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Host Cell and Ultraviolet Reactivation of Ultraviolet-Irradiated Mycoplasmaviruses

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The mycoplasma *Acholeplasma laidlawii* was shown to have mechanisms for both host cell and ultraviolet (UV) reactivation of UV-irradiated mycoplasmaviruses. Host cell reactivation was examined by comparing the survival abilities of UV-irradiated double-stranded deoxyribonucleic acid mycoplasmavirus plated on both untreated and on acriflavine-treated cells. Acriflavine treatment inhibited cell excision repair. Decreased survival on the acriflavine-treated cells demonstrated host cell reactivation. UV reactivation was studied by comparing the survival of UV-irradiated virus plated on untreated cells with its survival on cells that received a small UV dose before plating. The UV-irradiated cells gave increased virus survival, showing UV reactivation. Similar experiments with a single-stranded deoxyribonucleic acid mycoplasmavirus showed that this virus could be UV reactivated, but not host cell reactivated.

The mycoplasmas are a group of small procaryotic cells that do not have cell walls; instead, each cell is bounded by a single lipoprotein "unit" membrane (18). The various isolates have been classified into six genera (6). Of these, the biology of the genus *Acholeplasma*, in particular *A. laidlawii*, has been studied most extensively.

A. laidlawii deoxyribonucleic acid (DNA) is about 33 mol% guanine plus cytosine (19) and has a molecular weight of 1.0×10^9 (1). The only studies of ultraviolet (UV) sensitivity and repair capabilities of mycoplasmas have, thus far, involved *A. laidlawii*. These cells are capable of repairing UV damage to their DNA by photoreversal and dark repair, involving an excision repair mechanism (4, 8, 22).

Three groups of mycoplasmaviruses have been isolated that can infect *A. laidlawii* (17). The properties of these viruses are: (i) group 1 viruses are naked, bullet-shaped particles (about 15 by 80 nm) containing circular single-stranded DNA of 2×10^6 daltons (15); (ii) group 2 viruses are roughly spherical, enveloped particles (about 80 nm in diameter) containing double-stranded DNA (J. Maniloff, unpublished data); and (iii) group 3 viruses are short-tailed polyhedral particles (about 57 nm by 62 nm) containing double-stranded DNA (10). These viruses allow another approach for the study of the repair capablities of these small cells.

In *Escherichia coli*, UV-induced damage in phage DNA can be repaired by host cell reactivation (HCR) and ultraviolet reactivation (UVR). HCR is the repair of UV-damaged viral DNA by cellular enzymes (9) and is primarily dependent on host cell excision repair (3). UVR is the increased survival of UV-irradiated virus when the virus is assayed on a host that has been lightly UV irradiated before infection (23). The UVR mechanism of coliphages involves a UV-induced repair system that is distinct from either the pyrimidine excision repair responsible for HCR (3) or recombinational repair (2).

In this paper, we report studies using both single- and double-stranded DNA mycoplasmaviruses to examine mycoplasma UV repair capabilities. These results show HCR and UVR in *A. laidlawii*, demonstrating that these repair systems exist in the smallest free-living cells.

MATERIALS AND METHODS

Cells and viruses. *A. laidlawii* JA1 (16) was used in all experiments. This cell strain was chosen for these studies because it is an indicator host for all three mycoplasmavirus groups.

The single-stranded DNA mycoplasmavirus used was MVL51, a group 1 virus isolated by Liss and Maniloff (16). The double-stranded DNA mycoplasmavirus used was MVL2, a group 2 virus originally isolated by Gourlay (11). These viruses have been assayed as plaque-forming units on lawns of JA1 cells; although both are nonlytic viruses, plaques formed as a result of the slower growth of infected cells (17).

Media and buffers. The tryptose broth medium used contained 20 g of tryptose (Difco Laboratories, Detroit, Mich.), 5 g of NaCl, 5 g of tris(hydroxy-

methyl)aminomethane (Tris), 10 g of glucose, and 10 ml of heat-inactivated (60 min at 60°C) PPLO serum fraction (Difco) in 1,000 ml of water, adjusted to pH 8.0. Tryptose agar plates were made with tryptose broth containing 1% agar (Difco) and 0.025% thallium acetate (to inhibit bacterial contamination). The Tris-hydrochloride buffer contained 0.1 M NaCl, 0.01 M Tris, and 0.001 M ethylenediaminetetraacetic acid, adjusted to pH 8.0 with HCl.

Irradiation of virus. Viruses in tryptose broth were diluted 1:10 with Tris-hydrochloride buffer, and 2 to 3 ml of virus suspension in a 60-mm petri dish was irradiated at room temperature in the dark. The suspension was continuously agitated during irradiation. Irradiation was achieved with a Westinghouse germicidal lamp (257 nm) calibrated with a UV dosimeter. At the indicated doses, we diluted samples of the virus suspension with tryptose broth and assayed for plaque-forming units. The assays were done on lawns of *A. laidlawii* JA1, each of which was prepared by spreading and drying approximately 10^8 colony-forming units onto the surface of a 10-cm tryptose agar plate.

