Proc. Natl. Acad. Sci. USA
Vol. 78, No. 7, pp. 4480–4484, July 1981
Genetics

Indirect induction of mutagenesis of intact parvovirus H-1 in mammalian cells treated with UV light or with UV-irradiated H-1 or simian virus 40

(error-prone processes/thermosensitive virus mutants/oncogenesis/cycloheximide)

JAN J. CORNELIS*, ZAO ZHONG SU*, DAVID C. WARD†, AND JEAN ROMMELAERE*†‡

*Département de Biologie Moléculaire, Université libre de Bruxelles, 1640 Rhode St Genèse, Belgium; and †Department of Human Genetics, Molecular Biophysics and Biochemistry, Yale University School of Medicine, New Haven, Connecticut 06510

Communicated by Richard B. Setlow, April 6, 1981

ABSTRACT **The frequency of mutants among the descendants of intact parvovirus H-1 was increased when cells of human and rat origin were exposed to UV light prior to infection. An enhanced mutagenesis of intact H-1 could be induced to almost the same extent in unirradiated cells by preinfecting them with H-1 or simian virus 40 that had been irradiated with UV light. The expression of an enhanced mutagenesis of unirradiated H-1 after cell treatment with UV light or UV-irradiated virus was delayed, transient, and dose-dependent, and it was dependent on *de novo* protein synthesis.**

Increased yields of mutants are produced among the descendants of unirradiated herpes simplex virus or simian virus 40 (SV40) in UV-irradiated monkey kidney cells (1–3). The level of mutagenesis in the unirradiated viruses was maximal when a delay was allowed between cell irradiation and virus infection (1, 4). The data suggest that, after UV irradiation of the cells, a mutator activity is induced, modified, or available at a higher level or for a longer time. In this study, the autonomous parvovirus H-1 was used to probe mutator activity in human and rat cells. Autonomous parvoviruses contain single-stranded DNA genomes, multiply only in proliferating cells, and depend on their host for replication of their DNA (5).

An intriguing question is the nature of the signal activating the enhancement of mutagenesis. UV irradiation of cells might inactivate directly a specific cell target(s). Alternatively, UV-induced lesions in nonspecific targets might generate, or interact with, a diffusible intermediate. Consistent with the latter possibility, a mutator activity was shown to operate on UV-irradiated phage in undamaged *Escherichia coli* recipients after the transfer by conjugation of F-lac episomes from UV-irradiated donors (6). Therefore, it was of interest to determine whether mutations in an unirradiated parvovirus could be produced indirectly by providing undamaged cells with a second virus UV-irradiated extracellularly.

MATERIALS AND METHODS

Cell Lines and Virus Stocks. SV40-transformed newborn human kidney cells (NB-E) (7) and Harvey murine sarcoma virus-transformed rat liver cells (RL5E) (8) were cultivated in Eagle's minimal essential medium supplemented with 5% heat-inactivated fetal calf serum. Wild-type (wt) H-1 virus and a thermosensitive (ts) H-1 mutant termed ts6 (a generous gift of S. L. Rhode) were propagated in NB-E or RL5E cells at 37°C and 33°C, respectively. Virus stocks were purified according to Tattersall *et al.* (9) and diluted in phosphate-buffered saline containing Ca^{2+} and Mg^{2+}. Spontaneous revertant frequencies of the ts6 mutant stocks were about 1×10^{-5}. SV40 (wt strain 776) was propagated in BSC-1 monkey kidney cells as described (3).

UV Irradiation. Irradiation was carried out with a germicidal lamp with maximal output at 254 nm. Calibration was performed with a Latarjet dosimeter. Virus was irradiated in a liquid layer (1 mm depth) of phosphate-buffered saline (for H-1) or of culture medium (SV40). Cell monolayers were exposed to UV light after removal of the medium.

