DNA REPAIR CAPACITY AND RATE OF EXCISION REPAIR IN UV-IRRADIATED MAMMALIAN CELLS

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DNA repair capacity in UV-irradiated cultured mammalian cells has been demonstrated by colony-forming ability (Takebe *et al.* 1972; Takebe *et al.* 1974; Kraemer *et al.* 1976), host-cell reactivation (HCR) of UV-irradiated virus (Lytle 1971; Lytle *et al.* 1972; Takebe *et al.* 1974; Cleaver and Bootsma 1975), unscheduled DNA synthesis (UDS) using cellular autoradiography (Rasmussen and Painter 1966; Cleaver 1968; Clarkson and Evans 1972), pyrimidine dimer excision (Regan *et al.* 1968; Cleaver and Trosko 1970; Takebe *et al.* 1974), single strand breaks of DNA using density gradient centrifugation (Takebe *et al.* 1974; Paterson and Lohman 1975), and sister chromatid exchanges (Sasaki 1977; de Weerd-Kastelein *et al.* 1977).

In our previous work (Takebe *et al.* 1974), BSC-1 (African green monkey) cells showed the highest capacity of HCR but more sensitive colony-forming ability to UV-irradiation than other cell lines with normal repair capacity. A similar cell line, CV-1, has also been shown to have such high HCR capacity (Lytle 1971). This paper intends to extend the studies by Takebe *et al.* (1974) to elucidate these characteristics of CV-1 cells in comparison with other cell lines. Slow rate of excision repair could be one of the important factors involved in the unique characteristics of CV-1 cells.

MATERIALS AND METHODS

Cell strains

The cell strains used were N6 and N1AW, normal human skin fibroblast cells; XP10S, skin fibroblast cells of a xeroderma pigmentosum (XP) patient belonging to a genetic complementation group A (Takebe *et al.* 1977); CV-1, African green monkey kidney origin; FL, human amnion origin; and L, mouse connective tissue.

Human fibroblast cells, FL and CV-1 cells were obtained from Department of Fundamental Radiology, Faculty of Medicine, Osaka University. Mouse L cells were kindly supplied by Dr. M. Horikawa, Division of Radiation Biology, Faculty of Pharmaceutical Sciences, Kanazawa University.

Virus

Herpes simplex virus, type I (Takebe *et al.* 1974) grown on FL cells was used for HCR experiments.

Media

The growth medium was Eagle's MEM from GIBCO and Flow Laboratories supplemented with 15% fetal calf serum for human fibroblasts, 10% for CV-1 cells and 10% bovine calf serum for other strains. Vitamines and amino acids in the medium for human fibroblasts were doubled from the original formula.

Culture conditions

All cells were maintained in glass bottles with silicon rubber stoppers. The cells for experiments were incubated in plastic petridishes (Falcon, 6 cm, except for UV-irradiation; Lux, 6 cm Contur dishes for UV-irradiation) in 10% CO₂ for human fibroblasts and 5% CO₂ for other strains at 37°C. The Contur dishes had UV (254 nm) transmittancy of 49%. For autoradiography, cells were cultured either on a cover slip in a petri-dish or in Lab-Tek chambers (Miles). The plating efficiency was more than 90% for FL and L cells, about 50% for CV-1 cells, less than 10% for human fibroblasts.

Assay of virus

The virus suspension in PBS was added to the monolayer cells in petri-dishes. Adsorption of virus was carried out for 90 min at 37°C by continuous slow agitation of the culture. After adsorption, 4 ml of medium containing 0.25% human γ -globulin (Nutritional Biochemicals) was added for prevention of virus movement. After plaque formation, cells were fixed with methanol and stained with Giemsa solution (Merk). Plaques were scored on the 3 to 5th day of incubation.

UV irradiation

The UV light source was two 15 watt mercury vapor germicidal lamps (Mitubishi). The fluence was measured by a UV photometer (Topcon UVR-254) which was calibrated against a standard lamp.

