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Comparative studies of host-cell reactivation, cellular capacity and enhanced reactivation of herpes simplex virus in normal, xeroderma pigmentosum and Cockayne syndrome fibroblasts *

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Summary

Host-cell reactivation (HCR) of UV-irradiated herpes simplex virus type 2 (HSV-2), capacity of UV-irradiated cells to support HSV-2 plaque formation and UV-enhanced reactivation (UVER) of UV-irradiated HSV-2 were examined in fibroblasts from 4 patients with Cockayne syndrome (CS), 5 with xeroderma pigmentosum and 5 normals. All UV-survival curves for HSV-2 plaque formation showed 2 components. HCR was similar to normal for the XP variant strain and the 2 CS strains tested, but substantially reduced in the 4 excision-deficient XP strains. The capacity of UV-irradiated fibroblasts to support HSV-2 plaque formation was determined by UV-irradiating fibroblast monolayers with various doses of UV and 48 h later, infecting the monolayers with unirradiated HSV-2. The D_{37} values for the delayed-capacity curves so obtained were in the range 8.6–12.4 J/m^2 for the normal strains, 2.8–3.2 J/m^2 for the CS strains, 6.7 J/m^2 for an XP variant strain and between 0.3 and 1.5 for the XP excision-deficient strains tested. These results indicate that delayed capacity for HSV-2 plaque formation is a more sensitive assay than HCR in the detection of cellular DNA-repair deficiency for XP and CS. For the examination of UVER, fibroblasts were irradiated with various UV doses and subsequently infected with either unirradiated or UV-irradiated HSV and scored for plaque formation 2 days later. UVER expression was maximum when the delay between UV-irradiation of the cells and HSV infection was 48 h. The magnitude of UVER expression was also found to be dependent on the UV dose to the cells and increased with increasing UV dose to the virus. Using a UV dose to the virus resulting in a plaque survival of about 10^{-2} on unirradiated cells, the the maximum UVER factor had a mean value of 1.3 for the normal strains following a dose of 15 J/m^2 to the cells. Somewhat higher UVER values were found for all the patient strains tested and resulted from lower UV doses to the cells than for normal strains. Maximum UVER factors for the CS strains ranged from 2.2 to 3.3 at a dose of 5 J/m^2 to the cells, for the XP excision-deficient strains; 2.1 to 2.6 at doses of 0.5 to 2.5 J/m^2 to the cells and for the XP variant strain tested; 2.5 at UV dose of 10 J/m^2 to the cells.

Viruses have been widely used as probes to study a variety of host-cell functions. In particular, several nuclear replicating DNA viruses have been used to study the repair of DNA damage, recombination and mutagenesis in human cells (Defais et al., 1983). The use of a viral probe offers the possibility to separate the effects of cell treatment from those resulting from treatment of the virus.

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The survival of UV-irradiated virus has been used to investigate the levels of constitutive DNA repair in a variety of human cell strains. Reduced host-cell reactivation (HCR) of UV-irradiated SV40 (Aaronson and Lytle, 1970; Abrahams and Van der Eb, 1976), HSV (Lytle et al., 1972) or adenovirus (Day, 1974; Rainbow, 1980, 1981) has been reported for several XP excision-deficient cell strains. A reduced HCR for UV-irradiated SV40 (Abrahams and Van der Eb, 1976) as well as adenovirus (Day, 1975; Rainbow, 1981) has been reported in XP variants, whereas HCR of UV-irradiated HSV has been reported to be both normal (Lytle, 1978; Selsky and Greer, 1978) and at reduced levels (Coppey and Menezes, 1981; Lytle et al., 1982; Takebe et al., 1978) in such strains. There are also conflicting reports concerning the HCR of UV-irradiated virus in CS strains. Some (Day et al., 1981; Rainbow and Howes, 1982; Lytle et al., 1983) but not all (Hoar and Davis, 1979; Ikenaga et al., 1979) investigators have found a reduced HCR of UV-irradiated virus in CS strains.

It is evident that a number of cellular DNA-repair processes are involved in the expression of HCR in normal human cells: excision repair, post-replication repair as well as the repair mechanism which is deficient in CS cells, although it is not clear as to the relative importance of these mechanisms for different viral assay systems.

The capacity of UV-irradiated cells to support virus infection, as well as the recovery of such capacity, has also been employed to study differences in the DNA repair capacity of human cells. Lytle et al. (1976) and Coohill et al. (1981) showed a reduced capacity of UV-irradiated XP excision-deficient cells to support HSV plaque production as compared to UV-irradiated cells from normal individuals. Coppey et al. (1979) were able to detect a reduced recovery in the capacity of UV-irradiated cells to support HSV progeny production for XP variants and XP heterozygotes as well as XP excision-deficient strains compared to normal strains. These results suggest that the capacity for HSV production in UV-irradiated cells is a very sensitive assay for cellular-repair capacity.