Virus Infection. Cell monolayers (10^6 cells per 60-mm Petri dish) were rinsed with phosphate-buffered saline and infected for 1 hr at 37°C with 0.4 ml of virus inoculum in phosphate-buffered saline (H-1) or minimal essential medium (SV40). The inoculum was removed, and the cells were rinsed with phosphate-buffered saline.

Plaque Assay. Plaque assay was as described by Rhode (10). Cultures were stained with neutral red for plaque visualization 6–8 days after infection. Between 10 and 70 plaques were counted per dish. Sufficient dishes were incubated in order to limit the error of the plaque counts to less than 15%.

Virus Mutation Assays. Mutagenesis was studied by determining the reversion frequencies of H-1 ts6 to a wt phenotype at the nonpermissive temperature (39.5°C). At the restrictive temperature, H-1 ts6 is like ts1 (10) in that it is deficient in a late function: the processing of mature single-stranded DNA genomes from replicative intermediates (S. L. Rhode, personal communication).

Two mutation assays were used: a single-cycle and a direct-plating procedure. Asynchronous NB-E and RL5E cells (5–10 $\times 10^5$ cells per 60-mm plate) were treated with UV light or UV-irradiated virus and incubated for various times at 37°C. Cells were then infected with unirradiated H-1 ts6.

For single-cycle assay, the dishes of infected cells were placed at 33°C for 30 hr. Receptor-destroying enzyme (11), a preparation kindly provided by P. Tattersall, was added to the cultures (1% vol/vol) 20 hr after infection in order to prevent a second virus cycle. Cells and media were frozen and thawed (9), the supernatants were clarified by low-speed centrifugation, and the virus was titered at 39.5°C and 33°C, using NB-E cells as indicators.

The direct-plating assay was either a direct-plaque assay or an infectious-center assay. For the direct-plaque assay, infected NB cells were covered with overlay medium immediately after infection. For the infectious-center assay, primarily infected

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U. S. C. §1734 solely to indicate this fact.

Abbreviations: SV40, simian virus 40; wt, wild-type; ts, temperature-sensitive; MOI, multiplicity of infection; PFU, plaque-forming unit.

‡To whom reprint requests should be addressed at Dept. Molecular Biology, Université libre de Bruxelles, 1640 Rhode St Genèse, Belgium.

Table 1. Plaque formation of virus in UV-preirradiated cells at 33°C and 39.5°C

	Virus	Assay	Cells	Relative titers in UV-treated cells* 33°C	Relative titers in UV-treated cells* 39.5°C	Enhanced mutagenesis
1	H-1 ts6	Direct-plaque	NB-E	0.95	2.3	2.4 ± 0.4
2	H-1 ts6	Infectious-center	NB-E	1.0	2.4	2.3 ± 0.3
3	H-1 ts6	Infectious-center	RL5E	0.95	2.0	2.1 ± 0.2
4	H-1 ts6	Single-cycle	RL5E	1.0	1.9	1.9 ± 0.3
5	Revertant 3026	Direct-plaque	NB-E	1.1	1.0	0.9 ± 0.2
6	Revertant 3026	Infectious-center	RL5E	1.0	0.9	0.9 ± 0.2
7	H-1 wt	Direct-plaque	NB-E	1.05	1.0	0.9 ± 0.3
8	H-1 wt	Infectious-center	RL5E	1.0	1.0	1.0 ± 0.2

Cells were irradiated with UV light (0 or 4.5 J m^{-2}), incubated for 14 hr in medium at 37°C, and infected with virus. Cells were infected with H-1 ts6 at MOIs of 5, 10, and 0.7 for direct-plaque assay (39.5°C), infectious-center assay (39.5°C), and single-cycle assay (33°C), respectively. For the determination of plaque formation of H-1 ts6 at 33°C and for infection with revertant 3026 or H-1 wt at 33°C and 39.5°C, the MOIs were 10^{-5} to 10^{-4} for direct-plaque assay and 10^{-3} to 10^{-2} for infectious-center assay.

