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Direct and indirect effects of ultraviolet light on the mutagenesis of parvovirus H-1 in human cells

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U.v. radiation is directly mutagenic for the single-stranded DNA parvovirus H-1 propagated in human cells. Mutation induction in the progeny of u.v.-irradiated virus increased linearly with the dose and could be ascribed neither to an increased number of rounds of viral replication nor to the indirect activation of an inducible cellular mutator activity by the u.v.-damaged virus. The level of mutagenesis among the descendants of both unirradiated and u.v.-damaged H-1 was enhanced if the host cells had been exposed to sublethal doses of u.v. light before infection. This indirect enhancement of viral mutagenesis in pre-irradiated cells was maximal at multiplicities lower than 0.2 infectious particles/cell. The frequency of mutations resulting from cell pre-irradiation was only slightly higher for u.v.-irradiated than for intact virus. Thus, the induced cellular mutator appeared to be mostly untargeted in the dose range given to the virus. U.v.-irradiation of the cells also enhanced the mutagenesis of u.v.-irradiated herpes simplex virus, a double-stranded DNA virus (Lytle and Knott, 1982).

Key words: human cells/induced processes/mutagenesis/parvovirus/u.v. light

Introduction

The spontaneous mutation frequencies of single-stranded or double-stranded DNA viruses that replicate in the cell nucleus are enhanced if the mammalian host cells are irradiated with u.v. light or treated with some chemical mutagens before infection (Das Gupta and Summers, 1978; Lytle *et al.*, 1980; Cornelis *et al.*, 1980, 1981a, 1981b; Su *et al.*, 1981). This enhanced virus mutagenesis might result from the u.v. activation or induction of a mutator activity in the cells. The expression of the mutator phenotype is maximal after a delay following treatment of the cells, after which it drops to undetectable levels (Das Gupta and Summers, 1978; Cornelis *et al.*, 1981a, 1981b; Su *et al.*, 1981).

It was originally found in prokaryotes that the increase in phage mutagenesis induced by the pre-exposure of bacteria to a variety of DNA damaging agents, was concomitant with the activation of a set of responses including an increase in the survival of damaged phage (Defais *et al.*, 1982). Similarly, concomitant with the expression of a mutator activity (the mutator), induced mammalian cells express an increased capacity to produce virus (enhanced capacity) (Rommelaere *et al.*, 1981) and an increased ability to reactivate u.v.-damaged virus (enhanced reactivation) (Lytle, 1978). The enhanced virus mutagenesis and enhanced capacity and reactivation effects show similar time courses and dose responses (Das Gupta and Summers, 1978; Vos *et al.*, 1981; Cornelis *et al.*, 1981b); moreover, they are all depressed if irradiated cells are incubated with cycloheximide, an inhibitor of *de novo* protein synthesis (Lytle, 1978; Lytle and Goddard, 1979; Rommelaere *et al.*, 1981; Vos *et al.*, 1981; Su *et al.*, 1981).

U.v. light is strongly mutagenic for double-stranded DNA viruses propagated in unirradiated primate cells (Cleaver and Weil, 1975; Lytle *et al.*, 1980; Sarasin and Benoit, 1980; Cornelis *et al.*, 1980, 1981b, 1982b; Day and Ziolkowski, 1981). The mutants among the progeny of u.v.-irradiated SV40 may arise as a consequence of a constitutive error-prone process (Cleaver and Weil, 1975). Alternatively, the damaged virus itself might trigger the induction of a cellular mutator activity operating on its own progeny (Lytle *et al.*, 1980; Cornelis *et al.*, 1980, 1981a), in which case, the irradiated virus would be both an induction signal and a preferential substrate of the mutator activity. The signalling potency of irradiated virus is supported by recent evidence that rat cells infected with u.v.-damaged SV40 display a transient mutator activity towards superinfecting intact, unirradiated parovirus H-1 (Cornelis *et al.*, 1981a, 1982a). There is lack of agreement, however, about whether the induced mutator operates preferentially on damaged viral templates. Whereas some have reported that the mutagenesis of u.v.-damaged double-stranded DNA viruses increases drastically in cells pre-exposed to u.v. light or to certain chemicals (Sarasin and Benoit, 1980; Mezzina *et al.*, 1981), others found no such enhancement (Cornelis *et al.*, 1981b; Day and Ziolkowski, 1981). In one study, the induced increment of mutagenesis depended on the use of relatively high multiplicities of infection with irradiated virus (Lytle *et al.*, 1980). It is therefore questionable whether the indirect induction of a mutator activity accounts for the high mutation rates in u.v.-irradiated double-stranded viruses.

These uncertainties prompted us to investigate the mutagenic processes operating on u.v.-damaged templates using parvoviruses as probes. Parvoviruses contain a linear single-stranded DNA genome which is converted into duplex replicative forms in infected cells (Tattersall and Ward, 1976). Therefore, premutagenic lesions induced in viral DNA cannot be removed by the nucleotidyl excision repair mechanism before replication, nor can the replication block be alleviated by recombination with a sister DNA molecule. Moreover, the conversion of viral single-stranded DNA to replicative forms appears to rely entirely on cell functions (Rhode, 1978). Parvoviruses should, therefore, be sensitive probes for measuring misreplication of damaged DNA in mammalian cells.