Enhanced survival UV-irradiated virus in mammalian cells treated prior to infection with UV light or chemical carcinogens is strong evidence for the existence of inducible DNA-repair pathways in eukaryotic cells that are analogous to the inducible 'SOS' functions in *E. coli* (Defais et al., 1983). Such an enhanced survival of UV-irradiated virus in pre-UV-irradiated cells (UVER) has been observed in normal human fibroblasts for SV40 (Cornelis et al., 1982), adenovirus (Day and Ziolkowski, 1981; Jeeves and Rainbow, 1983) and HSV (Lytle et al., 1976; Coppey and Menezes, 1981; Abrahams et al., 1984). UVER and HSV plaque production (Lytle et al., 1976), HSV infectious center formation (Abrahams et al., 1984), HSV progeny production (Coppey and Menezes, 1981) and adenovirus V antigen formation (Jeeves and Rainbow, 1983a) has been reported for XP excision-deficient strains from several complementation groups, although the UV dose to the cells producing the maximum UVER response was considerably less than that required for normal strains. Levels of UVER in XP variant strains appear to be markedly dependent on the assay system employed: normal UVER levels in XP variants have been reported for HSV infectious center formation (Abrahams et al., 1984), substantially elevated levels have been reported using HSV progeny production (Coppey and Menezes, 1981) and reduced levels have been found using adenovirus V antigen production (Jeeves and Rainbow, 1983a). UVER has also been reported for several CS strains for HSV progeny production (Coppey and Menezes, 1981) and adenovirus V antigen formation (Jeeves and Rainbow, 1983a). The UV doses to the CS cells producing a maximum UVER response were found to be lower than that for normal strains but greater than that required for XP-excision-deficient strains.

Previous work in our laboratory has examined the relative levels of HCR and UVER of adenovirus in a number of normal and repair-deficient human fibroblast strains (Rainbow, 1981; Jeeves and Rainbow, 1983, 1983a). In the present work experiments were carried out to compare the relative levels of HCR, cellular capacity for viral expression and UVER for HSV plaque formation in several CS strains, cell strains from the XP complementation groups A, C and D known to be deficient to differing amounts in various hallmarks of UV-excision repair, as well as an XP variant

strain known to be deficient in some aspects of post-UV DNA processing (Lehmann, 1978; Friedberg et al., 1979).

The major reason for this study was to determine to what extent the expression of viral reactivation depends upon the constitutive DNA-repair mechanisms known to exist in human cells and to what extent this expression depends upon the particular viral probe employed. It was also considered of interest to determine the relative sensitivity of the various viral reactivation phenomena in detecting cellular DNA-repair deficiencies.

Materials and methods

Cells and virus

Stock monolayer cultures of diploid human fibroblasts were grown in screw cap bottles (Falcon Plastic) and placed in a CO_2 incubator at 37°C and 90–100% humidity. The growth medium was Eagle's alpha-minimal essential medium (alpha-MEM) supplemented with 10% fetal bovine serum together with antibiotics. Details of the human fibroblast strains used including their origin are given in Table 1. Cell cultures were generally confluent by 7–9 days following a split ratio of 1 : 3.

Vero (African green Monkey kidney) cells and an inoculum of the HSV-2 strain 333 used to prepare virus stocks were obtained from Dr. Silvia Bacchetti, Department of Pathology, McMaster University, Hamilton, Ont. (Canada). Stock cultures of vero cells were grown in F15-minimal medium containing 10% calf serum together with antibiotics. Virus stocks were prepared by infecting confluent monolayers of vero cells with virus at an input multiplicity of 0.1–0.5 pfu/cell at 37°C. 48 h after infection, the cells were scraped off into the medium, pelleted by low-speed centrifugation, and resuspended in a small volume of medium without serum. The cell suspension was sonicated on ice twice, for 30 sec each time, using a Biosonic III ultrasonic power unit. The cell debris was removed by centrifugation, and the supernatant containing the virus particles was di-

TABLE 1

DESCRIPTION OF FIBROBLAST STRAINS

Cell strain	Sex	Age [a]	Source
Normals			
GM969	F	2	HGMCR [b]
A2	U [c]	U	Dr. S. Goldstein, Hamilton, Ont.
Hff	M	infant	Mr. J. Kawamoto, Hamilton, Ont.
CRL1221	M	40	ATCC [d]
GM2803	M	27	HGMCR
Cockayne syndrome			
CS1BE	F	10	HGMCR (GM1629) [e]
CS3BE	M	13	HGMCR (GM1856)
CS278CTO	M	24	HGMCR (GM2838)
GM739	F	3	HGMCR
Xeroderma pigmentosum			
XP25RO (group A)	M	1.5	HGMCR (GM710)
XP8LO (group A)	F	2	ATCC (CRL1376)
XP4RO (group C)	F	16	ATCC (CRL1260)
XP6BE (group D)	F	19	ATCC (CRL1157)
XP4BE (variant)	M	27	ATCC (CRL1162)

[a] Age in years in biopsy.
[b] Human Genetic Mutant Cell Repository, Camden, NJ (U.S.A.)
[c] Unknown.
[d] American Type Culture Collection, Rockville, MD (U.S.A.).
[e] Cell culture repository number.

vided into 1-ml aliquots and stored frozen at −70°C until used. The titer of virus stock was generally about 2×10^7 pfu/ml, as determined by direct plaque assay on normal human fibroblasts.

Ultraviolet light irradiation

Irradiation of cell cultures and virus was performed using a General Electric Germicidal Lamp G8T5 emitting principally at a wavelength of 254 nm. Dose rates ranging from 0.05 to 1 J/m^2/sec for cells and 0.5 to 5.0 J/m^2/sec for virus were measured using a J-225 shortwave UV meter (Ultraviolet products, San Gabriel, CA, U.S.A.).

Prior to irradiation of cells at room temperature, the growth medium was aspirated off and the cell monolayers washed with sterile PBS. Immediately after irradiation, prewarmed growth medium was added to the cells and the cultures returned to the incubator.

For irradiation of the virus, stock virus was diluted 1 : 1 with cold alpha-MEM without serum and an aliquot of virus suspension no greater than 1.5 ml was irradiated in a 35-mm diameter petri dish (Falcon plastics), kept on ice, with constant swirling during the irradiation.

