*

presence of caffeine, loss of infectivity showed a two-component character which probably reflects a "multiplicity reactivation" (32) of SV40 after stronger UV irradiation.

Effect of HU on transformation. In the experiments above it was shown that inhibition of host cell DNA repair reduced transformation capacity of UV-irradiated SV40. Therefore, we wanted to see if the transformation capacity of UV-irradiated SV40 was affected when normal cellular DNA synthesis is inhibited but host cell repair of DNA is maintained. These conditions are fulfilled by the use of HU (3). When logarithmically growing Syrian hamster kidney cells were exposed to a 1 mM concentration of HU for 18 or 24 h, DNA synthesis as estimated by autoradiography and incorporation of [^{3}H]thymidine into acid-insoluble material was reduced by more than 90% of the controls. After virus adsorption, HU was added to the medium at a final concentration of 1 mM for 5 or 24 h. The medium was then changed and transformation frequency was estimated as previously described (24). Inhibition of normal cellular DNA synthesis for 5 or 24 h after virus adsorption only slightly decreased the transformation efficiency, and to the same extent, for UV-irradiated and nonirradiated SV40 (Fig. 5). These results indicate that the early events in the transformation process proceed also under conditions of highly depressed normal DNA synthesis.

DISCUSSION

The major photobiochemical action of UV irradiation appears to be the formation of thymidine dimers in the DNA (12), which prevents its transcription and therefore its functions. The survival of different functions after UV irradiation is dependent upon the molecular weight of the nucleic acid, its strandedness, and possible host factors (17). We have reported a difference in the photosensitivity of SV40 functions to UV light: an enhancement and a high resistance of transformation capacity in vitro (25, 27) and of

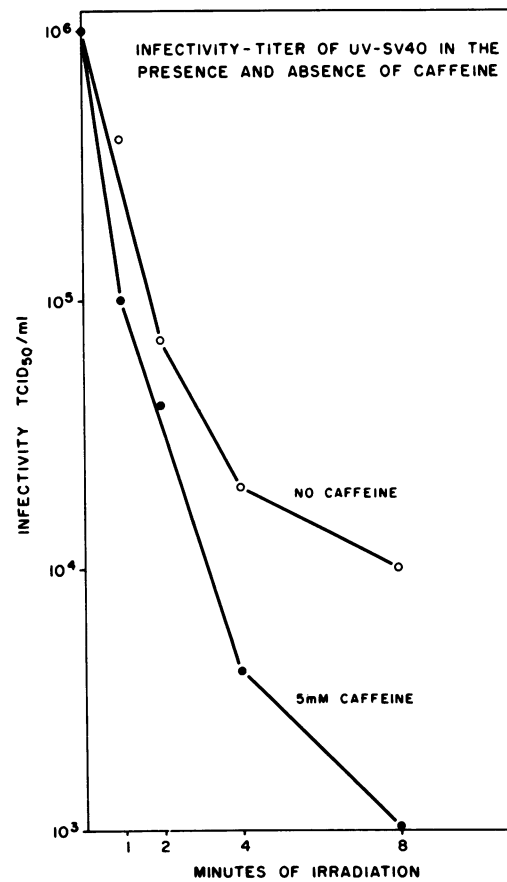

FIG. 4. *Survival of infectivity of UV-irradiated "defective" SV40 in CV-1 cells in presence and absence of caffeine (5 mM, 24 h postinfection).*

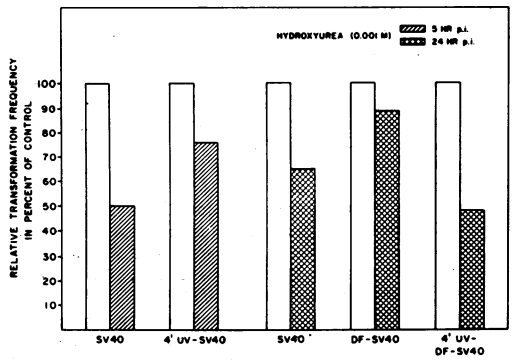

FIG. 5. *Relative transformation frequency of UV-irradiated and nonirradiated "complete" and "defective" SV40 in presence of hydroxyurea (1 mM, 5 and 24 h postinfection expressed as percent of control in the absence of the drug.*

