MOLECULAR AND CELLULAR BIOLOGY, June 1984, p. 1159–1162
0270-7306/84/061159-04$02.00/0

Vol. 4, No. 6

UV Irradiation Induces an Activity Which Stimulates Simian Virus 40 Rescue upon Cell Fusion

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UV irradiation of African green monkey cells greatly stimulated efficiency of simian virus 40 induction from simian virus 40-transformed Syrian hamster cells after cell fusion. The maximum inducing activity was observed at 15 to 20 h after irradiation but remained only transiently. The addition of cycloheximide after UV irradiation eliminated the stimulation of the activity.

When UV light-irradiated bacterial plasmids are transferred to lysogenized *Escherichia coli*, induction of the phage subsequently occurs from the recipient bacteria (1). This indirect induction, as opposed to direct induction in which induction of the phage occurs after direct UV irradiation of lysogenized bacteria, has provided a unique opportunity to study the molecular events leading to the induction of prophage. In mammalian cells, certain viruses which are persistently present in host cells are induced after treatment of the cells by DNA-damaging agents (2, 10), halogenated pyrimidines (4, 5), or tumor promoters (12). Although induction of simian virus 40 (SV40) by DNA-damaging agents is observed in semipermissive cells, such as Syrian hamster kidney cells (2, 6, 8, 10), the induction is not detected in nonpermissive cells, including a variety of SV40-transformed rat and mouse cell lines. On the other hand, SV40 can be induced (rescued) from SV40-transformed nonpermissive as well as semipermissive cells by cell fusion with permissive cells, such as African green monkey cells (3). This suggests the presence of a *trans*-acting factor in the monkey cells which somehow triggers the excision or expression of the SV40 genome integrated in the chromosome of semi- and nonpermissive cells. The nature and the regulation of synthesis of such a hypothetical inducing factor is still unknown. We undertook a series of experiments to examine whether the activity present in the monkey cells is further increased in response to DNA damage, as can be suggested from indirect induction of bacteriophage λ.

MATERIALS AND METHODS

Cell culture and UV irradiation. African green monkey cells (C7) were grown at 37°C with 5% CO_2 in air to a cell density of 10^6 per dish in plastic petri dishes (100 by 20 mm) which each contained 15 ml of Eagle minimum essential medium (MEM; Nissui Seiyaku) supplemented with 10% calf serum (Granite). All sera were heat inactivated before use. After removal of the medium by suction and rinsing the cells once with phosphate-buffered saline (137 mM NaCl, 4.2 mM KCl, 9.6 mM Na_2HPO_4, 1.1 mM KH_2PO_4), the plates were placed under a germicidal lamp (15 W; Toshiba-GL15) at a distance of 52 cm for 15 s, which gave a UV intensity of 20 J/m^2. After irradiation, 15 ml of fresh MEM (containing 10% calf serum) was added to each dish and further incubated for 16 h before cell fusion. SV40-transformed Syrian hamster kidney cells (E line, kindly provided by P. H. Black) were inoculated at a density of 3×10^5 per ml in plastic petri dishes (60 by 15 mm) which each contained 5 ml of fresh MEM supplemented with 10% fetal calf serum (Flow Laboratories, Inc.). The cultures were maintained at 37°C with 5% CO_2 in air for 3 days.

Cell fusion. For cell fusion (9), both C7 and hamster cells were each trypsinized and resuspended in 5 ml of MEM (containing 10% fetal calf serum) at cell densities of 1×10^6 and 5×10^6, respectively. The two samples were then mixed, centrifuged ($800 \times g$ for 10 min), and washed once with 5 ml of phosphate-buffered saline. To the pellet, 0.5 ml of MEM containing 50% (wt/wt) polyethylene glycol 6000 (J. T. Baker Chemical Co.) was added. After 2 min, cells were resuspended in MEM (2.5 ml) by gentle mixing and left for 3 min at room temperature. MEM (10 ml) supplemented with 10% fetal calf serum was then added, and the cells were collected by centrifugation ($500 \times g$ for 5 min). After the addition of 70 ml of fresh MEM containing 10% fetal calf serum, the fused cells were incubated in plastic petri dishes (100 by 20 mm) at 37°C in a CO_2 incubator. At days 0, 1, 2, 3, 4, 5, and 7, the cells were collected by a rubber policeman and disrupted by sonication (1.8 A for 5 min; model 200 M, Kubota), and the number of infectious SV40 virus particles in the extract was assayed.