Plaque assay

Plaque assays were carried out on either UV-irradiated or unirradiated monolayers of human fibroblasts using 24-well plastic Linbro tissue culture plates (Flow Laboratories Inc., Hamden, CN, U.S.A.). Growth medium was aspirated off the cells and each well was inoculated with 0.2 ml of an appropriate dilution of the virus in alpha-MEM so as to produce between 1 and 30 plaques per well. Duplicate wells were used for 3 serial dilutions of virus for each determination of either UV-irradiated or unirradiated virus titer. Virus was allowed to adsorb for 60–90 min at 37°C and then overlayed with alpha-MEM containing 10% fetal calf serum, antibiotics and 0.05% pooled immune human serum globulin to prevent reinfection from progeny virions other than that through cell to cell contact. After 48 h incubation, the growth medium was aspirated off and the monolayers were simultaneously fixed and stained by adding 0.5 ml of crystal violet solution (2 g crystal violet, 20 ml methanol, 36 ml formaldehyde and 144 ml of 1 × PBS filtered through Whatman No. 1 paper) for 5 min, then gently washed with distilled water. Plaque counts were scored in duplicate at 3 serial dilutions of the virus and the data points fitted to a straight line using least squares analysis. Taking into account the dilution factor, the slope of the regression line was used to determine the plaque titer in pfu/ml.

Results

Host-cell reactivation of HSV

Unirradiated and UV-irradiated suspensions of HSV were assayed for their plaque-forming ability on monolayers of several different human fibroblast strains. Fibroblasts were seeded such that they reached confluency 24 h later at which time they were infected with virus. Results obtained in typical experiments are shown in Figs. 1 and 2 for the survival of HSV plaque formation on the normal strain CRL 1221, the xeroderma pigmentosum strains XP40RO, XP8LO and XP4BE (variant) as well as the cockayne syndrome strains CS278CTO and CS3BE. The survival curves displayed the two-component nature typical for this virus on human fibroblasts and other mammalian cells (Ly-

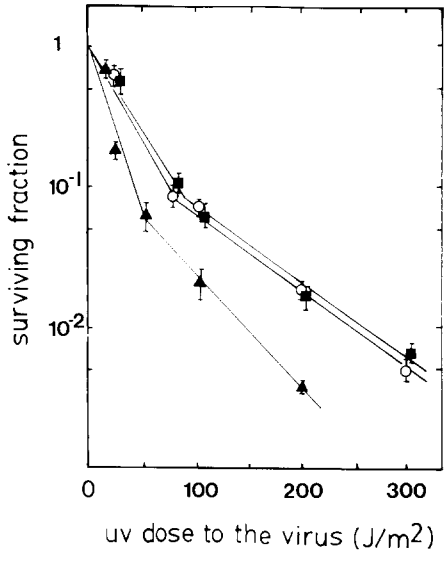

Fig. 1. Survival of plaque-forming ability of UV-irradiated HSV-2 in normal, Cockayne syndrome and xeroderma pigmentosum fibroblasts. Typical results for a single experiment. The error bars represent one standard error. Symbols: ○, normal strain CRL 1221; ■, Cockayne syndrome strain CS3BE; ▲, xeroderma pigmentosum group C strain XP4RO.

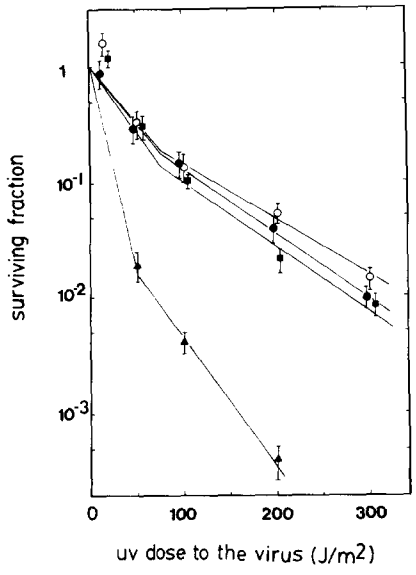

Fig. 2. Survival of plaque-forming ability of UV-irradiated HSV-2 in normal, Cockayne syndrome, xeroderma pigmentosum and xeroderma pigmentosum variant fibroblasts. Typical results for a single experiment. The error bars represent one standard error. Symbols: ○, normal strain CRL 1221; ■, Cockayne syndrome strain CS278CTO; ▲, xeroderma pigmentosum group A strain XP8LO; ●, xeroderma pigmentosum variant strain XP4BE.

tle et al., 1982). It can be seen that the UV survival of HSV was substantially reduced in the XP excision-deficient strains compared to normal cells as has been reported by others for other XP strains Lytle et al., 1972, 1982, 1983). However, UV survival of HSV in the CS strains and the XP variant strain was not significantly different from normal in this as well as subsequent experiments.

The survival points for each experiment were fitted by least squares analysis to obtain the D_0 value for each of the two components of the survival curve. Data points were analysed such that the greatest correlation was obtained for the 2 components of each curve. Such an analysis resulted in inflection points of 75 J/m^2 for normal, CS and XP variant strains and 50 J/m^2 for the excision-deficient XP strains.

