Transfection with Extracellularly UV-Damaged DNA Induces Human and Rat Cells to Express a Mutator Phenotype Towards Parvovirus H-1

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Human and rat cells transfected with UV-irradiated linear double-stranded DNA from calf thymus displayed a mutator activity. This phenotype was identified by growing a lytic thermosensitive single-stranded DNA virus (parvovirus H-1) in those cells and determining viral reversion frequencies. Likewise, exogenous UV-irradiated closed circular DNAs, either double-stranded (simian virus 40) or single-stranded (phiX174), enhanced the ability of recipient cells to mutate parvovirus H-1. The magnitude of mutator activity expression increased along with the number of UV lesions present in the inoculated DNA up to a saturation level. Unirradiated DNA displayed little inducing capacity, irrespective of whether it was single or double stranded. Deprivation of a functional replication origin did not impede UV-irradiated simian virus 40 DNA from providing rat and human cells with a mutator function. Our data suggest that in mammalian cells a trans-acting mutagenic signal might be generated from UV-irradiated DNA without the necessity for damaged DNA to replicate.

Repair and mutagenic functions expressed by mammalian cells can be identified through their action on viruses growing in those cells. The survival of UV-irradiated nuclear-replicating DNA viruses is enhanced if the host cells are exposed to radiation or certain chemicals before virus infection (7). Similarly, the mutagenesis of intact or UV-damaged viruses is increased, under certain conditions, in mammalian cells pretreated with physical or chemical agents (4, 7). The latter phenomenon, termed enhanced virus mutagenesis (EM), suggests that certain mutagens might act indirectly by conferring a mutator phenotype to treated cells. The expression of EM is maximal several hours after cell treatment, and it later decays to undetectable levels (20). EM is reminiscent of the transient induction of a mutagenic process(es) in bacteria as part of the pleiotropic SOS response to DNA damage or interrupted DNA replication (7).

An intriguing aspect of the EM response is the nature of the signal(s) which eventually activated the cellular mutator phenotype. This question could be investigated by supplying intact cells with exogenous effectors suspected of signalling capacity and testing whether these effectors led to the indirect induction of the EM response. We have shown previously that human and rat cells can be provided with the EM induction signal by inoculation with UV-killed single- or double-stranded DNA viruses (5). The magnitude and time course of EM expression were similar in cells exposed to the direct (UV irradiation) and indirect (UV-killed viruses) inducing treatments. Thus, the activation of EM did not appear to be mediated by direct damage in specific cellular genes. The nature of the EM activator transferred into intact cells by UV-irradiated viruses is not known. It has been shown that UV-damaged exogenous DNA triggered the indirect induction of mutagenesis in unirradiated bacteria (8). Products of DNA metabolism might also generate an induction signal in mammalian cells since chemicals which do not damage DNA directly but inhibit its replication have been found to enhance the cellular ability to reactivate damaged viruses (14, 17), a process expressed concomitantly with EM (20).

Altogether, these observations prompted us to investigate whether transfection with extracellularly UV-damaged DNA conferred a mutator phenotype to unirradiated human and rat cells. The cellular mutator activity was measured by using a temperature-sensitive mutant of the autonomous parvovirus H-1 (hamster osteolytic virus) (16) as a probe. Mutagenesis was assayed by determining viral reversion frequencies after lytic growth. Previous work has shown that replication of H-1 provides a sensitive assay for the determination of mutator functions expressed by host cells (5, 20).

MATERIALS AND METHODS

Cells and virus. Cultures of simian virus 40 (SV40)-transformed newborn human kidney cells (NB-E) and Harvey murine sarcoma virus-transformed rat liver cells (RL5E) were used. The virus used as a probe was a thermosensitive mutant of parvovirus H-1 termed H-1 ts6 (kindly provided by S. L. Rhode). The spontaneous backmutation frequency of the virus stock was about 2 × 10⁻⁵. Conditions of cell maintenance, virus preparation, and infection have been described previously (5).