This study was undertaken to determine whether u.v. light is mutagenic for the autonomous parvovirus H-1 (Hamster Osteolytic Virus) propagated in unirradiated cells and, if so whether the induction of a cellular mutator activity by the damaged virus itself might be responsible for that mutagenesis. We also investigated whether the sensitivity of the virus to the mutator induced in u.v.-irradiated cells depends on the presence of lesions in viral DNA and/or on the multiplicity of infection.

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Results

Single-cycle growth curves of intact and u.v.-irradiated virus

Unirradiated cultures of newborn human kidney cells transformed by SV40 (NB-E cells) produced similar yields after infection with intact and u.v.-irradiated H-1 virus, provided that the u.v.-induced loss of virus infectivity, measured by direct plaque assay, was compensated by a proportional increase of input virus (Figure 1, left curves and Table IA). Thus, virus surviving u.v. irradiation produced yields similar in size to unirradiated virus. Likewise, infectious centres produced equal numbers of progeny irrespective of the multiplicity of the input virus (Table IB).

The exposure of NB-E cells to subtoxic doses of u.v. light or 2-nitronaphthofuran derivatives before infection, enhances both the mutagenesis of intact H-1 and the survival of u.v.-irradiated H-1, as measured by infectious centre assays (Cornelis *et al.*, 1981a; Su *et al.*, 1981). These responses, designated enhanced virus mutagenesis and enhanced reactivation, respectively, were maximal if a 12–14 h interval was allowed between cell treatment and virus infection. Figure 1 and Table II show the effect of u.v.-pre-irradiation of cells on total virus production per culture. The growth curves of intact H-1 virus in unirradiated and u.v.-pre-irradiated cells were similar to each other and to those of the parvoviruses MVM and H-1 propagated in mouse and rat cells, respectively (Rommelaere *et al.*, 1981; Cornelis *et al.*, 1981a). Although in some experiments there was no difference between virus yields from control- and u.v.-pre-irradiated NB-E cultures infected with intact virus, when the results from several experiments were averaged the yield was slightly but significantly higher in u.v.-pretreated cells (Table II, right column). Comparable fluctuations between similar experiments have been noticed with intact SV40 in monkey cells (Cornelis *et al.*, 1981b). Pre-irradiation of the cells also enhanced the fraction of cells able to support productive infection by intact virus [Table II (middle column)]. The enhancement of total virus production per culture in the plateau phase (Figure 1) was very close to the enhanced capacity determined for the same cultures by infectious centre assays (Table II). Therefore, the increase in the fraction of virus producing cells appears to be the major cause of the higher yields of virus in u.v.-pretreated *versus* control cultures. Correcting for this effect, the average yield of virus per productively infected cell did not differ significantly in irradiated and control cells inoculated with unirradiated virus.

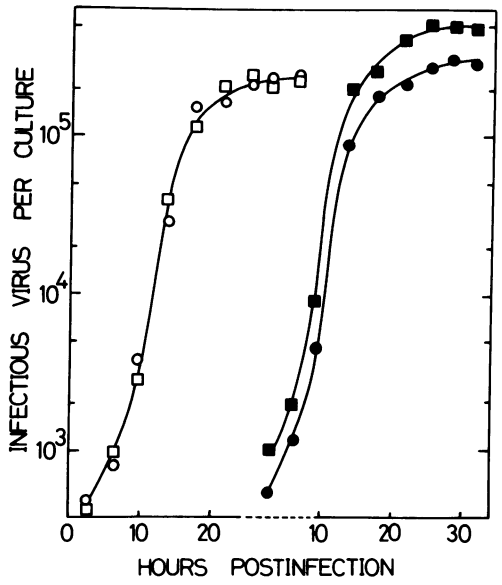

Fig. 1. One-cycle growth of intact and u.v.-irradiated H-1 in u.v.-irradiated and control NB-E cells. Cultures (4 x 10^5 cells) were irradiated (0 or 4.5 J/m^2), incubated for 14 h at 37°C, infected with intact or u.v.-irradiated (85 J/m^2; survival 2 x 10^{-4}) H-1 wt, and processed for single-cycle assays at 33°C. Multiplicities of infection were 10^{-2} and 50 p.f.u./cell for intact and u.v.-irradiated virus, respectively. Open symbols: unirradiated cells; closed symbols: u.v.-irradiated cells; circles: unirradiated virus; squares: u.v.-irradiated virus. Results are the average from three experiments (s.d. <20%).

Comparison of the left and right panels of Figure 1 indicates that u.v.-irradiated H-1 gave rise to ~2-fold more virus in pre-irradiated cells than in unirradiated cells. This increase in the total virus yield can be ascribed to a higher number of infected cells supporting a productive infection, as measured simultaneously by a direct infectious centre assay (Table II). Therefore, the higher yield of virus from u.v.-damaged virus in u.v.-irradiated compared to control cultures seems to be mainly due to the enhanced capacity of the irradiated cells and to an additional enhanced ability of these cells to reactivate u.v.-damaged virus. Thus, virus surviving u.v. irradiation produced bursts of similar size in pretreated and control cells.