The mean D_0 value of each component for a number of experiments was determined for each of the cell strains tested (see Table 2). The mean D_0 for the first component of the survival curve ranged from 26.4 to 31.1 J/m^2 for the 5 different normal fibroblast strains tested, with an average value of 28.2 J/m^2. The mean D_0 value obtained for the various CS and XP strains was expressed as a

TABLE 2

HOST-CELL REACTIVATION OF UV-IRRADIATED HSV IN NORMAL, XERODERMA PIGMENTOSUM AND COCKAYNE SYNDROME FIBROBLASTS

Cell strain	First component		Second component		Number of Expts.
	D_0 (J/m^2) [a]	%HCR	D_0 (J/m^2)	%HCR	
Normals					
GM969	26.8		76.9		6
A2	27.1		66.8		3
Hff	29.5		97.1		2
CRL1221	26.4		81.4		3
GM2803	31.1		100.1		2
Average ± S.E.	28.2 ± 1.0		84.4 ± 7.0		
Cockayne syndrome					
CS3BE	31.7	113	64.6	77	8
CS278CTO	27.8	98	71.1	85	8
Xeroderma pigmentosum					
XP25RO (group A)	12.1	43	23.5	28	6
XP8LO (group A)	11.3	40	38.2	45	2
XP4RO (group C)	14.2	50	51.2	61	2
XP6BE (group D)	14.9	53	28.2	33	2
XP4BE (variant)	34.0	121	81.8	97	3

[a] Mean D_0 value from a number of separate experiments as indicated in column 6.

percentage of the average value for the 5 normal strains and the percent HCR values for the first component of the survival curve so obtained are shown in Table 2. It can be seen that the HCR for the first component of the survival curve was similar to normal for the XP variant and the 2 CS strains tested, but substantially reduced for the 4 XP excision-deficient strains.

A similar calculation was also made using the second component of the survival curves and this too showed a substantial reduction in HCR for the 4 XP excision-deficient strains, but apparently normal HCR for the XP variant strain. HCR for the second component in the 2 CS strains, although somewhat reduced, was not significantly different from the normal strains used in these experiments. Thus only the XP excision-deficient strains exhibited a significant reduction in the repair of UV-irradiated HSV.

UV-inactivation of cell capacity for HSV plaque formation

UV-irradiation of human fibroblasts has been shown to reduce their capacity to support HSV infection (Lytle et al., 1976; Coppey et al., 1978).

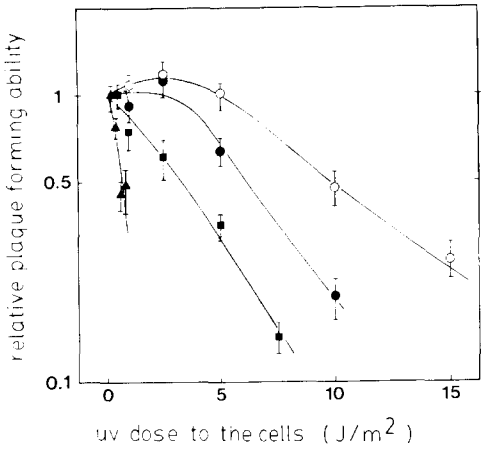

Fig. 3. Cellular capacity for HSV-2 plaque formation as a function of UV-dose to the cells for normal, Cockayne syndrome and xeroderma pigmentosum fibroblasts. Typical results for a single experiment. Fibroblast monolayers were UV-irradiated or left unirradiated and 48 h later infected with unirradiated HSV-2. Data points were fitted by eye and the error bars represent one standard error. Symbols: ○, normal strain GM2803; ■, Cockayne syndrome strain CS278CTO; ▲, xeroderma pigmentosum group A strain XP25RO; ●, xeroderma pigmentosum variant strain XP4BE.

Furthermore, recovery of the capacity of such UV-irradiated cells to support HSV infection has been shown to depend on the DNA-repair capacity of the cells themselves (Coppey et al., 1979). Since, in our experiments, HCR of UV-irradiated HSV had failed to reveal a significant reduction in the DNA-repair capacity of the CS cells and the XP variant tested, it was considered of interest to examine the UV-inactivation of cellular capacity for HSV plaque formation in these cells.

Fibroblast monolayers, which had just reached confluency, were UV-irradiated and infected 48 h later with HSV. Results for such a delayed-capacity experiment are shown in Fig. 3 for the normal strain GM 2803, the xeroderma pigmentosum strains XP25RO and XP4BE and the Cockayne syndrome strains CS278CTO. It can be seen that both the CS and the XP strains showed a reduced delayed capacity for HSV plaque formation compared to the normal strain.

TABLE 3

UV-INACTIVATION OF CELLULAR CAPACITY FOR HSV PLAQUE FORMATION IN NORMAL, XERODERMA PIGMENTOSUM AND COCKAYNE SYNDROME FIBROBLASTS

Cell strain	D_{37} value (J/m^2) [a]	Percent capacity	Number of Expts. performed
Normal			
GM969	11.0		4
Hff	8.6		4
CRL1221	10.8		5
GM2803	12.4		3
Mean normal ± S.E.	10.7 ± 0.9	100	
Cockayne syndrome			
CS3BE	2.8	26	6
GS278CTO	2.9	27	7
GM739	3.1	29	2
CS1BE	3.2	30	3
Xeroderma pigmentosum			
XP25RO (group A)	0.3	3	5
XP4RO (group C)	1.5	14	5
XP6BE (group C)	1.2	11	2
XP4BE (variant)	6.7	63	4

[a] Mean D_{37} value for the number of experiments indicated in column 4.