oncogenicity in vivo (5, 27) after light and especially after strong UV irradiation up to 80,000 erg/mm^2. The high resistance of transformation capacity led to the assumption that a host cell repair mechanism might be involved in the phenomenon (27). Since earlier experiments gave no indication of photoreactivation involved in the transformation process (N. Seemayer, unpublished observations), the validity of the host cell repair mechanism assumption was tested in the present experiments by incorporating caffeine in the system. We used this compound since it is known to inhibit the host cell repair mechanism of UV-irradiated bacteria (16) and of UV-irradiated bacteriophages (23) leading to a reduction of survival. Also, the survival of mammalian cells, such as mouse L (19) and Chinese hamster cells (21) is strongly reduced in the presence of caffeine after UV irradiation but not after X irradiation (19). In preliminary experiments we found similar effects of caffeine on the survival of Syrian hamster kidney cells after UV irradiation. The effect of caffeine appears to be due to an inhibition of repair enzymes (22, 23, 31) or to a direct interaction of the drug with the DNA (6, 29). Our present results demonstrate that the survival of transforming capacity of UV-irradiated SV40 is reduced in the presence of caffeine (1 to 5 mM). The enhancement of transformation observed with lightly UV-irradiated SV40 (25) is also sensitive to the action of caffeine, but to a lesser extent than UV-irradiated virus samples showing no enhancement.

The reduction of transforming capacity in the presence of caffeine was more evident with SV40 samples which had received strong UV irradiation. For example, after 12 min of UV irradiation (60,000 ergs/mm^2), the transforming capacity was 70% of the unirradiated controls in the absence of caffeine, but in the presence of caffeine it was reduced to 6%. This effect of caffeine could be due to some general effect on cell physiology, such as inhibition of phosphodiesterases which would produce an increased level of intracellular cyclic adenosine 5' monophosphate (14). However, this is improbable because the transforming capacity of nonirradiated SV40 or of SV40 photodynamically inactivated in the presence of toluidine blue 0 (26) or by beta-propiolactone treatment (Seemayer and Defendi, manuscript in preparation) was not affected by a similar concentration of the drug. We can conclude, therefore, that host cell repair is an important step in the transformation process of UV-irradiated SV40.

Of particular interest is the finding that the survival of replication of UV-irradiated SV40 in CV-1 cells is also caffeine sensitive and that it is reduced in the presence of the drug. The change in slope after 1 $\log_{10}$ reduction of UV-irradiated SV40 disappeared in the presence of caffeine, resulting in a straighter line in a semilogarithmic scale. However, even in the presence of the drug, the second component of the inactivation curve, which probably represents "multiplicity reactivation" (32), still remained. Similar reduction of survival in the presence of caffeine has been reported for UV-irradiated bacteriophage T1 in *Escherichia coli* (23) and for UV-treated pseudorabies virus in chicken fibroblasts (33). Some evidence of host cell repair in the induction of SV40 T antigen and transformation has been reported by Aaronson and Lytle (1) on the basis of experiments with normal human fibroblasts and fibroblasts from patients with xeroderma pigmentosum infected with UV-irradiated SV40. The variability of survival curves of UV-irradiated herpes simplex virus in cells of different species indicates that mammalian cells may express different degrees of host cell repair (15, 18). Thus, the differences between the rates of inactivation of the capacity for infectivity and transformation of UV-irradiated SV40 which still persist in the presence of caffeine may reflect differences in the size of the viral genome utilized for replication and transformation; it could also reflect, at least in part, different degrees of host cell repair of irradiation damage in these two cell systems.

It is thus apparent that exact inactivation curves of UV-irradiated viruses and the correctness of the estimate of the gene size necessary for a certain function can only be determined in mammalian cells which are defective in repair mechanisms (as has been done in the phage-bacterial system [8, 23]).