* For direct-plating assays, relative titers refer to plaque formation on treated versus control cells inoculated with the same dilutions of the virus stock (≈10^9 PFU/ml). For the single-cycle assay, relative titers refer to virus production by treated versus control cells, as measured by secondary titrations (≈2×10^4 PFU/ml). Data are from two or three experiments.

NB-E or RL5E cells were incubated for 4 hr in minimal essential medium/5% fetal calf serum (39.5°C and 33°C), harvested by trypsin treatment, transferred at appropriate dilutions to preseeded monolayers of untreated NB-E indicator cells (10^6 cells per dish), further incubated for 4 hr (39.5°C and 33°C) and covered with overlay medium. Overlaid dishes were incubated at 39.5°C and 33°C until staining. A maximum of 2×10^5 cells were transferred to an indicator dish in the infectious center assays.

One-Step Growth Curve. Asynchronous rat cells (10^5 cells per 60-mm dish) were irradiated with UV light and incubated for 14 hr at 37°C in minimal essential medium/5% fetal calf serum prior to infection with unirradiated H-1 wt [multiplicity of infection (MOI) = 10^{-2}]. After infection, cultures were incubated at 33°C or 39.5°C. Receptor-destroying enzyme was added to the culture 20 hr after infection. Cultures were harvested with their medium at various times after infection. At the conclusion of the experiments virus was released from the cells by freezing and thawing (9) and titered on NB-E cells at 37°C.

Definitions. The MOI refers to the number of infectious (plaque-forming) virus particles (PFU) per cell for an equivalent inoculum of unirradiated virus titered by direct-plaque assay on untreated NB-E cells. The mutation frequency is defined as the fraction of infectious particles (33°C) that form plaques at the nonpermissive temperature (39.5°C). Enhanced mutagenesis is expressed by the ratio of the mutation frequency in treated cells to that in control cells. Independent measurements of enhanced mutagenesis were used as variables to calculate average values and standard deviations.

RESULTS

Enhanced Mutagenesis in UV-Preirradiated Cells. As shown in Table 1 (lines 1–4), the relative titer of intact H-1 ts6—i.e., the ratio of the virus titer in UV-preirradiated cells to that in control cells, was about 2 at 39.5°C. UV irradiation of the cells induced little or no effect on their capacity to produce virus forming plaques at 33°C. Therefore, higher virus titers in UV-preirradiated cells at nonpermissive temperature are indicative of a 2-fold induction of viral enhanced mutagenesis in human and rat cells. This interpretation is supported by the following lines of evidence:

(*i*) As shown in Table 1 (lines 1–6, 33°C column), no significant effect of UV irradiation of the cells on their capacity to replicate intact virus was found either for H-1 ts6 or for a revertant picked at 39.5°C. Therefore, a preferential growth of revertants preexisting in the stock of H-1 ts6 does not account for a higher frequency of revertants in UV-irradiated cells.

(*ii*) Possible pitfalls of the direct-plating procedures for mutagenesis are: (*a*) the incubation of treated cells at 39.5°C, and (*b*) the higher MOI used at 39.5°C owing to the low reversion frequency. If the high temperature or MOI resulted in a greater enhancement of the capacity of treated cells to support virus growth at 39.5°C than at 33°C, a higher titer increase at restrictive temperature would arise independently of any mutagenesis. Data shown in Table 1 do not, however, support this possibility: (*a*) the effect of UV irradiation of the cells on their capacity to replicate H-1 wt or revertant H-1 was the same at 33°C as at 39.5°C (lines 5–8); (*b*) the same magnitude of enhanced mutagenesis of H-1 ts6 was found with the direct-plating as with the single-cycle procedures (lines 1–4).

(*iii*) Similar levels of enhanced mutagenesis of H-1 ts6 were obtained with the direct plaque assay and the infectious-center assay (Table 1, lines 1 and 2). This fact demonstrates that primarily infected cells rather than indicator cells are involved in enhanced mutagenesis.