The data points for each capacity-inactivation curve were fitted by eye and the D_{37} value of the curve determined. The mean D_{37} value for a number of experiments on each cell strain is shown in Table 3. It can be seen that the D_{37} value for the normal strains ranged from 8.6 to 12.4 J/m^2 with an average value of 10.7 J/m^2. The D_{37} values for the XP and CS strains were expressed as a percentage of the average value for the normal strains to obtained the percent capacity for each strain as shown in Table 3. It can be seen that the UV-inactivation of capacity was greater in all the XP and CS strains tested compared to normal strains. Percent capacity values ranged from 26 to 30% for the 4 CS strains, 3 to 14% for the XP deficient strains and was 63% for the XP variant strain. Capacity-inactivation curves for the normal and XP variant strain showed a shoulder region for UV doses less than about 2.5 J/m^2 as shown in Fig. 3, whereas capacity curves for the CS and XP excision-deficient strains showed no shoulder over the range of doses tested.

UV-enhanced reactivation of UV-irradiated HSV

Fig. 4 shows the result of a typical UVER experiment using the normal strain CRL 1221, the XP strain XP4RO and the CS strain CS3BE. Fibroblast monolayers were UV irradiated and 48 h later were infected with either UV-irradiated or unirradiated HSV. The UV dose given to the virus was such that the survival of HSV plaque-forming ability on the unirradiated cells was about 10^{-2} for all the fibroblasts tested. The relative plaque-forming ability of the virus was then determined for unirradiated virus (Fig. 4, upper curves) and UV-irradiated virus (Fig. 4, middle curves). The lower curves in Fig. 4 show the behaviour of the UVER factor as a function of UV dose to the cells, where the UVER factor is defined as the normalised ratio of the relative survival of UV-irradiated to unirradiated virus at each UV dose to the cells. It can be seen that UV-irradiation of the cells resulted in enhanced reactivation of the virus for all 3 strains, although the UV doses to the XP and CS strains resulting in the maximum UVER value were considerably lower than that yielding maximum UVER in the normal strain, as has been reported previously (Lytle et al., 1976; Coppey and Menezes, 1981).

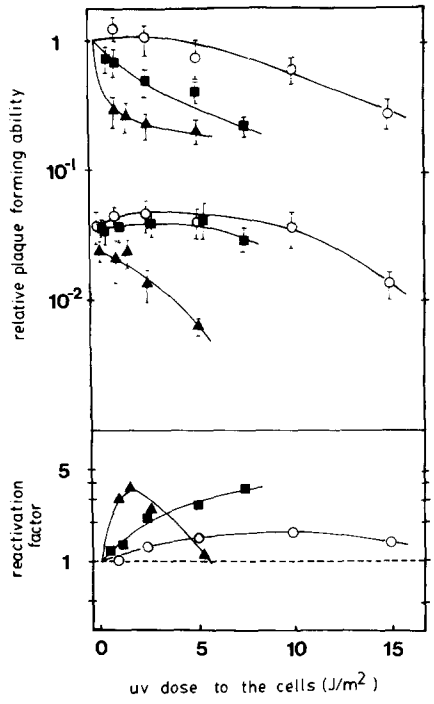

Fig. 4. UV-enhanced reactivation of HSV-2 plaque formation as a function of UV-dose to the cells for normal, Cockayne syndrome and xeroderma pigmentosum fibroblasts. Typical results for a single experiment. Fibroblast monolayers were UV-irradiated or left unirradiated and 48 h later infected with either unirradiated or UV-irradiated HSV-2. Data points were fitted by eye and the error bars for the relative plaque formation of unirradiated (top curves) and UV-irradiated (middle curves) HSV-2 represent one standard error. Symbols together with the UV dose given to the virus are: ○, normal strain CRL1221, 200 J/m^2; ■, Cockayne syndrome strain CS3BE, 200 J/m^2; ▲, xeroderma pigmentosum strain XP4RO, 75 J/m^2.

Factors affecting the UVER response

Several reports indicate that the UVER response is a function of the time between UV-irradiation of the cells and viral infection (Abrahams et al., 1984; Coppey and Menezes, 1981; Lytle et al., 1976). This would be expected if enhanced viral reactivation is a UV-induced cellular response. To determine the effects of delay between UV-irradiation and infection, cell monolayers were irradiated at 96, 48 and 0 h before infection. Fig. 5 shows the reactivation factors obtained plotted as a function of the delay between UV-irradiation of the cells and viral infection for a normal and 2 CS strains. It can be seen that the maximum expression of enhanced re-

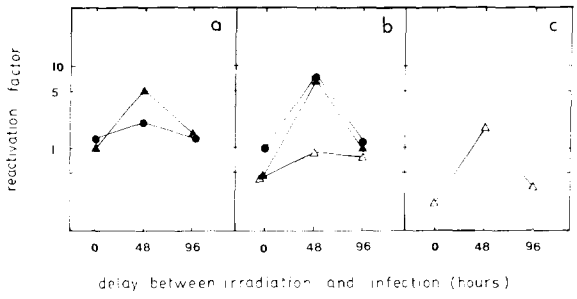

Fig. 5. UV-enhanced reactivation of HSV-2 as a function of time between irradiation and infection. Each point represents the logarithmic mean of 2 independent experiments. UV-dose to the virus was 200 J/m^2 for all fibroblast strains. UV-dose to the cells was (a), 2.5 J/m^2; (b) 5 J/m^2; and (c) 10 J/m^2. Symbols: normal strain Hff, △; Cockayne syndrome strains CS3BE, ●, and CS278CTO ▲.

activation was detected for infection of the cells 48 h after UV-irradiation.