HU inhibits replication of normal cell DNA, but allows unscheduled host cell repair DNA synthesis (2, 3). The transforming capacity of unirradiated and UV-irradiated "complete" and "defective" SV40 was reduced in the presence of HU at a similar rate. The partial reduction of transformation frequency is probably due to the fact that cells in DNA synthesis are lethally damaged, whereas other cells, blocked at the G1-S boundary of the cycle, become partially synchronized and survive the drug action (28). Our results indicate that a strong temporary depression of semiconservative DNA replication only moderately influences the in vitro transformation frequency of SV40 and UV-irradiated SV40, provided that DNA repair synthesis is permitted. The results complement the finding that SV40 DNA integration in host cell DNA can occur in the absence of cellular DNA synthesis (4, 11).

ACKNOWLEDGMENTS

This investigation was supported, in part, by an Eleanor Roosevelt International Cancer Fellowship of the American Cancer Society awarded by the International Union Against Cancer and a grant from the "Deutsche Forschungsgemeinschaft" to N.S., by no. VC-73N and no. ACS PRP-45 from the American Cancer Society Inc., and by Public Health Service research grant CA-10815 from the National Cancer Institute.

The skillful technical assistance of J. D. Weston and R. Walsh is gratefully acknowledged.

LITERATURE CITED

1. Aaronson, S. A., and C. D. Lytle. 1970. Decreased host cell reactivation of irradiated SV40 virus in xeroderma pigmentosum. Nature (London) **228**:359-361.
2. Brandt, W. N., W. G. Flamm, and N. J. Bernheim. 1972. The value of hydroxyurea in assessing repair synthesis of DNA in HeLa cells. Chem. Biol. Interact. **5**:327-339.
3. Cleaver, J. E. 1969. Repair replication of mammalian cell DNA: effects of compounds that inhibit DNA synthesis or dark repair. Radiat. Res. **37**:334-348.
4. Collins, C. J., and G. Sauer. 1972. Fate of infecting simian virus 40-deoxyribonucleic acid in nonpermissive cells: integration into host deoxyribonucleic acid. J. Virol. **10**:425-432.
5. Defendi, V., F. Jensen, and G. Sauer. 1967. Analysis of some viral functions related to neoplastic transformation. *In* J. S. Colter and W. Paranchych (ed.), Molecular biology of viruses, Academic Press Inc., New York.
6. Domon, M., B. Barton, A. Porte, and A. M. Rauth. 1970. The interaction of caffeine with ultraviolet-light irradiated DNA. Int. J. Radiat. Biol. **17**:359-399.
7. Fujiwara, Y., and T. Kondo. 1972. Caffeine-sensitive repair of ultraviolet light-damaged DNA of mouse L cells. Biochem. Biophys. Res. Commun. **47**:557-564.
8. Garen, A., and N. D. Zinder. 1955. Radiological evidence for partial genetic homology between bacteriophage and host bacteria. Virology **1**:347-376.
9. Girardi, A. J. 1965. Prevention of SV40 virus ocogenesis in hamsters. I. Tumor resistance induced by human cells transformed by SV40. Proc. Nat. Acad. Sci. U.S.A. **54**:445-451.
10. Green, M. 1970. Oncogenic viruses. Annu. Rev. Biochem. **39**:701-756.
11. Hirai, K., J. Lehman, and V. Defendi. 1971. Integration of simian virus 40 deoxyribonucleic acid into the deoxyribonucleic acid of primary infected Chinese hamster cells. J. Virol. **8**:708-715.
12. Howard-Flanders, P. 1968. DNA repair. Annu. Rev. Biochem. **37**:175.
13. Jensen, F., A. J. Girardi, V. Gilden, and H. Koprowski. 1964. Infection of human and simian tissue with Rous sarcoma virus. Proc. Nat. Acad. Sci. U.S.A. **52**:53-59.