The cells were exposed to UV through the bottom of Lux Contur petri-dishes. The virus suspension was exposed to UV from above in an open 10 cm petri-dish with continuous agitation at 0°C. The cells cultured in a Lab-Tek chamber for cellular autoradiography were washed with PBS and irradiated from above.

Autoradiography

For the detection of UDS, the cells in a Lab-Tek chamber were incubated in a culture medium containing 10 μ Ci/ml of ³H-thymidine (5 Ci/mmol, Radiochemical Centre, Amersham) for 3 h immediately after UV-irradiation. After labeling, the cells were washed twice in PBS, fixed in methanol, washed in cold 5% TCA and dehydrated with ethanol. After dried at room temperature, the cells were dipped in nuclear emulsion (Kodak NTB-3) at 41°C. After exposing in a dark box for 1 week at 4°C, they were developed by Kodak D-19 and stained with Giemsa solution.

For the measurement of the rate of UDS, the cells were pulse-labeled for 3 h in a culture medium containing 10 μ Ci/ml of ³H-thymidine at various intervals after UV-irradiation.

The rate of semi-conservative DNA synthesis was measured by pulse-labeling the cells for 15 min in a culture medium containing 0.2 μ Ci/ml of ³H-thymidine at various intervals after UV-irradiation. The procedures for pulse-labeling of unirradiated cells

Fig. 1. Survival curves for colony-forming ability of different cells. Cells were irradiated 24 h after inoculation in petri dishes.

were identical to the irradiated sample except for irradiation. This method was essentially the same as described by Rudé and Friedberg (1977). Mean rate of DNA synthesis per cell was measured by the number of silver grains in labeled nuclei, with mean background of 0.2 grains per nucleus in the cells not in S phase. The background may be due to UDS which was not detectable by this procedure because of the low activity and short period of labeling.

Measurement of thymine-containing dimers

The method described by Ikenaga *et al.* (1970) was used for the assay of thymine-containing dimers. Cells were labeled in a culture medium containing $1 \mu\text{Ci/ml}$ of ^3H -thymidine for 24 h before UV-irradiation. After UV-irradiation, the cells were washed with PBS, collected by trypsinization at various intervals during subsequent 24 h growth in a fresh medium. The collected cells were washed with PBS, sedimented in cold 10% TCA, washed with 3:1 ethanol-water, and then dried in vacuum. Each sample was hydrolyzed by trifluoroacetic acid for 90 min at 170°C in a flame-sealed Pylex tube. The hydrolyzed sample was put on a chromatographic paper (Whatman No. 1, 3×60 cm) in a line 12 cm from one end. The paper was developed by descending chromatography for about 16 h using a solvent composed of 86:16 butanol-water. After drying

Fig. 2. Survival curves for UV-irradiated Herpes simplex viruses in different cells.

the paper was cut into pieces 6 mm wide, and the radioactivity was measured by an Aloka liquid scintillation counter using Bray's solution.

RESULTS

Colony-forming ability and HCR

Comparison of UV survivals in different cell strains measured by colony-forming ability is shown in Fig. 1. FL and L cells were the most resistant to UV, whereas CV-1 cells were intermediate and XP cells were the most sensitive.

Comparison of UV survival of Herpes simplex virus assayed in different cell strains is shown in Fig. 2. CV-1 cells, FL and normal human fibroblast cells were efficient in HCR of UV-irradiated virus, while L and XP cells were reduced in HCR.

The UV inactivation of colony-forming ability was not always consistent with the HCR. As shown in Figs. 1 and 2, the XP cells had the highest sensitivity for both, while mouse L cells showed greatly reduced HCR and normal UV survival of colony-forming ability. CV-1 cells with high HCR capacity were intermediate in UV survival.

UDS

The amount of UDS in four cell strains after UV-irradiation with various doses

Fig. 3. The amount of UDS in different cells after UV-irradiation with various doses. The grains per nucleus on ordinate are the average of 100 nuclei.

is shown in Fig. 3. Relative amount in UDS appears to reflect the relative UV sensitivity in different cells as measured by colony-forming ability in Fig. 1. Fig. 3 represents UDS during the first 3 h after UV-irradiation.