(*iv*) Data shown in Table 2 indicate that virus generated in UV-irradiated cells and forming plaques at 39.5°C display a wt phenotype. Therefore plaques scored at nonpermissive temperature cannot be ascribed to the leakiness of the ts6 mutation.

Enhanced mutagenesis might be due to an increased number of generations during the first virus cycle in UV-treated cul-

Table 2. Virus growth in control cells at 33°C and 39.5°C

Virus	Ratio of titers (33°C/39.5°C)
Revertant 3026	1.1
Revertant 3031	2.1
H-1 wt	1.6
H-1 ts6	1.7×10^5

Virus isolates were obtained by picking well-separated plaques grown at 39.5°C (revertants), at 37°C (H-1 wt), or at 33°C (H-1 ts6). Virus stocks were obtained by multiplication of the isolates in NB-E cells at 33°C. The revertants were derived from unirradiated ts6 grown in NB-E cells that had been irradiated with 3.0 J m^{-2} of UV light 12 hr prior to infection. Five other revertants, not included in the table, gave ratios of titers between the values for the revertants shown. The virus stocks were titrated in unirradiated NB-E cells at 33°C and 39.5°C. The results are the average of two titrations.

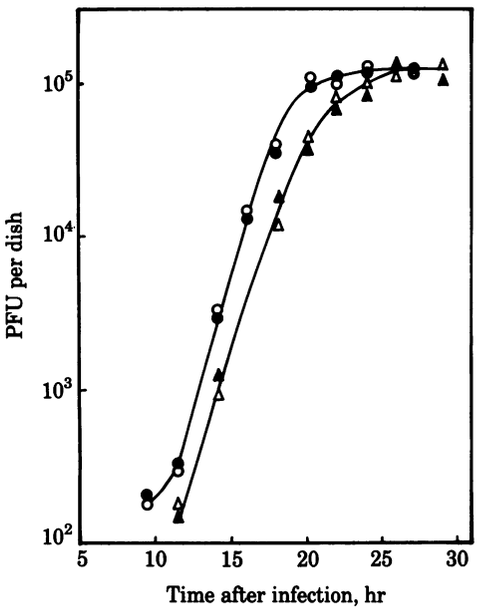

FIG. 1. One-step growth for H-1 wt in UV-pretreated rat cells at 39.5°C (○, ●) and 33°C (△, ▲). ○ and △, Control cells; ● and ▲, UV-irradiated cells.