DNA transfection. Two transfection protocols were used. (i) The calcium-phosphate coprecipitation technique was essentially as described by van der Eb and Graham (23). The DNA to be tested was mixed with highly polymerized carrier DNA from calf thymus to give a total DNA concentration of 20 μg/ml. After DNA coprecipitation with calcium-phosphate, the suspension was inoculated onto a cell monolayer (0.5 ml per 5 × 10⁵ cells in a 60-mm petri dish). After 10 min of incubation at room temperature, 4.5 ml of complete medium was added to the inoculum, and the cultures were kept for 6 h (RL5E) or 18 h (NB-E) at 37°C. These intervals were selected to minimize the cytotoxic effect of the procedure. Cells were rinsed and further incubated for 14 h.

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(RL5E) or 1 h (NB-E) in complete medium before virus infection. (ii) The DEAE-dextran transfection technique was used as described by McCutchan and Pagano (15). Treated cultures were incubated in complete medium for 14 h at 37°C before infection.

**UV irradiation.** Cells and DNA were irradiated with UV light (254 nm) as described (5). Calibration was performed with a Latarjet dosimeter.

**Assays for viral mutagenesis.** Viral mutagenesis was assayed by scoring reversions of H-1 ts6 to the wild-type phenotype (lytic growth at the nonpermissive temperature). Reversion frequencies were measured by either an infectious center assay or a single-cycle assay, as described previously (5). The multiplicities of infection with H-1 ts6 were $10^{-3}$, 10, and 0.1 PFU per cell for infectious center assays at 33 and 39.5°C and for single-cycle assays, respectively.

**Origin of DNAs.** Highly polymerized DNA from calf thymus was purchased from Sigma Chemical Co. and further purified by phenol extraction. Single-stranded circular φX174 DNA was kindly provided by O. P. Doubleday or was obtained from Bethesda Research Laboratories, Inc. Double-stranded closed circular SV40 DNA was purified from SV40-infected CV-1 cells essentially as described (19), except that the cesium chloride step was replaced by DNA deproteinization with proteinase K (100 μg/ml, 37°C, 1 h), followed by alkaline sucrose (15 to 30%) gradient centrifugation. The recovered DNA contained more than 95% form I molecules as determined by gel electrophoresis. Transforming SV40 DNA fragments with (ori') or without (ori) a functional replication origin were derived from wild-type strain 776 and a subclone of mutant 6-17 lacking nine nucleotides at the BglII site (9), respectively. The ori' EcoRI-BamHI large fragment and the ori EcoRI-linked HpaII-BamHI large fragment (1) were cloned in EcoRI- and BamHI-digested plasmid pAT153 (22). Plasmid DNA was digested with BamHI, EcoRI, and HpaII, and the SV40 fragment containing the transforming region and the origin of DNA replication was isolated either on a Sepharose 2B column or by agarose gel electrophoresis (24).

**Definitions.** The mutation frequency is defined as the fraction of infectious particles (33°C) that form plaques at the nonpermissive temperature (39.5°C). The relative mutation frequency is expressed by the ratio of the virus mutation frequency in DNA-transfected cells to that in mock-transfected cultures. Mock transfection had no significant mutagenic effect on H-1 ts6 (data not shown). EM is defined as the ratio of the virus mutation frequency in cells transfected with UV-irradiated DNA to that in cells transfected with unirradiated DNA.

**RESULTS**

**Signalling capacity of exogenous DNA.** Calf thymus DNA was introduced into NB-E human cells and tested for its effect on the mutagenesis of parvovirus H-1 replicating in those cells. The inoculum contained either increasing amounts of DNA exposed to the same dose of 1,500 J m⁻² of UV light (Fig. 1a) or a fixed amount of DNA irradiated with increasing UV doses (Fig. 1b). It is apparent that DNA-transfected cells achieved higher yields of virus revertants than mock-transfected cultures.

The magnitude of the mutator effect increased with the absolute number of UV-induced lesions present in the DNA supplied to the cells up to a saturation level. A similar dose response of EM expression was found when UV-irradiated SV40 virions were used as the inducing agent (3). The plateau may correspond to saturating amounts of signal DNA, although the levelling off of lesion formation at high UV doses (25) could partly account for it. Unirradiated DNA displayed limited, but significant, triggering activity, in agreement with our previous finding that undamaged SV40 was able to elicit EM expression only at high multiplicities and low spontaneous backmutation frequencies of the virus stock (3, 4).