14. Jost, J. P., and H. V. Rickenberg. 1971. Cyclic AMP. Annu. Rev. Biochem. **40**:741-774.
15. Lytle, C. D. 1971. Host-cell reactivation in mammalian cells. I. Survival of ultraviolet-irradiated herpes virus in different cell-lines. Int. J. Radiat. Biol. **19**:329-337.
16. Metzger, K. 1964. On the dark reactivation mechanism in ultraviolet irradiated bacteria. Biochem. Biophys. Res. Commun. **15**:101-109.
17. Proctor, W. R., J. S. Cook, and W. Tennant. 1972. Ultraviolet photobiology of Kilham rat virus and the absolute ultraviolet photosensitivities of other animal viruses: influence of DNA strandedness, molecular weight and host-cell repair. Virology **49**:368-378.
18. Rabson, A. S., S. A. Tyrell, and F. Y. Legallais. 1969. Growth of ultraviolet-damaged herpesvirus in xeroderma pigmentosum cells (34312). Proc. Soc. Exp. Biol. Med. **132**:802-806.
19. Rauth, A. M. 1967. Evidence for dark-reactivation of ultraviolet light damage in mouse L cells. Radiat. Res. **31**:121-138.
20. Reed, L. J., and H. Muench. 1938. A simple method of estimating fifty per cent endpoints. Amer. J. Hyg. **27**:493-497.
21. Rommelaere, J., and M. Errera. 1972. The effect of caffeine on the survival of UV-irradiated diploid and tetraploid Chinese-hamster cells. Int. J. Radiat. Biol. **22**:285-291.
22. Roulland-Dussoix, D. 1967. Degradation par la cellule hôte du DNA du bactériophage lambda irradié par le rayonnement ultraviolet. Mutat. Res. **4**:241-252.
23. Sauerbier, W. 1964. Inhibition of host cell reactivation in phage T1 by caffeine. Biochem. Biophys. Res. Commun. **14**:340-346.
24. Seemayer, N. 1968. DNA synthesis and T-antigen formation during oncogenic transformation of hamster kidney cells by SV40 in vitro. *In* J. L. Melnick (ed.), p. 180 First Int. Congr. Virol., Helsinki **7**:14-20.
25. Seemayer, N., and V. Defendi. Analysis of minimal functions of simian virus 40. II. Enhancement of oncogenic transformation in vitro by UV-irradiation. J. Virol. **12**:1265-1271.
26. Seemayer, N., K. Hirai, and V. Defendi. 1973. Analysis of minimal functions of simian virus 40. I. Oncogenic transformation of Syrian hamster kidney cells *in vitro* by photodynamically inactivated SV40. Int. J. Cancer **12**:524-531.
27. Seemayer, N., G. Seemayer, and R. Haas. 1969. Untersuchungen über die onkogene Transformation, Induktion der DNS-Synthese und T-Antigenbildung durch UV-bestrahltes simian Virus (SV-) 40. Z. Med. Mikrobiol. Immunol. **155**:123-132.
28. Sinclair, W. K. 1965. Hydroxyurea: differential lethal effects on cultured mammalian cells during the cell cycle. Science **150**:1729-1731.
29. Tso, P., and P. Lu. 1964. Interaction of nucleic acids. I. Physical binding of thymine, adenine, steroids and aromate hydrocarbons to nucleic acids. Proc. Nat. Acad. Sci. U.S.A. **51**:17.
30. Uchida, S., S. Watanabe, and M. Kato. 1966. Incomplete growth of simian virus 40 in African green monkey

kidney cultures induced by serial undiluted passages. Virology **27**:135-141.
31. Wragg, J. B., J. V. Carr, and V. C. Ross. 1967. Inhibition of DNA polymerase activity by caffeine in a mammalian cell line. J. Cell Biol. **35**:146A-147A.
32. Yamamoto, H., and H. Shimojo. 1971. Multiplicity reactivation of human adenovirus type 12 and simian virus 40 irradiated by ultraviolet light. Virology **45**:529-531.
33. Zavadova, Z., and J. Zavada. 1968. Host-cell repair of ultraviolet-irradiated pseudorabies virus in chick embryo cells. Acta Virol. **12**:507-514.