Mutagenesis of unirradiated parvovirus H-1

Since the H-1 burst size is independent of the multiplicity of infection (see previous section) the total number of virus generations per infectious cycle is likely to be maximal at low input multiplicities when each cell is infected by a single infectious particle. Because the viral mutation frequency increases

Table I. Virus yields from H-1 infected cultures

A				B	
U.v. dose to virus (J/m^2)	Expected virus survival (%)	Multiplicity of infection	Virus production (p.f.u. x 10^{-6}/culture)	Multiplicity of infection	Virus production (p.f.u./infectious centre)
0	100	0.1	22.0	0.2	66.2
15	22	0.45	19.6	1.0	71.2
23	10	1.0	19.2	5.0	77.3
35	3	3.3	23.8	25.0	78.7
46	1	10.0	21.6	60.0	69.3
55	0.4	25.0	21.7		

A. Cell cultures (3 x 10^6 cells/dish) were infected with H-1 ts6 irradiated with various u.v. doses. Knowing the virus survival (see Figure 5) virus input was varied so that the number of virus survivors in the different inocula was similar at all u.v. exposures. The cultures were incubated at 33°C and processed for single-cycle assays. Total virus yields were determined by direct plaque assays at 33°C. B. Cell cultures of various sizes (3 x 10^6 – 1 x 10^4 cells) were inoculated with 6 x 10^5 p.f.u. of intact H-1 ts6 and processed for single-cycle assays at 33°C.

Table II. Comparison of the levels of enhanced capacity and reactivation as determined by infectious centres or by viral yields after single-cycle growth

U.v. irradiation of cells	U.v. irradiation of virus	Number of infectious centres/4 x 10^3 cells	Virus production (p.f.u. x 10^{-5}/4 x 10^5 cells)
−	−	30.0 ± 3.1	2.3 ± 0.3
+	−	39.2 ± 4.1	3.05 ± 0.5
−	+	27.5 ± 6.2	2.1 ± 0.5
+	+	55.4 ± 7.8	4.5 ± 1.0

Experimental conditions as described in the legend of Figure 1, except that infectious centres were assayed at 33°C simultaneously with the single-cycle assays. Average values and s.d. from six experiments. Infectious centre and single-cycle assays gave an enhanced capacity value of 1.3 and enhanced reactivation of 1.5 and 1.6, respectively.

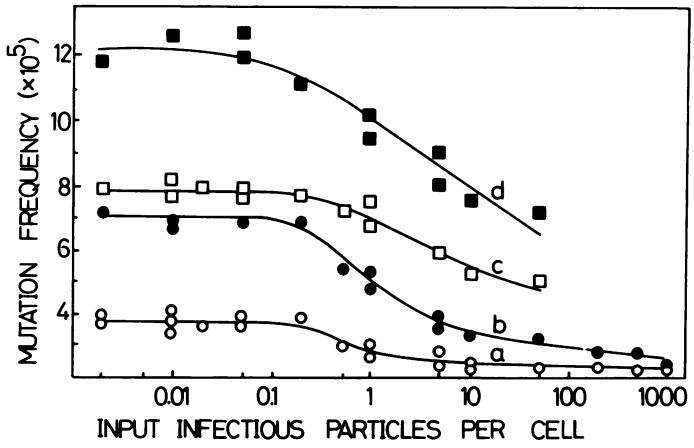

Fig. 2. Effect of the input multiplicity on direct and indirect mutagenesis of H-1 ts6. Cells were irradiated (0 or 4.5 J/m²), incubated at 37°C for 14 h, and infected with intact or u.v.-irradiated (46 J/m²; survival 10^{-2}) H-1 ts6. Infected cultures were processed for single-cycle mutation assays. The number of infectious particles per cell is equivalent to the multiplicity of infection for unirradiated virus and to 1% of the multiplicity for u.v.-damaged virus. For symbols see Figure 1. Results are compiled from six experiments with the same virus stock.

with the number of rounds of replication before the cells burst (Drake, 1970), highest mutation frequencies are to be expected at low multiplicities of infection. Data shown in Figure 2 (curve a) indicate that spontaneous H-1 mutagenesis was indeed inversely related to the multiplicity of infection in the range 0.1 − 5 p.f.u./cell.

Comparison of curves a and b in Figure 2 shows that viral mutagenesis was increased in u.v.-pre-irradiated cells. This effect will be referred to as the u.v. induction of a cellular mutator activity, although the enhancement of a normal mutagenic function is also conceivable. The cellular mutator increased almost 2-fold the mutation frequency at low virus inputs, but was barely detectable at the highest multiplicities used. Since the average burst size of H-1 in NB-E cells is small (~50 − 80 p.f.u./cell), it is conceivable that at these high multiplicities each infecting virus particle replicates very few times and is, therefore, a poor target for the mutator. Thus, intact virus was more likely to be mutated either spontaneously or by the mutator when the multiplicity of infection was lowered, and the number of virus generations was presumably increased.

A possible pitfall in measuring mutation induction by single-cycle assays is that pre-existing revertants have a growth advantage over the temperature-sensitive (ts) particles

Table III. Relative growth of H-1 ts6 and wt revertant virus at 33°C

Inoculum	Ratio of titres 39.5°C/33°C x 10^2
Mixture of revertant and H-1 ts6	2.01 ± 0.11
Mixture after one-cycle growth in:	
unirradiated cells	2.04 ± 0.08
u.v.-irradiated cells	2.19 ± 0.16

Cells were irradiated (0 or 4.5 J/m²), incubated for 14 h at 37°C, and infected (multiplicity of infection = 2) with H-1 ts6 containing 2% of the H-1 ts6 revertant 3026 (Cornelis *et al.*, 1981). After infection, the dishes were kept at 33°C and processed for a single-cycle mutation assay. Values are given with their s.d.

at the permissive temperature. To ascertain that the increase in mutation frequency after one virus passage was the result of mutations actually generated during that growth cycle, reconstruction experiments were performed with an inoculum consisting of a mixture of H-1 ts6 and wild-type (wt) revertants derived from H-1 ts6. Table III shows that there was no significant growth difference between revertant and ts virus during one lytic cycle at the permissive temperature.