Previous reports have shown that highly contact inhibited cells display lower levels of UVER than non-contact inhibited cells (Lytle et al., 1976, 1978; Hellman et al., 1974.) Therefore, it was considered of value to determine the effects of contact inhibition on the UVER response of fibroblasts. Fibroblasts monolayers were seeded such that they reached confluency 1 day after seeding. Cells which had been seeded in this way 1, 2 and 3 days previously were irradiated with a range of UV doses from 0 to 20 J/m^2 and then, 48 h later, were infected with unirradiated virus or virus irradiated to a survival level of about 10^{-2} in unirradiated cells. Plaques were counted 48 h after infection and UVER factors were calculated. The maximum reactivation factors obtained are plotted as a function of the number of days between cell seedling and UV-irradiation in Fig. 6. It is clear that increasing the time of cellular contact inhibition reduced the expression of UVER. Maximum expression of UVER was obtained by UV-irradiating cells which had just reached a minimum confluency and infecting with HSV-2 48 h later.

UVER as a function of UV-dose to cells

Using the optimum conditions for maximum expression of UVER as determined in the previous section, the UVER response of a number of diploid human fibroblast strains was determined as a function of UV-dose to the cells for a UV-survival

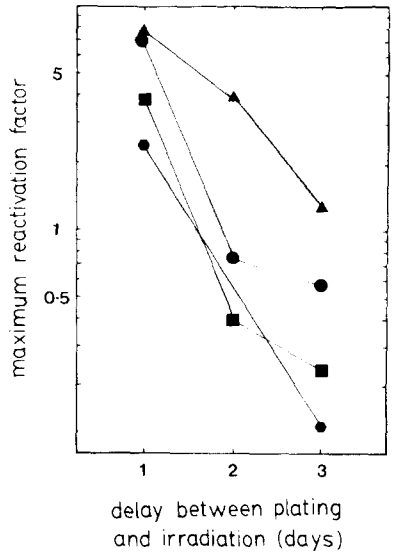

Fig. 6. UV-enhanced reactivation of HSV-2 as a function of time between cell plating and UV-irradiation. Fibroblasts were UV-irradiated at 1, 2 and 3 days after seeding and 48 h later infected with either unirradiated or UV-irradiated HSV-2. UV-dose to the virus was 200 J/m^2 for infection of both normal and CS strains and 75 J/m^2 for infection of the XP strains. Plaques were counted 48 h after infection and the maximum UVER factors obtained for each cell strain were ploted as a function of the time delay between plating and irradiation. UV-dose to the cells which resulted in maximum UVER factors for the various Hff, ◆; Cockayne syndrome strain CS278CTO, ■; xeroderma pigmentosum strains XP25RO, ▲, and XP6BE, ●.

level of about 10^{-2} to the virus. Experiments similar to that shown in Fig. 4 were carried out using 4 normal strains, 4 CS strains and 4 XP strains, including an XP variant strain. Results are summarised in Table 4. Maximum reactivation factors obtained for the normal strains were relatively low (average of 1.3) and resulted from a UV-dose to the cells of 15 J/m^2 for most strains. CS strains showed somewhat higher levels of UVER ranging from 2.1 to 3.2 at a lower UV-dose to the cells of 5 J/m^2. Maximum UVER factors for the three XP excision-deficient strains were also somewhat greater than that obtained for the normal strains and resulted from even lower UV-doses to the cells. The XP variant showed a maximum UVER value of 2.5 at a UV-dose to the cells of 10 J/m^2. UV-doses to the cells greater than that required to produce the maximum UVER expression often resulted in deterioration of the cell

TABLE 4

PARAMETERS DESCRIBING UVER OF UV-IRRADIATED HSV PLAQUE FORMATION IN HUMAN FIBROBLASTS

Cell strain	Dose to produce maximum reactivation factor (J/m^2)	Maximum reactivation factor [a]	Number of Expts. performed
Normals			
GM969	15	1.0	4
CRL1221	15	1.5	4
GM2803	15	1.4	3
Hff	10	1.3	4
Cockayne syndrome			
CS3BE	5	3.2	6
CS278CTO	5	3.2	5
GM739	5	2.4	2
CS1BE	5	2.1	2
Xeroderma pigmentosum			
XP25RO (group A)	0.5	2.1	3
XP4RO (group C)	2.5	2.3	5
XP6BE (group D)	1.0	2.6	2
XP4BE (variant)	10	2.5	2

[a] Mean maximum reactivation factor for the number of experiments indicated in column 4. UV dose to the virus for each cell strain was such that the plaque survival on unirradiated cells was about 10^{-2}.

monolayers such that plaque counts could not be made.

UVER as a function of UV-dose to the virus

Survival curves for plaque formation of UV-irradiated HSV were obtained on UV-irradiated as well as unirradiated cell monolayers for the normal strain, CRL 1221, CS 3BE, XP 25RO and XP 4BE. The UV-dose to the cells for each of the strains was that which produced the maximum UVER response as shown in Table 4, and the cell monolayers were infected 48 h later with HSV. It can be seen from Fig. 7 that UV-irradiation of the cells resulted in an increased survival of HSV, indicating enhanced reactivation, for all the strains tested.