The signalling capacity of UV-irradiated exogenous DNA was compared with that of direct UV irradiation of cells. We have shown previously that a 14-h interval between cell UV irradiation and virus infection is necessary to allow maximal expression of EM in this system (5). Exogenous DNA is detectable inside virtually all of the recipient cells within a few hours after inoculation (13). Therefore, infection of DNA-treated NB-E cells with H-1 ts6 was delayed until 15 to 20 h after the beginning of the transfection procedure. Under these conditions, cells transfected with UV-irradiated DNA displayed a similar level of EM to that of cells directly exposed to UV light (Table 1, lines 1, 3, and 5). It was calculated that for a given amount of irradiated DNA, the UV dose necessary to achieve the plateau level of EM was ca. 1,000 times higher for inoculated exogenous DNA (cell transfection) than for endogenous DNA (cell irradiation). This discrepancy may result from the low fraction of exogenous damaged DNA which is taken up by the cells and
reaches its intracellular site of action (13). Alternatively, the transfection process may restrict the spectrum of signalling DNA lesions to UV damage which only occurs at a low rate (11).

**Effect of cell type and experimental conditions.** Table 1 (lines 3 and 4) shows that human (NB-E) and rat (RL5E) cells displayed similar levels of EM after transfection with UV-irradiated calf thymus DNA. Moreover, no significant difference between the abilities of the calcium-phosphate and DEAE-dextran transfection techniques to supply cells with signalling DNA could be detected in these experiments (Table 1, lines 1 and 3). Single-cycle and infectious center mutation assays were equally effective to reveal the EM phenomenon (Table 1, lines 1 and 2). Thus, UV irradiation of exogenous DNA enhanced the fraction of recipient cells which produced viral revertants, irrespective of the temperature and MOI used. This result strongly suggests that damaged DNA triggered the generation of new revertants rather than selectively enhanced the amplification of preexisting revertants.

**Effect of the structure and origin of DNA on its signalling capacity.** Double-stranded (SV40) and single-stranded (φX174) closed circular DNAs of similar size were compared for their ability to elicit a mutator effect in RL5E cells. As shown in Fig. 2 (open symbols), transfection with unirradiated DNA caused only a limited increase in the H-1 mutation frequency, irrespective of whether the DNA was single or double stranded. In contrast, UV irradiation of both types of DNA enhanced their signalling capacity and resulted in an EM response the amplitude of which increased with the amount of transfected DNA up to a saturation level (Fig. 2, closed symbols). No major difference in the mutator effects of single- and double-stranded DNAs could be detected.

**Influence of replication of signalling DNA on the mutator response.** As shown in Fig. 2a, UV-irradiated SV40 DNA conferred a mutator phenotype to RL5E cells which are unable to support productive SV40 infection. The replication of UV-damaged SV40 DNA might still be involved in the generation of the EM signal since limited viral DNA synthesis occurs in nonpermissive cells (2). Therefore, the role of DNA replication was further investigated by testing whether the signalling capacity of SV40 DNA was influenced by a mutation which abolishes normal initiation of viral DNA synthesis. A mutant of SV40 carrying a deletion in the region of the genome comprising the origin of DNA replication and the major binding site of large T antigen initiator molecules, termed ori− (9), was used for that purpose. Such ori− mutants of SV40 are unable to replicate in permissive host cells (10). Data presented in Table 2 show that UV-irradiated ori+ DNA elicited similar levels of EM expression as ori+ DNA in both rat and human cells. This result strongly suggests that semiconservative replication of damaged DNA is not a prerequisite for the induction of the mutator activity.

**DISCUSSION**

We observed that transfection with UV-irradiated double- or single-stranded DNA of eucaryotic or procaryotic origin induces human and rat cells to express mutator function(s) responsible for EM of parovirus H-1. Therefore, an EM signal appears to be generated from UV-damaged DNA. A parallel may be drawn between these results and the indirect

**FIG. 2.** Dose-response curves of the relative mutation frequency of H-1 ts6 in RL5E cells pretreated with (a) double-stranded SV40 DNA or (b) single-stranded φX174 DNA. Cells were transfected with increasing amounts of SV40 or φX174 closed circular DNA (form I), using the calcium-phosphate technique. Before inoculation, SV40 and φX174 DNAs were either UV irradiated (1,500 J m−2) or not and they were then supplemented with unirradiated carrier DNA to achieve a total DNA concentration of 20 µg/ml. After incubation, treated cells were infected with H-1 ts6 and processed for infectious center assays. Average values from five experiments are given with their standard deviations. Data relating to both types of DNA were obtained in the same experiments. Closed symbols, UV-irradiated DNA; open symbols, unirradiated DNA.