Mutagenesis of u.v.-irradiated parvovirus H-1

The mutagenesis of intact and u.v.-irradiated virus was compared in cells infected with the same number of infectious particles. Because survival of parvovirus is independent of the multiplicity of infection (Rommelaere *et al.*, 1981), the loss of infectivity of u.v.-damaged virus was compensated by a proportional increase of the virus input. Since virus surviving u.v. irradiation produced bursts of similar average size to intact virus (see above), the progenies of the intact and u.v.-irradiated virus whose mutation frequencies were compared, presumably arose from the same number of virus generations under these conditions.

As with intact virus, survivors of u.v.-irradiated H-1 generated more mutants at low multiplicities (Figure 2, curve c). At all multiplicities of infection tested, mutation frequencies among the descendants of u.v.-damaged virus grown in unirradiated cells were higher than those of intact virus in similar cultures (Figure 2; compare curves a and c). Moreover, mutation frequencies among the progeny of u.v.-irradiated virus were higher in u.v.-pre-irradiated than in control NB-E cells at all multiplicities used (Figure 2, curves c and d). The mutator triggered by cell pre-irradiation thus appeared to act on u.v.-irradiated virus as well as intact virus. As with unirradiated virus, the mutator induced more mutants in the progeny of irradiated virus at low multiplicities of infection.

Mutation induction curves for virus irradiated at different doses and propagated in control and u.v.-irradiated cells are shown by Figure 3 (direct plaque assays) and 4 (single-cycle assays). The number of virus killed by radiation was compensated by increasing the multiplicity of infection, so that a similar fraction of the cells produced infectious virus at all u.v. exposures.

In unirradiated cells, the mutation frequency among the progeny of u.v.-irradiated H-1 ts6 increased linearly with the u.v. dose (lower curves of Figures 3 and 4). For each u.v. dose given to H-1 ts6, the number of revertants was higher in u.v.-pre-irradiated than in control cultures, a result of the increased activity of the cellular mutator. As in control cells, the induction of virus mutations in u.v.-irradiated cells was related

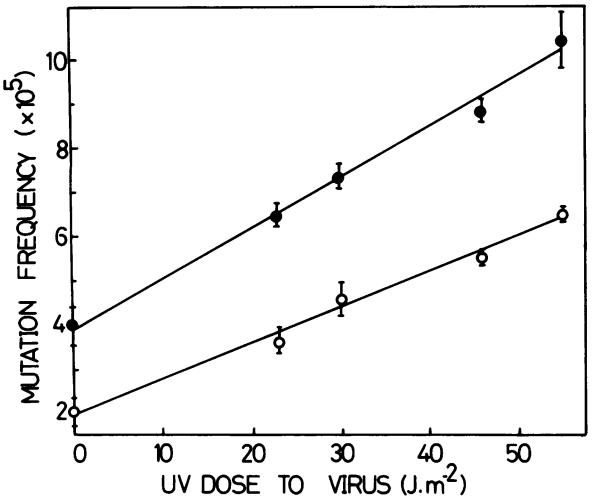

Fig. 3. Direct plaque assay of mutation induction in u.v.-irradiated parvovirus H-1 ts6. Viral mutagenesis was measured using unirradiated (○) or u.v.-pre-irradiated (●) cells as indicators. Pretreated cultures were exposed to 4.5 J/m² 14 h prior to infection. The proportion of productively infected cells was 0.25 for all u.v. exposures to the virus. The degrees of confidence for the regression lines shown were >99%. Results are given with standard deviations of the mean of two independent experiments with the same mutant stock.

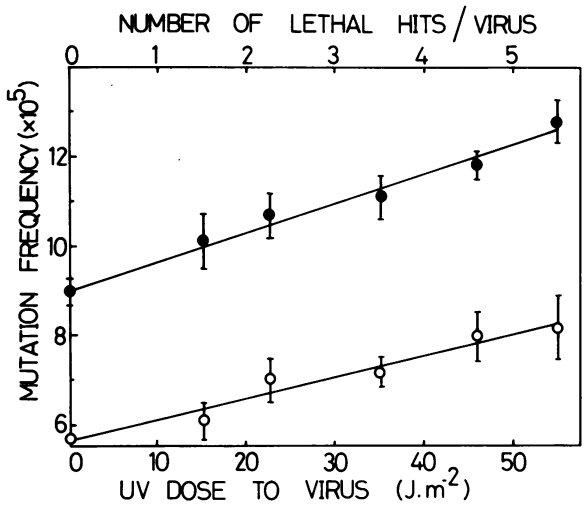

Fig. 4. Single-cycle assay of mutation induction in u.v.-irradiated parvovirus H-1 ts6 propagated in unirradiated (○) or u.v.-pre-irradiated (●) NB-E cells. Pretreated cultures were exposed to 4.5 J/m² 14 h before infection. The proportion of productively infected cells was adjusted to 0.1 with the variously irradiated virus. Average values and s.d. bars are from three experiments with the same H-1 ts6 stock. Regression lines are shown and can be fitted to the induction curves with a degree of confidence >98%.