The reactivation factor increased with increasing dose to the virus for all strains tested. It can also be seen that the expression of UVER was

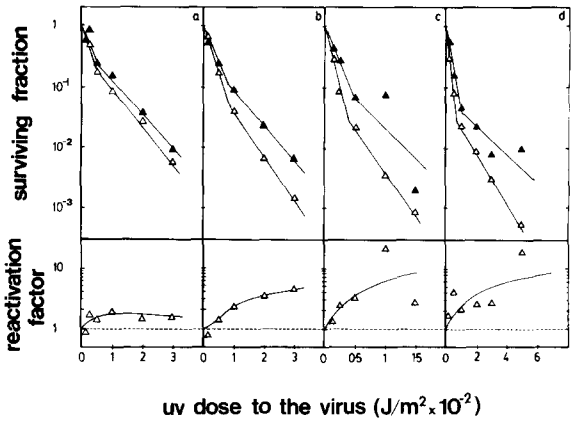

Fig. 7. UV-enhanced reactivation of HSV-2 as a function of UV-dose to the virus. The top panels show plaque survival on unirradiated (open symbols) and UV-irradiated (closed symbols) cells. Each point on the top panels represents the log mean of the plaque survival using pooled data from at least 3 separate experiments. Bottom panels show the reactivation factor (open symbols). (a) Pooled data for normal strains CRL1221, GM969 and A2, 15 J/m^2 to cells, (b) Cockayne syndrome strain CS278CTO, 5 J/m^2 to cells, (c) xeroderma pigmentosum strain XP25RO, 0.5 J/m^2 to cells, and (d) xeroderma pigmentosum variant strain XP4BE, 10 J/m^2 to cells.

somewhat greater in the XP and CS strains compared to the normal strains at comparable levels of virus survival, consistent with the reactivation factors shown in Table 4.

Discussion

In this study, the UV-survival curves for HSV-2 plaque formation in the various human fibroblast strains tested showed two components as has been found by others for HSV (Lytle et al., 1982). However, some investigators have reported single-component survival curves for UV-irradiated HSV in human cells (Takebe et al., 1974; Selsky and Greer, 1978).

The mean D_0 value for the normal strains was 28.2 and 84.4 J/m^2 for the first and second components of the HSV-2 survival curves respectively, somewhat larger than the values reported by others for HSV-1 in normal human fibroblasts (Lytle and Benane, 1974; Lytle et al., 1983). This difference presumably results from differences in the irradiation conditions for the virus, the time at which

HSV plaques were counted, UV dosimetry, an inherent difference between HSV-1 and HSV-2, or a combination of these factors. For the normal strains, the mean D_0 of the second component was 3 times greater than the mean D_0 for the first component, which agrees with earlier reports (Lytle and Benane, 1974).

A significant reduction in survival of UV-irradiated HSV was found in the four XP excision-deficient strains as compared to normal. Percent HCR values for the second component of the survival curves were 28% and 45% for the XP25RO and XP8LO group A strains respectively, 61% for the XP4LO group C strain and 33% for the XP6BE group D strain. Similar results for the survival of UV-irradiated HSV have been reported for other XP excision-deficient strains (Lytle et al., 1972; Selsky and Greer, 1978; Takebe et al., 1978; Lytle et al., 1983). However, percent HCR values for UV-irradiated adenovirus V antigen production (Rainbow, 1981) or plaque formation (Day, 1974) and SV40 DNA infectious center production (Abrahams and van der Eb, 1976) have been reported to be much smaller than those for HSV when comparing the same XP strain. This suggests that the reactivation of HSV is less dependent of host-cell excision repair than that of either SV40 or adenovirus.

A reduced HCR for UV-irradiated SV40 DNA (Abrahams and van der Eb, 1976) as well as adenovirus (Day, 1975; Rainbow and Howes, 1979) has been reported for XP variant cells. HCR of UV-irradiated HSV in XP variants has been reported to be normal by some investigators (Lytle, 1978; Selsky and Greer, 1978), whereas other reports suggest reduced levels in such strains (Coppey and Menezes, 1981; Lytle et al., 1982; Takebe et al., 1978). In the present investigation we found normal HCR of UV-irradiated HSV for the one XP variant strain tested.

There are also conflicting reports concerning HCR of UV-irradiated virus in CS strains. Some (Day et al., 1981; Rainbow and Howes, 1982), but not all (Hoar and Davis, 1979) investigators have found a reduced HCR of UV-irradiated adenovirus in CS strains. Ikenaga et al. (1979) reported normal levels of HCR for UV-irradiated HSV in one of the CS strains reported by us to show reduced HCR for UV-irradiated adenovirus. However, the results of Coppey and Menezes (1981) using HSV progeny production suggest a reduced HCR for some CS strains and more recently, Lytle et al. (1983) showed percent HCR values of 83 and 71% for the CS2BE and CS3BE strains respectively using the second component of the survival curves for UV-irradiated HSV plaque formation. Results of the present study show similar percent HCR values of 84 and 77% for the CS278CTO and CS3BE strains respectively (see Table 2). However, due to the range of D_0 values found for the different normal strains used in this study, the percent HCR values for the CS strains were not significantly different from normal.

A reduced capacity of UV-irradiated cells to support HSV plaque formation was found for all the XP and CS strains tested compared to normal strains. The percent capacities for the XP strains compared to normal were 3%, XP25RO group A, 14%, XP4RO group C, 11%, XP6BE group D and 63% XP4BE variant. The relative capacities of the different XP complementation groups are in good agreement with those previously reported (Lytle et al., 1976; Coppey et al., 1979). Percent capacity values for the CS strains ranged from 26 to 30% compared to normal. The relative capacities obtained for the XP and CS strains also parallel the UV-sensitivity of colony-forming ability of the cells themselves (Andrews et al., 1978, 1978a) suggesting that loss of capacity for HSV expression may result, in part at least, from cell death.

Comparison of capacity and HCR values for the various repair-deficient strains suggests that the capacity for HSV expression is a more sensitive indicator of cellular repair capacity for UV damage than HCR of HSV. The relative survival of UV-irradiated HSV in the XP excision-deficient and CS strains compared to normal (Table 1) is considerably greater than the relative UV survival of the cells themselves (Andrews et al., 1978, 1978a) as well as the relative capacity of UV-irradiated cells to support HSV production (Table 3). Assuming that UV survival of the cells themselves as well as the cellular capacity of UV-irradiated cells for HSV expression (Coppey et al., 1979) are a reflection of the repair of cellular DNA, these results suggest that the repair of HSV has some independence of cellular repair mechanisms. This independence could result from an inability of HSV to

fully utilize certain cellular DNA-repair pathways or because HSV codes for some of its own repair enzymes.