The rate of UDS during post-UV incubation was shown in Fig. 4. The rate for CV-1 cells was slower than other cells.

Excision of pyrimidine dimers

Excision of thymine-containing pyrimidine dimers in various cells after UV-irradiation is shown in Fig. 5. The rate of pyrimidine dimer excision was higher in FL and normal human fibroblast cells than in CV-1 cells, and no excision was observed in XP cells. This also corresponds to UV survivals and the UDS.

Suppression and recovery in DNA synthesis after UV-irradiation

DNA synthesis after UV-irradiation measured by cellular autoradiography is shown in Fig. 6. The DNA synthesis was suppressed until 4 h after UV-irradiation for FL and normal human fibroblast cells, and subsequently recovered. CV-1 cells recovered slowly from 6 h followed by quick recovery after 12 h after UV-irradiation, but no recovery was observed for XP cells.

Fig. 4. Rate of UDS during post-UV incubation in different cells. Cells were pulse-labeled for 3 h in a culture medium containing $10 \mu\text{Ci/ml}$ of ^3H -thymidine at various intervals after UV-irradiation with 30 J/m^2 . Rate of UDS determined by the mean grain counts per nucleus was plotted against incubation time as a percentage of grain counts at zero-time after UV-irradiation.

DISCUSSION

HCR of UV-irradiated phages in bacteria, in general, has been regarded to reflect the excision repair capacity of the host cell (Boyle and Setlow 1970). The results in Fig. 2 are consistent with this correlation; XP and mouse L cells, both reduced in excision repair (Cleaver 1968; Cleaver and Trosko 1970; Lehman 1972; Fujiwara and Kondo 1974) showed low HCR capacity. Normal UV sensitivity of L cells in UV survival as shown in Fig. 1, despite the reduced excision repair, suggests that an additional repair system may be functioning in mouse L cells, possibly gap-filling post-replication repair (Lehman 1972; Fujiwara and Kondo 1974). CV-1 cells, which had a high capacity of HCR, was intermediate for UV sensitivity in colony formation. CV-1, GMK and BSC-1 cells, all originating from African green monkey kidney, have been reported to have a high efficiency of HCR (Lytle 1971; Takebe *et al.* 1974). As shown

Fig. 5. Rate of pyrimidine dimer excision during post-UV incubation in different cells. Relative amount of pyrimidine dimer remaining in acid insoluble fraction was plotted against post-incubation times after UV-irradiation with 22.5 J/m².

in Fig. 3, the dose response of UDS for CV-1 cells has an intermediate value between XP and other normal cells. This also reflected the UV sensitivity of CV-1 cells.

CV-1 cells had an intermediate level of excision of thymine-containing dimers between XP and other cells of normal repair capacity (Fig. 5). This corresponded to the results of UDS and UV sensitivity measured by colony-forming ability.

The time course of the repair replication after UV-irradiation was reported in HeLa cells by Edenberg and Hanawalt (1973), and in human leucocyte by Clarkson and Evans (1972). Our results (Fig. 4) of the rate of UDS after UV-irradiation for FL and normal human fibroblast cells are not significantly different from their results. The rate of UDS for CV-1 cells, however, is slower than FL and normal human fibroblast cells during early period of incubation, and the repair synthesis continued as long as 24 h after UV-irradiation. This could correspond to the previous report (Takebe *et al.* 1974) that the rejoining of DNA strands in BSC-1 cells, the same origin as CV-1 cells, were slower than FL cells after UV-irradiation using alkaline sucrose gradient centrifugation. The slow rate of UDS in CV-1 cells appears to coincide with the slower rate of dimer excision than FL and normal human fibroblast cells (Fig. 5).