Table IV. Wt revertant induction from u.v.-damaged H-1 ts6 propagated in unirradiated and u.v.-pre-irradiated cells

Experiment	u.v. dose to cells = 0 J/m²		u.v. dose to cells = 4.5 J/m²		
	Mutation frequency (x 10^5/10 J/m²)	Correlation coefficient	Mutation frequency (x 10^5/10 J/m²)	Correlation coefficient	Degree of confidence
1	0.87	0.94	1.14	0.95	> 80%
2	0.77	0.99	1.15	0.99	> 99%
3	0.75	0.99	1.02	0.99	> 99%
4	0.42	0.92	0.56	0.83	> 50%
5	0.30	0.84	0.36	0.87	> 40%
6	0.96	0.99	1.35	0.96	> 95%
7	0.46	0.97	0.87	0.98	> 99%
Average	0.65		0.91		

Experimental conditions are given in the legends of Figures 3 and 4. Experiments were performed independently using direct plaque assays (experiments 1, 2) or single-cycle assays (experiments 3–7). Induced mutation frequencies per 10 J/m² (10 J/m² gives 1 lethal hit; see Figure 5) were calculated for each u.v. exposure to virus and were averaged for each experiment (columns 2 and 4). Correlation coefficients (columns 3 and 5) give the probability of fitting of regression lines with mutation induction curves. The averaged induced reversion frequencies in irradiated and control cells were compared using the Student-Fisher test. The degree of confidence (column 6) expresses the probability that H-1 mutation induction per unit dose is higher in u.v.-pre-exposed than in control host cells.

linearly to the u.v. dose given to the virus (upper curves of Figures 3 and 4). In every experiment, the slope of the induction curve was somewhat steeper for virus propagated in u.v.-irradiated cells compared with control cultures (Table IV). Therefore, the mutator activity present in pre-irradiated cells seemed to operate with a higher probability of error on some u.v.-damaged sites than on intact portions of the viral genome. However, it is apparent from Table IV and Figures 3 and 4, that the difference in the slopes of the dose responses was small and that the action of the mutator was mostly untargeted for the range of u.v.-exposures given to the virus. Thus, induced mutagenesis of u.v.-damaged H-1 in u.v.-pre-irradiated cells was close to the sum of: (i) the mutations induced indirectly in intact virus by the activated mutator; and (ii) the mutations which arose directly from the processing and/or replication of damaged viral templates.

Concomitant expression of enhanced reactivation and enhanced mutagenesis

Survivals of the virus in the experiments illustrated by Figures 3 and 4 were averaged (see Figure 5). Survival was increased if the cells were exposed to u.v. light prior to virus infection, a phenomenon known as enhanced reactivation. The ratio of virus survival in irradiated *versus* control cells was 1.5 at the highest u.v. dose to the virus (55 J/m²; survival = 4 x 10^{-3}). The number of wt revertants in the progeny of intact H-1 ts6 and the number of infectious centres from H-1 wt given 85 J/m² u.v. light varied with the u.v. dose delivered to the host cells (Figure 6; lower panel). The dose responses of enhanced reactivation and the mutator were parallel and very similar to those obtained with RL5E rat cells exposed to u.v. light or with NB-E or RL5E cells pretreated with 2-nitro-

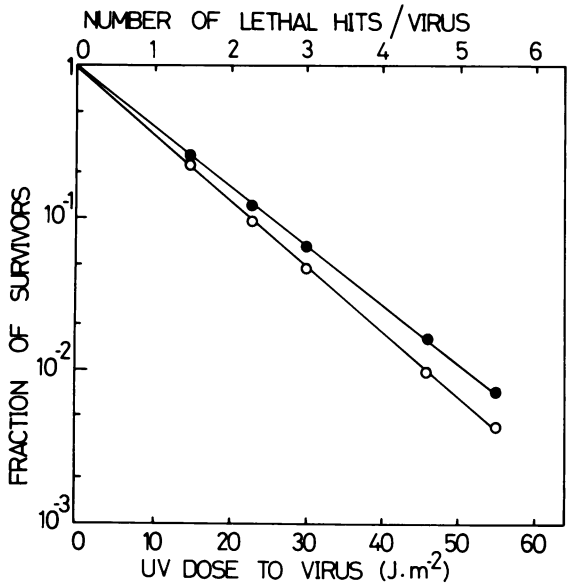

Fig. 5. Survival of u.v.-irradiated H-1 in normal and u.v.-pre-treated NB-E cells. The surviving fractions of u.v.-irradiated H-1 grown at 33°C were averaged from five experiments (two direct plaque assays and three single-cycle assays) whose mutation data are shown in Figures 3 and 4. S.d. was <25%. Open symbols: control cells; closed symbols: u.v.-pre-irradiated cells.