In the present study, UVER of HSV plaque formation was detected in a number of different human fibroblast strains. Consistant with the idea that enhanced viral reactivation is a UV-induced cellular response, the UVER factor was found to be a function of the time between UV irradiation of the cells and viral infection, as has been reported by others (Lytle et al., 1976; Coppey and Menezes, 1981; Abrahams et al., 1984). It was also found that increasing the time during which the cells were in contact inhibition prior to their UV-irradiation reduced the expression of UVER, consistent with the reports of others (Lytle et al., 1976, 1978; Hellman et al., 1974).

The magnitude of UVER expression was also found to be dependent on the UV-dose to the cells as well as the UV-dose to the virus. For a UV-dose to the virus corresponding to a plaque survival of about 10^{-2}, the maximum reactivation factor had a mean value of 1.3 for the 4 normal strains tested. Similar reactivation factors for normal human fibroblast strains have been reported for HSV plaque production (Lytle et al., 1976) and HSV infectious center formation (Abrahams et al., 1984) and somewhat higher UVER levels have been reported for HSV progeny production (Coppey and Menezes, 1981). The higher UVER levels reported for HSV by Coppey and Menezes may be explained by the differences in experimental protocol. The UV-dose to the cells resulting in maximum UVER was found to range from 10 to 15 J/m^2 for the normal strains similar to that reported previously for HSV (Lytle et al., 1976; Coppey and Menezes, 1981; Abrahams et al., 1984), SV40 (Cornelis et al., 1982) and adenovirus (Jeeves and Rainbow, 1983).

The 4 CS strains tested showed somewhat higher levels of UVER compared to normal with maximum reactivation factors ranging from 2.1 to 3.2 for a UV-dose to the cells of 5 J/m^2. The same UV-dose was reported to produce similar reactivation factors for HSV progeny production (Coppey and Menezes, 1981) as well as adenovirus V antigen production (Jeeves and Rainbow, 1983a).

Maximum UVER expression for the XP excision-deficient strains was also somewhat greater than that obtained for the normal strains (reactivation factors ranged from 2.1 to 2.6) and resulted from even lower UV-doses to the cells. The UV-dose to the cells producing maximum UVER expression was 0.5 J/m^2 for the XP25RO group A strain, 1.0 J/m^2 for the XP6BE group D strain and 2.5 J/m^2 for the XP4RO group C strain. Similar UV-doses producing a peak UVER response in the different complementation groups of XP excision-deficient strains have been reported for other studies using HSV (Coppey and Menezes, 1981; Lytle et al., 1976; Abraham et al., 1984) as well as adenovirus (Jeeves and Rainbow, 1983a).

Coppey and Menezes (1981) report a more pronounced than normal UVER expression for HSV progeny production in 3 different XP variant strains; the reactivation factors ranged from 17.5 to 26, almost 10 times the value for the normal strains. Such markedly elevated values of UVER were not found for XP variant strains in other studies using HSV infectious center production (Abrahams et al., 1984) and a reduced UVER expression compared to normal has been reported for adenovirus V antigen production in 2 XP variant strains (Jeeves and Rainbow, 1983a). In the present work using HSV-2 plaque production, the reactivation factor for the one XP variant strain tested was almost 2 times the mean value for the normals, similar to that found for the CS and XP excision-deficient strains in this study. These discrepancies in UVER expression for XP variants may result from differences in experimental protocol such as the multiplicity of infection, the level of virus survival in unirradiated cells as well as the viral endpoint being scored. The fact that adenovirus shows reduced UVER levels in XP variants (Jeeves and Rainbow, 1983a), may suggest differences in the relative contribution of virally coded enzymes to the UVER process in these cells. The aberrations reported in the UVER response for XP variants may in some what relate to the delayed expression of enhanced untargeted viral mutagenesis reported for these strains (Abrahams et al., 1984).

Treatment of *E. coli* with various chemical or physical agents that damage DNA and/or inhibit DNA synthesis is known to induce a coordinately regulated set of 'SOS' functions including Weigle reactivation and Weigle mutagenesis of phage

lambda (Radman, 1980). Many features of UVER in human cells appear similar to Weigle reactivation suggesting that the mechanism responsible for human UVER may be analagous to the 'SOS' mechanism which is related to error-prone, mutagenic repair in bacteria. One of the inducing signals for SOS functions in bacteria appears to be unrepaired DNA lesions, and results in some mammalian virus systems suggest that a similar signal may be responsible for the putative 'SOS' functions in mammalian cells (Cornelis et al., 1981, 1982; Van der Lubbe et al., 1983). It is also of interest that plasminogen activator (PA) synthesis is induced by a uv damage in human cells (Miskin and Reich, 1980) and the UVER profiles for normal and XP strains in this and other work (Jeeves and Rainbow, 1983a; Coppey and Menezes, 1981; Abrahams et al., 1984) are very similar to those obtained for the induction of PA in these cells. More recent results show that both UVER and enhanced viral mutagenesis of HSV (Abrahams et al., 1984) and adenovirus (Rainbow et al., 1983) are expressed in human cells. Taken together, these results suggest there are a number of human 'SOS'-like responses which may be triggered by the same cellular event.

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