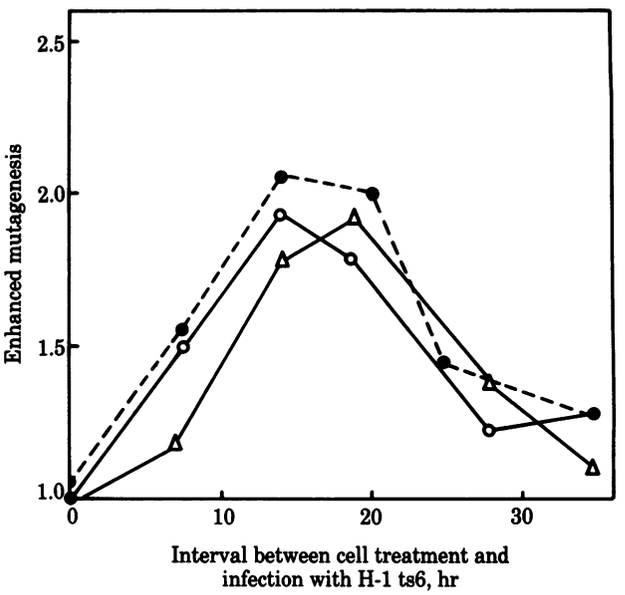

FIG. 2. Time course of enhanced mutagenesis. RL5E cells were irradiated with UV light (4.5 J m^{-2}) or preinfected with UV-damaged SV40 (1500 J m^{-2}; MOI = 10) and incubated at 37°C. At distinct times after treatment, the dishes were inoculated with H-1 ts6 and processed for infectious-center or single-cycle assays. For infectious-center assay the MOI of H-1 ts6 was 10 at 39.5°C and 10^{-2} at 33°C. For single-cycle assay, the MOI was 0.7. Average values from two experiments are presented; SD was less than 20%. Cell UV irradiation: ●, infectious center assay; ○, single-cycle assay. Cell pretreatment with SV40: △, single-cycle assay.

tures. However, Fig. 1 shows that production of H-1 wt in UV-irradiated and control cells was identical, at both 33°C and 39.5°C. Therefore, irradiated and control cells are unlikely to differ with respect to the number of viral DNA replication rounds, suggesting that an enhanced mutation frequency in pretreated cells can be ascribed to a higher mutation rate. The results thus suggest that UV-irradiated cells display mutator activities acting on intact templates.

Enhanced Mutagenesis in Cells Pretreated with Extracellularly UV-Irradiated Virus. As shown in Table 3 (lines 1–4), the mutation enhancement in unirradiated H-1 virus could also be obtained indirectly—i.e., by preinfecting unirradiated cells with UV-irradiated virus. The "signaling" virus in these indirect induction experiments was either UV-inactivated H-1 ts6 or UV-irradiated SV40.

Enhanced mutagenesis induced by UV-treated SV40 was assayed in rat cells, which are nonpermissive for this virus. SV40 had to be irradiated in order to show its inducing capacity (Table 3, lines 4 and 5). UV-irradiated SV40 is therefore likely to act by activating mutagenic functions that are not normally elicited by undamaged SV40.

Irradiated H-1 ts6 was exposed to a UV dose giving a surviving fraction lower than 10^{-8} as extrapolated from the survival curve (data not shown). Under the conditions used, UV-irradiated H-1 ts6 alone did not form plaques at 39.5°C or at 33°C. Given the spontaneous revertant frequency of the mutant stock of 1×10^{-5}, rescue of irradiated viruses in cells coinfected with intact H-1 ts6 is very unlikely to account for the increased frequency of revertants.

Similarly to direct UV irradiation, cell pretreatment with UV-irradiated virus (*i*) triggered a level of enhanced mutagenesis of about 2, whether measured with the direct-plaque assay or the infectious-center assay (Table 3, lines 1 and 2), but (*ii*) did not affect the average size of the burst produced by intact H-1 ts6 (data not shown). The slight decrease in virus titers in pretreated cells (Table 3, 33°C column) might result from a low level of interference of signaling viruses with the multiplication of intact H-1 ts6.

Expression of Enhanced Mutagenesis. Time courses of enhanced mutagenesis triggered by cell UV irradiation or preinfection with UV-irradiated SV40 indicate that the expression of

Table 3. Effect of cell pretreatment with UV-irradiated virus on plaque formation by intact H-1 ts6 at 33°C and 39.5°C

	Inducing treatment	Assay	Cells	Relative titers in pretreated cells* 33°C	Relative titers in pretreated cells* 39.5°C	Enhanced mutagenesis
1	UV-H-1 ts6	Direct-plaque	NB-E	0.9	1.7	1.9 ± 0.2
2	UV-H-1 ts6	Infectious-center	NB-E	0.8	1.7	2.1 ± 0.3
3	UV-H-1 ts6	Infectious-center	RL5E	0.95	1.9	2.0 ± 0.4
4	UV-SV40	Infectious-center	RL5E	0.9	1.7	1.9 ± 0.3
5	SV40	Infectious-center	RL5E	0.85	0.8	0.9 ± 0.1

Cells were infected with UV-damaged viruses (UV-H-1 ts6, UV-SV40, or intact SV40) at a MOI of 10 and incubated for 14 hr at 37°C in medium prior to infection with intact H-1 ts6 (MOIs as given in the legend of Table 1). Dose to UV-H-1 ts6 was 200 J m^{-2}. SV40 was irradiated with a dose of 1500 J m^{-2}, which reduced infectivity to 8.5% in a direct-plaque assay on BSC-1 cells (3).