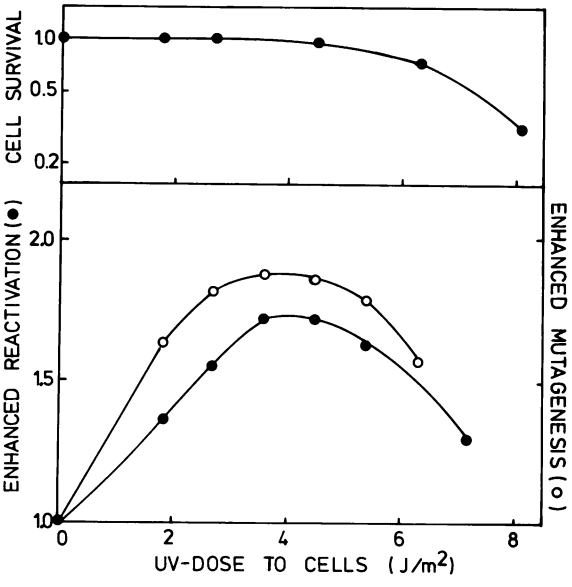

Fig. 6. Dose-responses of cell killing and of u.v.-enhanced H-1 reactivation and mutagenesis. **Lower panel**: NB-E cells were exposed to various doses of u.v. light, incubated for 14 h at 37°C, infected with intact H-1 ts6 for mutagenesis measurement or with intact or u.v.-irradiated H-1 wt for survival measurement. Infected cultures were processed for infectious centre assays. The u.v. dose to wt virus was 85 J/m^2, giving a survival of 2 x 10^{-4}. The relative survival of u.v.-irradiated virus and the relative mutagenesis of intact virus in u.v.-pre-irradiated versus untreated cells are shown as enhanced reactivation (●) and enhanced mutagenesis (○), respectively. Average values from three experiments. S.d. was <20%. **Upper panel**: cells were exposed to various doses of u.v. light, and tested for their colony forming ability. Average values from three experiments are shown relative to unirradiated cells.

naphthofuran derivatives (Cornelis *et al.*, 1981a; Su *et al.*, 1981). Maximal mutagenic and repair activities were obtained around 4 J/m^2, a dose which barely affected cell survival as measured by colony forming ability (Figure 6; upper panel). As for nitrofurans (Su *et al.*, 1981), the time courses of the expression of mutagenic and repair functions were parallel in u.v.-pre-irradiated NB-E cells (data not shown). The expression of these two effects seems, therefore, to be triggered coordinately in human NB-E cells exposed to various genotoxic agents.

Discussion

Parvovirus mutagenesis in human cells could be induced either by damaging the virus or by pre-irradiating the host cells, or by both. Virus or cell irradiation did not affect detectably the size of the viral burst per productively infected cell. Therefore, a higher number of rounds of virus replication is unlikely to account for the increase in mutagenesis. The data rather suggest that u.v. irradiation of virus and cells both affect the operation of cellular mutator activities acting on intact and/or u.v.-damaged virus.

Direct mutagenic effect of virus u.v. irradiation

The mutation frequency among the progeny of u.v.-irradiated H-1 increased linearly with the dose. It was calculated from Table IV that u.v. irradiation of H-1 ts6 induced revertant frequencies of $\sim 6.5 \times 10^{-6}$ and 6.5×10^{-7} per lethal hit and per unit dose (J/m^2), respectively. Such a linear induction might be the result of premutagenic lesions in u.v.-damaged DNA. If so, fixation of mutation would result from the replication and/or processing of these lesions by mechanism(s) expressed constitutively in human cells. Biochemical studies performed with the parvovirus MVM suggested that most pyrimidine dimers, the major u.v.-induced DNA lesions, permanently block the elongation of the strand complementary to the u.v.-damaged viral genome (Rommelaere *et al.*, 1982). However, mutagenesis of u.v.-irradiated virus in untreated cells might be directed by minor lesions in single-stranded genomes devoid of dimers or by a biochemically undetected fraction of the dimers which do not block replication. However, an alternative explanation is conceivable, based on recent evidence that intact H-1 ts6 can be mutagenized indirectly by pre-infecting the host cells with u.v.-irradiated SV40 or with u.v.-killed H-1 ts6 (Cornelis *et al.*, 1981a). The magnitude of this indirect induction of mutagenesis in intact virus depended on the absolute number of lesions introduced in the cells by the damaged pre-infecting virus (Cornelis *et al.*, 1982a). Accordingly, the dose-dependency of mutation in the progeny of u.v.-irradiated virus grown in untreated cells might be accounted for, at least in part, by the increase with dose of the ability of damaged virus to induce a cellular mutator acting on the virus progeny. It can be calculated from Cornelis *et al.* (1981a, 1982a) that infection with H-1 ts6 exposed to 46 and 55 J/m^2 u.v. light at multiplicities of infection of 25 and 62.5, respectively (Figure 3), would introduce enough lesions into NB-E cells to activate such a mutator. However, several lines of evidence strongly suggest that an induced mutator activity is not likely to be responsible for the formation of revertants in u.v.-damaged H-1 ts6 propagated in unirradiated NB-E cells. First, u.v.-killed H-1 ts6 was not able to enhance significantly the mutagenesis of intact H-1 unless superinfection with intact virus was delayed for several hours (Cornelis *et al.*, 1981a). If the signalling (irradiated) virus was inoculated simultaneously with the probe (intact) virus, the latter apparently replicated before the mutator was activated. Second, the mutation frequencies in the progeny of H-1 exposed to the largest u.v. dose used were higher than the reversion frequencies of intact