Assay of infectious SV40 particles. For assaying infectious SV40 virus particles, the cell extracts (200 μl each) after appropriate dilution were mixed with C7 (African green monkey kidney) cells (4×10^5 to 5×10^5 cells) which had been grown to confluence in Eagle MEM supplemented with 10% calf serum (Granite) at 37°C. After 2 h of virus absorption with occasional gentle rocking, the samples were transferred to 5 ml of solid medium (MEM supplemented with 10% calf serum and 0.9% Noble agar [Difco Laboratories]). After 5 days at 37°C with 5% CO_2 in air, 3 ml of the same medium containing 5% calf serum was overlaid on top of the solid medium. The samples were further incubated for 3 days under the same conditions, and 2 ml of MEM with 0.9% agar was again overlaid. After 2 to 3 days, 3 ml of MEM (0.9% agar) containing neutral red (0.016%) was added to each dish, and the number of plaques was scored the next day. Usually, the dilution of the original extracts was made to give between 10 and 100 plaques per dish, and samples with standard infectious SV40 viruses were always included in the assay.

RESULTS AND DISCUSSION

When African green monkey cells (C7), which are permissive to SV40 infection, were irradiated with UV light (20 J/m^2) before cell fusion, the induction (rescue) of SV40 infectious particles from the fused SV40-transformed Syrian

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TABLE 1. Effect of UV irradiation of C7 cells on SV40 induction after cell fusion with Syrian hamster kidney cells

Day after fusion	No. of plaques[a]		
	−UV (A)	+UV (B)	(B)/(A)
0	<10	<10	
1	<10	<10	
2	7.2×10	1.5×10^2	2.1
3	2.6×10^3	8.4×10^3	3.2
4	1.9×10^5	9.2×10^5	4.8
5	3.7×10^6	1.6×10^7	4.3
7	4.2×10^6	2.1×10^7	5.0

[a] Total number of the infectious virus particles produced in each fusion experiment. Details of the experiments are described in the text.

hamster kidney cells was greatly increased. Table 1 presents experimental results in which the number of SV40 infectious particles was measured as a function of time of incubation after cell fusion between monkey cells (C7) and SV40-transformed Syrian hamster kidney cells (E line). UV irradiation of the monkey cells before cell fusion increased the production of the infectious SV40 particles severalfold (Table 1). A similar stimulatory effect of UV irradiation was observed when SV40-transformed nonpermissive cells such as rat W3Y cells (derived from 3Y1 rat embryo) or mouse W2K cells (derived from C3H2K mouse fibroblast cells)

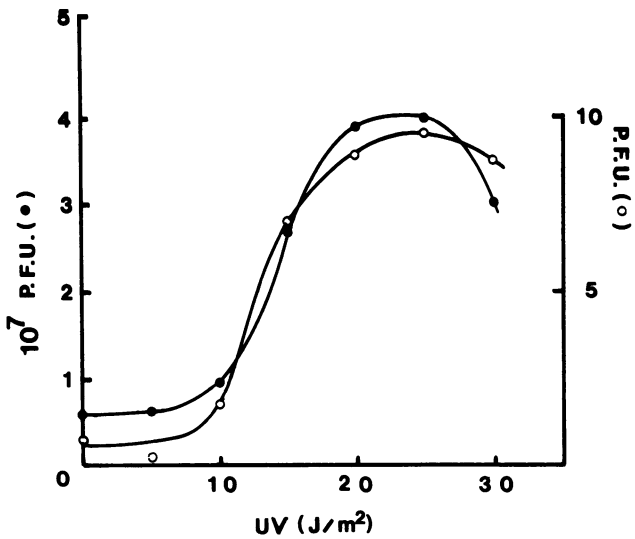

FIG. 1. Effect of UV dose on SV40 induction. For indirect induction (rescue) (●), C7 cells were irradiated by UV light as described in the text but with various intensities as shown on the abscissa. After cell fusion with the hamster cells, the cells were disrupted at day 7, and infectious SV40 particles were scored. PFU are the total number of the infectious virus particles produced in each fusion experiment. For direct induction (○), Syrian hamster kidney cells were inoculated at a density of 3×10^5 cells per ml into plastic petri dishes (60 by 15 mm) each with 5 ml of fresh MEM supplemented with 10% fetal calf serum. After 2 days at 37°C, the medium was removed by suction, and the cells were rinsed once with phosphate-buffered saline. The samples were then irradiated by UV light with various intensities as shown on the abscissa. After adding fresh medium (5 ml), the samples were incubated for 5 days, and infectious virus particles in the disrupted cell extracts were assayed as described in the text. PFU are expressed as the number per 0.1 mg of protein of the UV-irradiated cells. For details, see the text.