**TABLE 1.** EM of H-1 ts6 in cells pretreated with UV-irradiated DNA: effect of cell type and experimental conditions.

<table>
<thead>
<tr>
<th>Cell pretreatment</th>
<th>Transfection procedure</th>
<th>Mutation assay</th>
<th>Cells</th>
<th>EM*</th>
</tr>
</thead>
<tbody>
<tr>
<td>UV-DNA</td>
<td>Ca2+-phosphate</td>
<td>Infectious center</td>
<td>NB-E</td>
<td>2.9 ± 0.6</td>
</tr>
<tr>
<td>UV-DNA</td>
<td>Ca2+-phosphate</td>
<td>Single-cycle</td>
<td>NB-E</td>
<td>2.6 ± 0.4</td>
</tr>
<tr>
<td>UV-DNA</td>
<td>DEAE-dextran</td>
<td>Infectious center</td>
<td>NB-E</td>
<td>3.0 ± 1.3</td>
</tr>
<tr>
<td>UV-DNA</td>
<td>DEAE-dextran</td>
<td>Infectious center</td>
<td>RL5E</td>
<td>3.0 ± 1.4</td>
</tr>
<tr>
<td>UV light</td>
<td>—</td>
<td>Infectious center</td>
<td>NB-E</td>
<td>2.2 ± 0.4</td>
</tr>
</tbody>
</table>

*Cells were either transfected with UV-irradiated (1,500 J m−2) calf thymus DNA (UV-DNA) or exposed directly to UV light (4.5 J m−2) before virus infection.

EM is the ratio of the virus mutation frequency in cells transfected with UV-irradiated DNA to that in cells transfected with unirradiated DNA. Results are the average values and standard deviations from four experiments.

**TABLE 2.** Comparison between the abilities of UV-irradiated ori+ and ori− SV40 DNA fragments to trigger mutator activities in human and rat cells.

<table>
<thead>
<tr>
<th>Cells</th>
<th>SV40-DNA fragment*</th>
<th>Relative mutation frequency</th>
<th>EM</th>
</tr>
</thead>
<tbody>
<tr>
<td>RL5E</td>
<td>ori+</td>
<td>1.7 ± 0.4</td>
<td>1.4</td>
</tr>
<tr>
<td>RL5E</td>
<td>ori−</td>
<td>1.7 ± 0.5</td>
<td>1.4</td>
</tr>
<tr>
<td>NB-E</td>
<td>ori+</td>
<td>2.0 ± 0.8</td>
<td>1.8</td>
</tr>
<tr>
<td>NB-E</td>
<td>ori−</td>
<td>2.2 ± 1.2</td>
<td>2.2</td>
</tr>
</tbody>
</table>

*Cells were transfected (calcium-phosphate technique) with UV-irradiated (1,500 J m−2) ori+ or ori− SV40 DNA (2 μg/10⁶ cells) for 6 h (RL5E) or 18 h (NB-E).

**TABLE 3.** Differences in the frequency of H-1 ts6 revertants in UV-irradiated and unirradiated DNA sources.

<table>
<thead>
<tr>
<th>Cells</th>
<th>SV40-DNA fragment</th>
<th>Relative mutation frequency</th>
<th>EM</th>
</tr>
</thead>
<tbody>
<tr>
<td>RL5E</td>
<td>ori+</td>
<td>1.7 ± 0.4</td>
<td>1.4</td>
</tr>
<tr>
<td>RL5E</td>
<td>ori−</td>
<td>1.7 ± 0.5</td>
<td>1.4</td>
</tr>
<tr>
<td>NB-E</td>
<td>ori+</td>
<td>2.0 ± 0.8</td>
<td>1.8</td>
</tr>
<tr>
<td>NB-E</td>
<td>ori−</td>
<td>2.2 ± 1.2</td>
<td>2.2</td>
</tr>
</tbody>
</table>