virus in the presence of the mutator (Figures 3 and 4). Under optimal conditions of infection the mutator induced at most a 2-fold increase of the spontaneous back-mutation frequency in the range $5 \times 10^{-6} - 3 \times 10^{-5}$ (Figures 2, 3, 4; Cornelis *et al.*, 1981a; Su *et al.*, 1981). Third, lowering the multiplicity did not decrease induction of mutation in u.v.-irradiated H-1 (Figure 2, curve c). For virus exposed to 46 J/m² u.v. light (survival = 10^{-2}) a maximum and constant level of mutagenesis was attained at multiplicities lower than 20 (0.2 infectious particles/cell). Decreasing the multiplicity from 20 to 0.2 did not detectably affect mutagenesis. Moreover, it can be calculated that the small number of lesions introduced per cell at the lowest multiplicities is very likely to be devoid of any significant signalling capacity (see Cornelis *et al.*, 1982a). Fourth, u.v. irradiation of the cells induced equal reversion in the progeny of u.v.-damaged and intact virus (see below). U.v.-irradiated virus, therefore, did not appear to compete with direct u.v. irradiation of the cells for the induction of the mutator.

Indirect mutagenic effect of cell u.v. pre-irradiation

The mutagenesis of intact and u.v.-damaged parvovirus H-1 was increased if the host cells were exposed to mild doses of u.v. light before infection. Thus, u.v. irradiation of the human cells induced or enhanced a mutator activity operating on both intact and u.v.-damaged virus. This activity was stimulated concomitantly with a recovery process responsible for an enhanced survival of u.v.-damaged virus. The mutator was most effective when low multiplicities were used.

Virus growth curves showed that u.v. irradiation of the cells did not affect the onset and rate of H-1 production. These results are similar to those reported for parvovirus MVM propagated in mouse cells (Rommelaere *et al.*, 1981). The enhancement of parvovirus mutagenesis by cell irradiation is therefore unlikely to be the mere consequence of an alteration of the time course of virus multiplication in pretreated cells.

U.v. irradiation caused a delay in the multiplication of the double-stranded DNA viruses SV40 and HSV-1 (Sarasin, 1978; Coppey and Menezes, 1981), but it did not alter significantly the growth curve of parvovirus MVM (Rommelaere *et al.*, 1981) or H-1. Since the progeny of intact and u.v.-irradiated parvovirus suffered the same exposure to the mutator induced in the pretreated cells, we could study the effect of u.v. damage to the virus on its sensitivity to the mutator. For the u.v. dose range used, the absolute number of revertants induced by the mutator in the progeny of irradiated virus was only slightly greater than the number of mutant descendants of intact virus. Thus, little synergism was found between cell and virus irradiation with respect to viral mutagenesis. Most of the mutator action was untargeted, i.e., unrelated to u.v.-induced damage in the viral DNA, although a minor component of its activity appeared to be directed by u.v. lesions.

A u.v. dose of 3 – 5 J/m² to the cells was optimal to trigger the cellular mutator. The exposure of the virus to such a dose had no significant effect on its mutagenesis in pre-irradiated or control cells. These results raise the possibility that at least a fraction of cell mutations in cultures exposed to low u.v. doses might be the untargeted result of the activation of a mutator.

Although unirradiated virus might contain spontaneous lesions (Lindahl and Nyber, 1972), its sensitivity to the mutator raises the possibility that enhanced mutagenesis results from misreplication of viral templates rather than from lesion-directed, error-prone repair or recombination. The lack of susceptibility of parvoviruses to pre-replicative events, such as nucleotidyl excision repair or multiplicity reactivation, is consistent with this. Thus, the cellular mutator might conceivably result from the induction of either a temporary decrease in the accuracy of the DNA replication machinery or an enhanced or longer availability of a misreplication activity normally expressed by the cells. Interestingly, pre-irradiation of cells increased the fraction of single-stranded parvoviral genomes which were converted to duplex replicative forms after infection with u.v.-damaged virus (Rommelaere *et al.*, 1982). Whether the increases in viral mutagenesis and in viral DNA replication occur by related mechanisms is, however, unknown. Damaged virus was only slightly more responsive to the mutator than intact virus. The minor contribution of the damaged sites to the action of the mutator suggests that the lesions did not provide a sufficiently preferential target to compensate for their low frequency relative to intact sites, in the range of doses given to the virus.