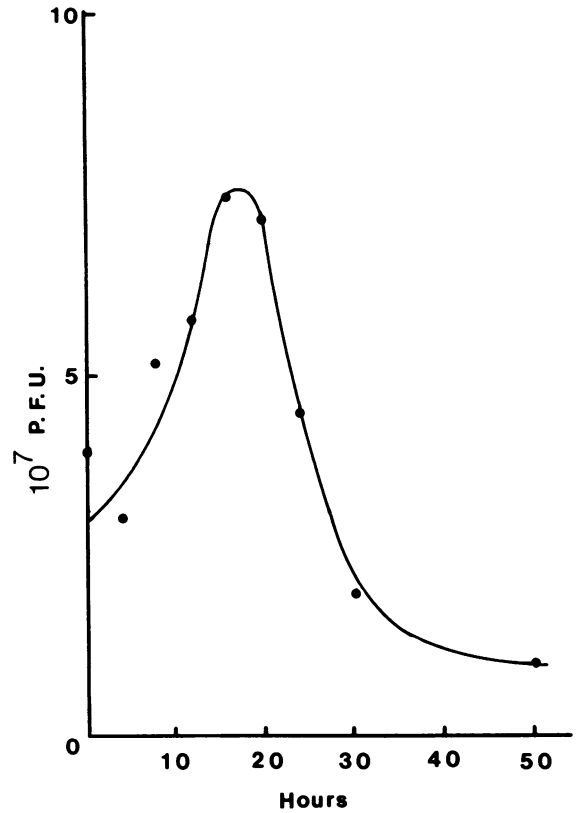

FIG. 2. SV40 induction as a function of time intervals between UV irradiation and cell fusion. C7 cells were irradiated by UV light at a UV dose of 20 J/m² as described in the legend to Fig. 1. The cells were then incubated for different lengths of time (see abscissa) before cell fusion. At day 7 after fusion, the cells were disrupted, and infectious SV40 particles were scored. PFU are the total number of infectious virus particles produced in each fusion experiment. The number of plaques in a control (no UV irradiation) experiment was 6.2×10^6. For details, see the text.

were used in place of the hamster cells. Furthermore, treatment of the monkey cells with other DNA-damaging agents such as mitomycin C (0.5 μg/ml for 16 h) or bleomycin (1 μg/ml for 16 h) also increased the rescue of SV40 from the hamster cells upon cell fusion (data not shown).

The stimulatory effect of UV irradiation on SV40 induction by cell fusion was affected by UV dosage as had been expected. The maximum stimulatory effect was observed between 20 and 25 J/m², whereas almost no stimulatory effect was seen at UV dosages below 10 J/m² (Fig. 1). This dose-response curve has a shape similar to the curve obtained in SV40 induction by direct UV irradiation of the SV40-transformed hamster cells, although in the latter case the induction efficiency was much lower than that obtained by cell fusion (Fig. 1). This suggests that the increased activity induced by UV irradiation in monkey cells may be closely related to the reaction which leads to SV40 induction by direct irradiation of Syrian hamster cells. We could not observe any synergistic effect by irradiating both monkey and hamster cells.

The stimulatory effect of UV irradiation on SV40 rescue was greatly affected by the timing of cell fusion after irradiation (Fig. 2). Although the stimulatory activity was already seen with the cells which were fused immediately after UV irradiation, the maximum stimulatory activity was

observed when the irradiated cells were fused at 15 to 20 h after irradiation, suggesting an inducible nature of the activity. Interestingly, the activity decreased sharply thereafter and reached near the control value (no UV irradiation) when fusion was performed at more than 30 h after UV irradiation. Thus, the stimulatory activity induced by UV irradiation in monkey cells exhibits a transient nature which lasts for ca. 30 h after the irradiation. The substantial activity demonstrated with the cells immediately after UV irradiation does not necessarily mean that the activity was produced immediately after the irradiation. It is possible that the activity was induced in the fused cells by damaged chromosomes which had been introduced from the irradiated C7 cells.

This apparent inducible and transient nature of the stimulatory activity in C7 cells prompted us to examine whether de novo protein synthesis is required for the increase of the activity after UV irradiation. The presence of cycloheximide during postirradiation incubation (16 h) before cell fusion completely eliminated the increase of the activity by UV irradiation, and cycloheximide had no effect on the SV40-inducing activity originally present in C7 cells (Fig. 3). These results suggest that de novo protein synthesis is required for the induction of the stimulatory activity by UV irradiation. It should be noted that the inhibitory effect of cycloheximide was not reversed even after the drug was removed from the medium before cell fusion (Fig. 3). This suggests that lesions in DNA produced by UV irradiation were either completely repaired by constitutive repair enzymes or became inoperative in expressing the stimulatory effect during a 16-h period after irradiation.

