Ultraviolet Inactivation of the Cytocidal and Transforming Activities of Human Adenovirus Type 1

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The transforming activity of adenovirus type 1 was more resistant to ultraviolet irradiation than was the cytocidal activity.

Human adenovirus type 1 (Ad-1) forms plaques in cultures of human cells (cytotoxic interaction) and foci of transformed cells in cultures of rat embryo cells (6). It was of interest to compare the size of the Ad-1 viral genome required for plaque formation with that required for focus formation. To make this comparison, we determined, by use of ultraviolet (UV) irradiation, the inactivation curves of the plaque-forming and the focus-forming abilities of the virus. Previous studies of UV-irradiated human Ad-12 indicated that the virus adsorbs to KB cells and uncoats normally; these studies also indicated that the target size of T-antigen production and of virus-induced chromosome aberrations is 25% of the target size for plaque formation (4, 7).

The Ad-1 virus stock and methods of adenovirus plaque and focus assay were described previously (5; R. M. McAllister et al., *J. Gen. Virol.*, *in press*). Samples (1.5 ml) of virus stock (in Eagle's medium, 5% fetal calf serum) in a 5-cm petri dish were irradiated at a distance of 6.4 cm with a model R-51 lamp (Ultraviolet Products Inc., San Gabriel, Calif.) for various lengths of time. The dose rate of irradiation (253.7 nm) at the liquid surface was 1.98×10^5 ergs per min per mm² as measured by a UV intensity meter (model J-225; Ultraviolet Products, Inc.). Each sample was assayed for plaque formation in cultures of primary human embryo kidney cells and for focus formation in cultures of primary rat embryo cells; multiplicities of 0.38 to 15 plaque-forming units (PFU)/cell were used. The plaques were counted after 10 days and the foci were counted after 47 days.

Table 1 presents the results obtained in two experiments. The survivor fractions are plotted

as a function of dose rate in Fig. 1. The curve for focus formation, apart from the initial shoulder, appears to obey single-hit kinetics. The curve for plaque formation shows a very slow inactivation after 4 min, presumably as a result of multiplicity reactivation. These data suggest that the transforming activity of Ad-1 is more resistant to UV irradiation than is the plaque-forming activity and therefore requires less of the virus genome.

Three objections to this interpretation can be raised. (i) Different multiplicities were used in the two assay systems, possibly leading to different rates of multiplicity reactivation. (ii) Different cell species were also used (with possibly different UV repair mechanisms and "code dictionaries").

TABLE 1. Effect of UV irradiation on plaque-forming and focus-forming capacities of Ad-1^a

Length of irradiation	Expt 1 (survivors)		Expt 2 (survivors)	
	PFU/ml	FFU/ml ^b	PFU/ml	FFU/ml
min				
0	1.5×10^8	1.3×10^3	7.5×10^7	2.8×10^3
1	NT ^c	NT	10^6	4×10^2
2	NT	NT	4.25×10^4	3×10^2
4	4×10^2	1.6×10^2	4×10^2	8×10^2
6	2×10^2	1.5×10^2	10^2	2.6×10^2
8	6.5×10^1	3.5×10^1	6.5×10^1	5×10^1

^a Infectivity assays were carried out in 5-cm petri dish cultures of human embryo kidney cells (four cultures per 10-fold dilution of virus stock). Transformation assays were carried out in 5-cm petri dish cultures of rat embryo cells (four cultures each; undiluted and 1:5, 1:10, and 1:20 dilutions of virus stock).

^b FFU, focus-forming units.

^c Not tested.

FIG. 1. Inactivation of plaque-forming and transforming abilities of Ad-1 by UV light. The logarithms of the surviving fractions are plotted on the ordinate, and the dose is plotted on the abscissa (experiment 2).

(iii) It is also possible that, in the transformation assay, more repair of damage in viral deoxyribonucleic acid occurs because the system survives longer. This hypothesis has never been adequately tested.

That virus multiplicity reactivation did not appear to be responsible for the increased survival of the transforming activity is suggested by the results shown in Table 2. As can be seen, reasonable linearity occurred with input multiplicities of 0.38 to 7.5. No data are available on the second objection; however, when UV-irradiated adenovirus SA-7 was assayed in hamster and monkey cells, a transformation to cytotoxic target size ratio of 0.3 was obtained (3), a result consistent with those obtained in the present studies with human and rat cells. The first two objections have also been raised and are probably not valid for studies of target sizes of transforming and cytotoxic activities of polyoma virus (1, 2).

TABLE 2. Effects of dilution of UV-irradiated samples on the numbers of Ad-1 transformed foci (experiment 2)

Virus input (PFU/cell)	Irradiation (min)				
	0	1	2	6	8
7.5	ND ^a	146 ^b	75	35	9
1.5	31	63	27	7	1
0.75	22	35	15	5	0.25
0.38	14	21	15	1.3	0.5

^a None detected.

^b Average number of foci per culture when stained 47 days after infection.

In summary, the rate of inactivation of the transforming activity of human Ad-1 was found to be lower than the rate of inactivation of the cytotoxic activity. These results have been interpreted to mean that transformation requires less of the viral genetic information than does plaque formation.

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