*Cells were transfected with UV-irradiated (1,500 J m−2) or unirradiated SV40 DNA (2 μg/10⁶ cells) for 6 h.
induction phenomenon demonstrated in *Escherichia coli*. Certain SOS functions, including enhanced mutagenesis, can be induced indirectly in unirradiated bacteria after conjugational transfer of UV-damaged DNA (8). However, this analogy does not necessarily imply that similar mechanisms are involved in the induction (see below) and operation (4) of mutator functions in bacteria and mammalian cells.

The EM signal is probably produced by the structure itself rather than by the expression of the exogenous DNA since it was obtained with DNAs of various origins and occurred at a higher level when the DNA was UV irradiated. Modified DNA structures resulting from UV damage might have signalling properties. Alternatively, the inducing signals might be by-products of the repair or replication of irradiated DNA, such as oligodeoxyribonucleotides, gaps, or relaxed nucleoids. DNA degradation could also confer a mutator phenotype to cells by disturbing the pools of DNA precursors. Although the actual signal has not been identified, the present study provides data restricting the spectrum of candidate structures.

**Role of single-stranded DNA.** Unirradiated DNA displayed little triggering capacity, irrespective of whether it was single or double stranded. Thus, in this system, single-strandedness does not seem to be sufficient to confer a signalling capacity to DNA. In contrast, a variety of single-stranded polynucleotides were found to act as cofactors in the pathway leading to prophage induction in bacteria, a function belonging to the same SOS regulatory system as enhanced capacity for mutagenesis (12).

**Role of DNA lesions.** The signalling capacity of DNA, whether single or double stranded, increased with its exposure to UV irradiation. However, the induction process might be saturable because the EM response levelled off when irradiation was administered to increasing fractions of the DNA inoculum. Although its magnitude was low, the mutator effect of unirradiated DNA was significant and might account, at least in part, for the small increase of mutagenesis of cellular genes after transfection with intact SV40 DNA (21).

**Role of DNA replication.** The EM response could be activated by UV-damaged DNA under conditions in which the DNA is unlikely to replicate. These conditions include transfection of nonpermissive rat cells with either bacteriophage DNA or SV40 DNA deprived of a functional origin of DNA replication. Moreover, we have reported previously that a UV-irradiated thermosensitive early mutant of SV40 was able to confer a mutator phenotype to unirradiated rat cells at the restrictive temperature (3). Together, these results strongly suggest that replication of damaged DNA is not a prerequisite for the formation of an EM signal. Similarly, D’Ari and Huisman (6) have identified a pathway leading to the induction of a bacterial gene belonging to the SOS regulatory system, in the absence of DNA replication. However, this pathway constitutes a minor component of overall SOS induction, most of which results from the attempt to replicate damaged DNA. In contrast, our data indicate that DNA replication does not contribute significantly to EM activation by exogenous DNA in mammalian cells. Indeed, the signalling capacity of UV-irradiated SV40 DNA in NB-E cells were independent of the presence of a functional viral replication origin, although these cells continuously express the large T antigen required for the initiation of SV40 DNA replication (18). It remains to be determined whether the lack of a role of signalling DNA replication in EM induction is specific for exogenous DNA or also applies to endogenous cellular DNA.

**ACKNOWLEDGMENTS**

We are indebted to J. Hertoghs for help and to M. Errera for support. This work was supported by grants 3.4514.80 (Fonds National Belge de la Recherche Médicale), G/826/81 (Ministère Belge de la Santé Publique), and BIO-359-B and ENV-355-B (European Communities) and by an Action de Recherche Concertée (Services Belges de Programmation de la Politique Scientifique). B.K. and A.J.v.d.E. were supported by Euratom Grant BIO-E-405-N and by the Institute of Radiopathology and Radiation Protection (Leiden). J.R. is a “Chercheur Qualifié” of Fonds National de la Recherche Scientifique de Belgique.

**LITERATURE CITED**

18. Schaffhausen, B. 1982. Transforming genes and gene products of...