* Titers are shown relative to untreated cells incubated at the same temperature. Data are from two or three experiments.

Table 4. Effect of cycloheximide on enhanced mutagenesis of unirradiated H-1 ts6

Treatment	Cells	Mutation frequency*			Enhanced mutagenesis	
		CH	UV	UV and CH	Without CH	With CH
UV	NB-E	0.8	1.9	0.7	1.9	0.9
UV	RL5E	0.9	2.0	1.1	2.0	1.2
UV-SV40	RL5E	0.9	1.8	1.1	1.8	1.1

Cells were UV irradiated (0 or 4.5 J m^{-2}) or preinfected with UV-irradiated SV40 (1500 J m^{-2}; MOI = 10), incubated for 14 hr in the absence or presence of cycloheximide (CH, 7.5 μg/ml), infected with intact H-1 ts6 (MOI = 10 at 39.5°C; MOI = 10^{-2} at 33°C), and processed for infectious center assays.

* Mutation frequencies are given relative to those from unirradiated controls without cycloheximide. The inhibition by cycloheximide of plaque formation on unirradiated cells was insignificant at 33°C and 14% at 39.5°C. Data are from two experiments (SD less than 25%).

the phenomenon is transient and is maximal after a 12- to 15-hr delay between cell treatment and infection with intact H-1 ts6 (Fig. 2). The time available for expression will actually be longer because the parvovirus will not initiate its replication until cells enter into S phase (12).

The data presented in Table 4 show that expression of enhanced mutagenesis requires *de novo* protein synthesis after cell treatment. Conditions of exposure to cycloheximide were selected that inhibited protein synthesis more than 85% but had no or little effect on plaque formation on unirradiated cells. The magnitude of the enhanced mutagenesis effect was about 2-fold in the absence of the inhibitor but was almost abolished in its presence.

Dose Effect Curve for Enhanced Mutagenesis. Enhanced mutagenesis of intact H-1 ts6 was measured at increasing UV doses given either to the cells or to signaling SV40. As shown in Fig. 3, a broad plateau was attained after both treatments, indicating a saturation of the induction process. Treatment with

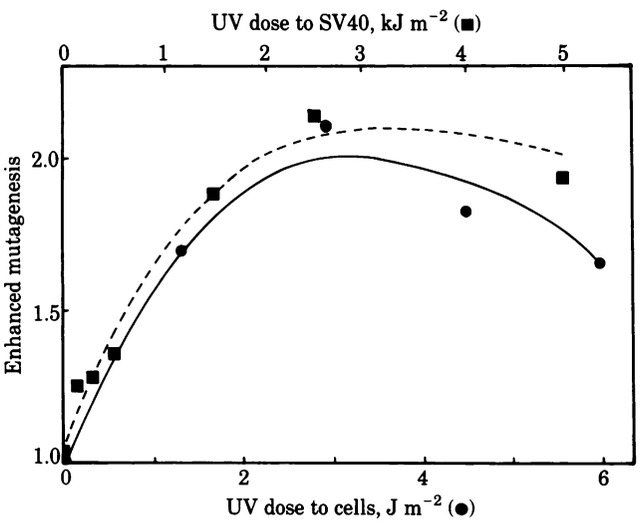

FIG. 3. Dose response of enhanced mutagenesis. Cells were exposed to increasing doses of UV light or preinfected with SV40 (MOI = 10) irradiated with different UV doses. Treated cells were incubated for 12 hr at 37°C prior to infection with H-1 ts6. For direct-plaque assay (NB-E cells) the MOI was 5 at 39.5°C and 10^{-3} at 33°C. For infectious-center assay (RL5E cells) MOIs were 12.5 and 10^{-2} at 39.5°C and 33°C, respectively. Average values from three (UV) or two (UV-irradiated SV40) experiments are shown; SD was less than 25%. ●, UV-irradiated NB-E cells; ■, RL5E cells preinfected with UV-irradiated SV40.