Comparison of mutation induction in H-1 and in double-stranded DNA viruses

The search for an induced cellular mutator operating on double-stranded DNA viruses led to contradictory results with both unirradiated and u.v.-damaged viral probes (Das Gupta and Summers, 1978; Cornelis *et al.*, 1980, 1981b, 1982b; Lytle *et al.*, 1980; Mezzina *et al.*, 1981; Day and Ziolkowski, 1981). Using single-stranded parvovirus H-1, we found that both types of probes revealed an enhanced mutagenic activity in cells pre-exposed to u.v. light, provided that specific experimental conditions were fulfilled. The definition of these conditions might resolve some of the discrepancies found with double-stranded DNA viruses, although it is questionable whether all the mutagenic processes operating on double-stranded viruses apply to single-stranded viruses (Cornelis *et al.*, 1982b). Several parameters affected the enhanced mutagenesis of H-1. (i) The background mutation frequency of the virus stock. It can be calculated from Table IV and Figures 3 and 4 that cell u.v.-irradiation induced an average mutation frequency of $6.5 \times 10^{-5} + 3 \times 10^{-7} \times D$, where D is the u.v. dose to virus in J/m². If the frequency of pre-existing revertants in the viral stocks was much higher than that of induced revertants, the effect of the mutator was masked, as shown by reconstruction experiments. (ii) The u.v. dose to virus. Overall u.v.-induced mutations in the progeny of u.v.-damaged H-1 propagated in u.v.-pre-irradiated cells can be considered as the sum of untargeted mutations induced indirectly by the activated cell mutator at intact viral sites and of targeted mutations induced at damaged viral sites either directly by constitutive processes or indirectly by the mutator. Targeted mutagenesis increases with the dose administered to virus and will eventually mask the untargeted action of the mutator whose relative contribution to overall u.v.-induced mutagenesis will decline. Consequently, the enhanced mutagenesis response will fade as the dose to the virus is increased, unless the mutator is also more efficient than constitutive processes for the induction of targeted mutations. NB-E cells appear to fulfill the latter condition to a greater extent for double-stranded herpes simplex virus (see accompanying paper by Lytle and Knott) than for single-stranded H-1 (Table IV). It can be calculated from Figure 3 that the magnitude of enhanced mutagenesis of H-1 decreased from 1.9 to 1.6 when

the dose given to the virus was increased from 0 to 55 J/m². (iii) Multiplicity of infection. The virus input was found to affect drastically reversion in the H-1 progeny produced in either control or u.v.-induced cells. The direct and indirect inductions of viral mutagenesis by virus and cell irradiation respectively, were maximal at low multiplicities. With double-stranded DNA viruses, this effect might be obscured by the occurrence of multiplicity reactivation which increases with the virus input and might participate in the enhanced mutagenesis (Lytle *et al.*, 1980). No evidence for multiplicity reactivation of parvoviruses was found in our study (Rommelaere *et al.*, 1981).

Comparison of mutation induction in H-1 and in single-stranded bacteriophages

Our results point to some interesting discrepancies between u.v. induction of mutations in parvovirus H-1 and in single-stranded DNA bacteriophages such as ϕX174 and M13 (Bleichrodt and Verhey, 1974; Yatagai *et al.*, 1981; Brandenburger *et al.*, 1981). First, u.v. irradiation of H-1 directly induced 6.5×10^{-6} reversions per lethal hit in the progeny produced in unirradiated cells. In contrast, the exposure of ϕX174 or M13 to u.v.-light induced a much smaller or even undetectable level of viral mutagenesis in unirradiated bacteria. Second, the synergism between irradiation of the host and of the virus with respect to the induction of viral mutagenesis was much more pronounced in *Escherichia coli* than in the human cells inoculated with single-stranded viruses. Thus, although seemingly analogous inducible mutagenic pathways have been identified in eukaryotic and prokaryotic cells, their contribution to overall mutagenesis appears to be different.

Materials and methods

Cell culture and virus stocks

NB-E cells were grown in Eagle's minimal essential medium supplemented with 5% heat-inactivated foetal bovine serum. Wt H-1 virus and a ts H-1 mutant termed ts6 (kindly provided by S.L. Rhode) were propagated in NB-E cells at 37 and 33°C respectively.

Assays for viral mutagenesis and survival

Mutagenesis in H-1 was measured as reversion of ts6 to wt phenotypes at the non-permissive temperature (39.5°C). At 39.5°C, H-1 ts6 is deficient in a late step of the virus cycle – the production of progeny single-stranded genomes – although infecting single-stranded DNA is converted to duplex replicative forms which are amplified (S.L. Rhode, personal communication).

Asynchronous NB-E cells were treated with u.v. light and incubated at 37°C. Cells were infected with unirradiated or u.v.-irradiated virus. Two assays for virus mutagenesis and survival were used: a single-cycle and a direct plating procedure. The direct plating assay was either a direct plaque assay or an infectious centre assay (Cornelis *et al.*, 1981a, 1982a; Su *et al.*, 1981). At least four pre-existing revertants were present in each individual virus inoculum in order to minimize their contribution to fluctuations in mutation frequency. Virus survival was calculated from total virus yields (single-cycle assays) or from plaque formation after infection with low input multiplicities (direct plating assays) performed at 33°C.

Miscellaneous

Procedures of virus purification, infection, plaque assay, irradiation of cells or virus with u.v. light (254 nm) have been described previously (Cornelis *et al.*, 1981a). The cytotoxic effect of u.v. light was determined by measuring colony-forming ability of the irradiated cells (Su *et al.*, 1981).

Definitions

The multiplicity of infection refers to the number of infectious virus particles (p.f.u.) per cell for an equivalent inoculum of unirradiated virus titered by direct plaque assay on unirradiated indicator cells. The mutation frequency of a virus population is defined as the fraction of the total number of infectious particles (33°C) able to form plaques at the non-permissive temperature (39.5°C). Enhanced virus mutagenesis is the ratio of the virus mutation frequency in irradiated (induced) cells to that in non-irradiated cells. Enhanced reactivation is the ratio of virus survival in induced *versus* that in non-induced cells. The enhanced capacity is the ratio of the fraction of inoculated cells giving rise to infectious centres in pretreated *versus* untreated cultures.

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