The quick decline in the rate of UDS as shown in Fig. 4 is not reflected in the rate of excision of pyrimidine dimers in Fig. 5. If we assume the process of excision repair as excision of dimers followed by patches (UDS), the rate of excision should be identical or faster than the rate of UDS. Since this is not the case with all three cell

Fig. 6. DNA synthesis during post-UV incubation in different cells. Cells were pulse-labeled for 15 min in a culture medium containing $0.2 \mu\text{Ci/ml}$ of ^3H -thymidine at various intervals after UV-irradiation with 15 J/m^2 . Relative amount of DNA synthesis in UV-irradiated cells and control (NO UV) cells as measured by mean number of grains per nucleus was plotted against incubation times after UV-irradiation.

lines tested in our experiments, the patching of the excised gaps may proceed faster than the removal of the dimers from the acid insoluble fractions which are likely to be DNA strands. Therefore, "patch and cut" process is more likely than "cut and patch" as an explanation for the data as shown in Figs. 4 and 5.

Kinetic studies of DNA replication in UV-irradiated mammalian cells have been reported by Edenberg (1976), Rudé and Friedberg (1977) and Meyn *et al.* (1977). Edenberg (1976) reported that the inhibition of DNA synthesis in UV-irradiated cells could be explained by replication fork halting at pyrimidine dimer regions using DNA fiber autoradiography and density labeling techniques. Rudé and Friedberg (1977) suggested that the recovery of DNA synthetic capacity after UV-irradiation is critically dependent on a cell's capacity to excise pyrimidine dimers. In synchronized Chinese hamster

ovary cells, Meyn *et al.* (1977) have demonstrated that the rate of DNA replication after UV-irradiation is depressed during the first S phase, but completely recovered in the second S phase. And they supposed that the recovery in the second S phase is closely related to post-replication repair.

Our results generally support these observations. Our comparative studies using different cell strains, however, suggest that more complicated mechanisms could be involved. As demonstrated clearly by the data on XP cells, excision repair should be responsible for the all recovery processes observed in this study. Additional factors should be taken into account in the case of CV-1 cells which behaved differently although they are basically proficient in excision repair as shown by dimer excision experiments as well as by HCR. The rate of excision repair, although the difference is small as shown in Fig. 4, appears to be one of the important factors which influence recovery of semi-conservative DNA replication after UV-irradiation. The possible involvement of post-replication repair in this phenomenon may be not ruled out since our experimental procedures were not aimed to investigate it.

A high HCR capacity in CV-1 cells, despite the low excision repair capacity might be due to the factors concerning the replication of viral DNA in the host cells after infection as discussed by Takebe *et al.* (1974).

SUMMARY

Repair capacities of five mammalian cell strains were measured by colony-forming ability, HCR of UV-irradiated virus, UDS, pyrimidine dimer excision, and semi-conservative DNA replication. Colony-forming ability of UV-irradiated cells was high for human amnion FL cells and mouse L cells, slightly low for African green monkey CV-1 cells, and extremely low for xeroderma pigmentosum cells. HCR of UV-irradiated Herpes simplex virus was high in CV-1 cells, FL and normal human fibroblast cells, low in both XP and L cells. The amount of UDS was high in FL and normal human fibroblast cells, considerably low in CV-1 cells, and essentially no UDS was observed in XP cells. Rate of UDS after UV-irradiation was slower for CV-1 cells than FL and human fibroblast cells. Rate of the excision of thymine-containing dimers from the acid-insoluble fraction during post-irradiation incubation of the cells was rapid in FL and normal human cells and slow in CV-1 cells, and no excision took place in XP cells.

Semi-conservative DNA synthesis was reduced after UV-irradiation in all cell lines, but subsequently recovered in FL, normal human and CV-1 cells. The onset of recovery was 4 h after UV-irradiation for FL and normal human cells, but about 6 h for CV-1 cells. The apparent intermediate repair capacity of CV-1 cells except for HCR may be related to the slow rate of excision repair. "Patch and cut" model is more favorable than "cut and patch" model to elucidate these results.

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