UV doses up to 5 J m^{-2} or with UV-irradiated SV40 did not detectably affect cell survival as measured by colony-forming ability (data not shown).

DISCUSSION

UV irradiation of human and rat cells leads to a 2-fold increase in the mutation frequency of an intact parvovirus. A similar phenomenon has been reported for other viruses propagated in UV-irradiated monkey cells (1–4). In addition, this paper shows that mutagenesis of undamaged H-1 is also enhanced in intact cells preinfected with UV-irradiated H-1 or SV40 virus. Although SV40 has been reported to be mutagenic for nonpermissive cells (13), unirradiated SV40 did not trigger any detectable enhanced mutagenesis of intact H-1 virus. Because much higher input multiplicities were used for the generation of cell mutations, the mutagenic process involved may be basically different.

Assuming that the same process is responsible for the increased frequency of mutations occurring in cells treated with UV light or with UV-irradiated signaling virus, these data indicate that enhanced mutagenesis does not require the direct hitting of specific cell targets. Indeed, the results indicate that UV-generated lesions are important *per se* and that a diffusible effector, whose relationship with the lesions is unknown, mediates enhanced mutagenesis. Our data suggest that UV lesions might modify the specificity, increase the availability, or induce the expression of a mutagenic function(s). It cannot be excluded, however, that UV light acts in a more direct way. For instance, lesions in cell or viral DNA might promote its recombination with undamaged H-1 DNA. Such recombinations might generate mutations in intact H-1, although the similar signaling efficiencies of homologous H-1 and heterologous SV40 viruses do not favor this possibility.

Although mutation studies with UV-irradiated viruses in mammalian cells are scarce, all data agree on the point that UV-damaged viruses are mutagenized at high rates in unirradiated hosts (2–4). This might be due to the presence in mammalian cells of a constitutive error-prone process acting on the site of the lesions. Alternatively, the data reported in this paper raise the possibility that UV-damaged virus itself might activate a mutagenic pathway operating on intact and possibly also on damaged parts of the viral genome.

The UV dose of 4.5 J m^{-2} given to the cells introduced about 5×10^5 pyrimidine dimers per cell (14), whereas the UV doses administered to the signaling H-1 and SV40 produced about 10 (15) and 80 (16) dimers per viral genome, respectively. The particle-to-infectivity ratios are 100 for SV40 (17) and 1000 for H-1 (S. L. Rhode, personal communication). Knowing the MOIs, it can be estimated that signal virus introduced about 10^5 dimers per cell. Therefore, the numbers of lesions present in cells after both the direct and the indirect UV treatments were of the same order of magnitude. It thus appears that a similar level of enhanced mutagenesis could be achieved irrespective of whether the lesions were induced intracellularly or administered after exogeneous induction.

In this study, experimental conditions that induced enhanced mutagenesis of unirradiated virus did not significantly affect the virus titer at the permissive temperature. In other circumstances, however, UV light has been shown to enhance cell capacity to support replication of an intact parvovirus (18). The possibility is thus open that a mutagenic function(s) activated in treated cells participate(s) in processes regulated coordinately with cell capacity. Enhanced mutagenesis of irradiated virus is accompanied by enhancement of virus survival (enhanced reac-

tivation) (1, 19), and may therefore be analogous to the SOS mutator function in *E. coli* (20, 21). However, this enhanced mutagenesis is sometimes undetectable under conditions producing reactivation (2, 3). The relationship between survival and mutagenic processes is thus not obvious and deserves further analysis. Similarly, it should be considered that the activation of an error-prone process acting on undamaged DNA may also be responsible for a significant fraction of the mutations produced in the genes of cells exposed to low doses of UV light.