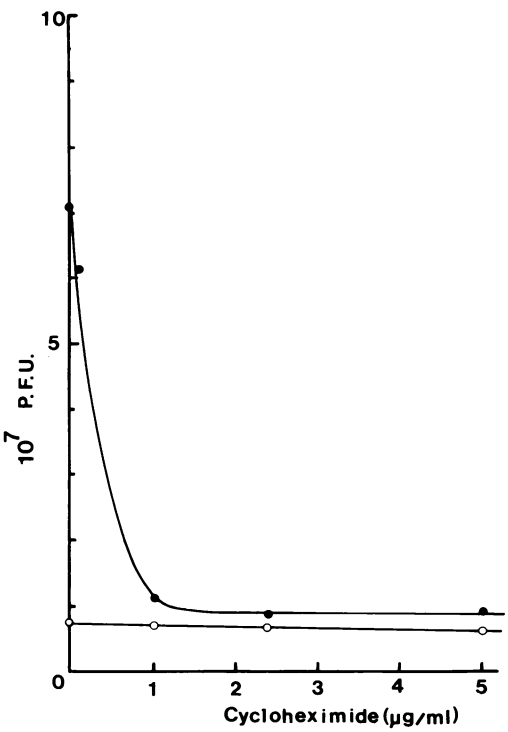

FIG. 3. Effect of cycloheximide on SV40 induction. C7 cells were irradiated by UV at an intensity of 20 J/m^2 as described in the legend to Fig. 1. Immediately after irradiation, various concentrations (see abscissa) of cycloheximide were added to the medium and incubated for 16 h. After cell fusion with hamster cells, the cells were disrupted at day 7, and infectious SV40 virus particles were scored. PFU are the total number of infectious virus particles produced in each fusion experiment. For details, see the text.

Two possibilities may be considered concerning the nature of the UV-inducible factor in C7 cells. A new factor may be induced in C7 cells after UV irradiation. This factor acts independently from or in concert with the preexisting monkey factor in inducing SV40 from fused cells. Alternatively, the intracellular level of the factor already present in monkey cells may be further increased in response to DNA damage. However, the stimulatory activity induced by UV irradiation was decreased to the level of the original activity 30 h after irradiation. It is more likely, therefore, that a new factor with a short life-span is induced in response to DNA damage. This may be a unique factor present only in monkey cells or a factor identical to replication or excision enzymes normally induced during virus replication in the recipient cells. However, from the experimental results described above alone, it is difficult to depict the precise mechanism of the stimulatory effect of UV irradiation on SV40 rescue upon cell fusion. For example, since we measured only the number of infectious particles, we could not conclude whether the stimulatory effect was a result of enhancement of excision or replication (or both) of SV40.

In similar experiments, Watkins reported that UV irradiation of CV-1 cells had no effect on their SV40 rescue ability in transformed baby rabbit kidney cells, but the irradiation apparently destroyed the ability in mouse 3T3 cells (11). Although his results are not consistent with ours, he used a much higher UV dosage (40 and 100 J/m^2), and the cells were fused immediately after irradiation. Those conditions were quite distinct from our optimum conditions to obtain stimulating activity (Fig. 1 and 2). It is also possible that the apparent discrepancy is due to the difference of transformed cell lines used. In cell fusion experiments similar to ours, Lambert et al. (7) recently reported that synthesis of polyoma DNA in rat fibroblast cells was stimulated by cell fusion with the fibroblast cells which had been treated with carcinogens such as benzo[*a*]pyrene. Although their conclusion is generally consistent with ours, the induced potential seemed to have a much longer half-life than ours. This may reflect either an induction of a different factor or differences in the cells employed.

In any event, the experimental results presented in this paper indicate considerable similarities between indirect induction of bacteriophage λ and SV40 induction by cell fusion with UV-irradiated cells. Both inductions are triggered or stimulated by DNA-damaging agents, the induced activity exhibits a transient nature, and de novo protein synthesis is required for the maximum induction of the activity. One may be tempted to suggest that a mechanism similar to λ induction is also exercised in SV40 induction. However, the fundamental mechanism of virus induction in mammalian cells is different from that of bacteriophage induction. Virus induction is likely to be controlled by positive factors, such as DNA replication enzymes, rather than by negative factors (repressors) which control bacteriophage induction.

ACKNOWLEDGMENTS

We thank N. Shobu for technical assistance and M. Harada for help during preparation of the manuscript. We are grateful to K. Oda for advice and to P. H. Black for sending us the Syrian hamster clone E used in this study.

This research was supported by grants from Nissan Science Foundation, Ministry of Education, and Ministry of Health and Welfare of Japan.

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