We thank Helen McGuire for technical assistance and Prof. M. Errera for his interest and support. We acknowledge Dr. S. L. Rhode III for his gift of the H-1 mutant and for many helpful suggestions and Dr. P. Tattersall for critical reading of the manuscript. The work was supported by an agreement between the Belgian Government and the Université libre de Bruxelles, Actions de Recherche Concertée, by the European Communities (contracts 156-76-1 BIOB and ENV/355/B), the Ministère de la Santé Publique (contract 0392/1980), and the Fonds National de la Recherche Médicale (contract 3.4514.80). J.R. is Chercheur Qualifié du Fonds National Belge de la Recherche Scientifique.

1. DasGupta, U. & Summers, W. C. (1978) *Proc. Natl. Acad. Sci. USA* **75**, 2378–2381.
2. Lytle, C. D., Goddard, J. G. & Lin, C. H. (1980) *Mutat. Res.* **70**, 139–149.
3. Cornelis, J. J., Lupker, J. H. & van der Eb, A. J. (1980) *Mutat. Res.* **71**, 139–146.
4. Cornelis, J. J., Lupker, J. H., Klein, B. & van der Eb, A. J., *Mutat. Res.*, in press.
5. Tattersall, P. & Ward, D. C. (1978) in *Replication of Mammalian Parvoviruses*, eds. Ward, D. C. & Tattersall, P. (Cold Spring Harbor Laboratory, Cold Spring Harbor, NY), pp. 3–12.
6. George, J., Devoret, R. & Radman, M. (1974) *Proc. Natl. Acad. Sci. USA* **71**, 144–147.
7. Ledinko, N., Hopkins, S. & Toolan, H. (1969) *J. Gen. Virol.* **5**, 19–31.
8. Bomford, R. & Weinstein, I. B. (1972) *J. Natl. Cancer Inst.* **49**, 379–386.
9. Tattersall, P., Cawte, P. J., Shatkin, A. J. & Ward, D. C. (1976) *J. Virol.* **20**, 273–289.
10. Rhode, S. L. (1976) *J. Virol.* **17**, 659–667.
11. House, W. (1967) *Lab. Pract.* **16**, 321.
12. Wolters, S., Richards, R. & Armentrout, R. W. (1980) *Biochim. Biophys. Acta* **607**, 421–431.
13. Theile, M., Strauss, M., Luebbe, S., Scherneck, S., Krause, H. & Geissler, E. (1980) *Cold Spring Harbor Symp. Quant. Biol.* **44**, 377–382.
14. Rothman, R. H. & Setlow, R. B. (1979) *Photochem. Photobiol.* **29**, 57–61.
15. Proctor, W. R., Cook, J. S. & Tennant, R. W. (1972) *Virology* **49**, 368–378.
16. Williams, J. I. & Cleaver, J. E. (1976) *Radiat. Res.* **67**, 619 (abstr.).
17. Uchida, S., Yoshiike, K., Watanabe, S. & Furono, A. (1968) *Virology* **34**, 1–8.
18. Rommelaere, J., Vos, J. M., Cornelis, J. J. & Ward, D. C. (1981) *Photochem. Photobiol.*, in press.
19. Sarasin, A. & Benoit, A. (1980) *Mutat. Res.* **70**, 71–81.
20. Radman, M. (1975) in *Molecular Mechanisms for Repair of DNA*, eds. Hanawalt, P. C. & Setlow, R. B. (Plenum, New York), pp. 355–367.
21. Bridges, B. A. (1980) in *Progress in Environmental Mutagenesis*, ed. Alacevic, M. (Elsevier, Amsterdam), Vol. 7, pp. 131–140.