Acriflavine treatment. Acriflavine was either included in the tryptose agar plates (at a concentration of 0.5 μg/ml), or the *A. laidlawii* indicator culture was treated before plating. For the latter procedure, acriflavine was added to the culture (10 μg of acriflavine per ml of culture) and, after 10 min, 1 ml was spread to make an indicator lawn with a final acriflavine concentration of 0.5 μg/ml of tryptose agar.

UVR. This repair mechanism was examined by plating UV-inactivated virus on *A. laidlawii* JA1 lawns that had been irradiated with 14 J of UV per m^2.

Survival curves. From the inactivation data, we draw survival curves according to a least-squares data analysis, as in the program described by Das et al. (4).

RESULTS

HCR. Treatment of UV-irradiated *E. coli* with the amino acridine dye acriflavine reduced the ability of the cells to excise pyrimidine dimers and repair their DNA (21, 24). The repair of UV-damaged bacteriophage DNA was also reduced when the phage was plated on acriflavine-treated cells (7), showing HCR of the irradiated phage. Hence, in the presence of acriflavine, hcr^+ strains phenotypically resemble hcr^- strains.

HCR of UV-irradiated mycoplasmaviruses was examined by noting differences in virus survival on untreated and acriflavine-treated *A. laidlawii* JA1 lawns. The dark-repair capability of *A. laidlawii* JA1, the mycoplasmavirus indicator host, was reduced by acriflavine treatment after UV irradiation (A. Ghosh, J. Das, and J. Maniloff, manuscript in preparation). Since treatment of *A. laidlawii* JA1 with up to 10 μg of acriflavine per ml had no effect on the ability of the cells to serve as an indicator host, acriflavine-treated cells could be used to study HCR of mycoplasmaviruses.

Survival of the double-stranded DNA mycoplasmavirus MVL2 was decreased when the UV-irradiated virus was plated on acriflavine-treated cells (Fig. 1). The shoulder of the survival curve was almost completely abolished by the acriflavine treatment; the 37% survival dose was reduced from 175 J/m^2 when the irradiated virus was plated on untreated cells to 80 J/m^2 on acriflavine-treated cells. Acriflavine also increased the slope of the linear part of the survival curve from 11 m^2/kJ for viruses plated on untreated cells to 15 m^2/kJ on acriflavine-treated cells. The dose reduction factor, at several values of the survival fraction, is given in Table 1 for the acriflavine effect.

In contrast, UV survival of the single-stranded DNA mycoplasmavirus MVL51 was not affected by treating the indicator cells with acriflavine (Fig. 2). With or without acriflavine treatment, the slope of the survival curve (the inactivation cross section) was 29 m^2/kJ, which agrees with previously published studies (14). Since an excision repair mechanism cannot operate on a single-stranded DNA, the lack of an acriflavine effect on the UV survival of the

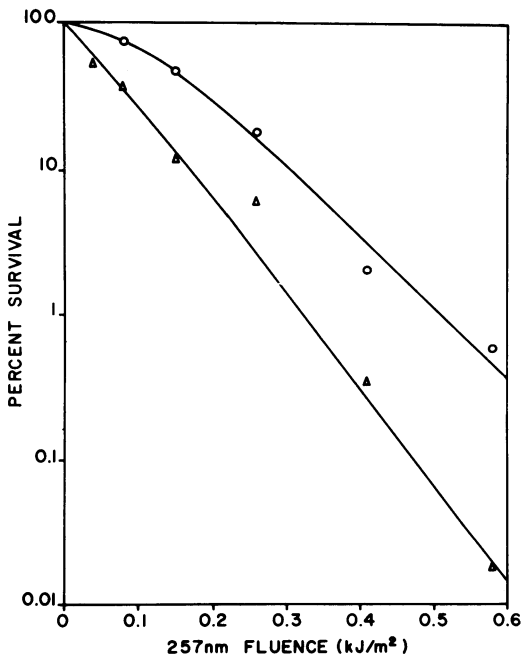

FIG. 1. *Survival curves for UV-irradiated MVL2, a double-stranded DNA mycoplasmavirus, plated on untreated* (○) *or acriflavine-treated* (△) *cells.*

TABLE 1. *Dose reduction factors and reactivation efficiencies for the acriflavine effect*[a]

Survival fraction	Dose reduction factor[b]	Reactivation efficiency[c]
0.500	0.37	0.63
0.100	0.56	0.44
0.050	0.59	0.41
0.010	0.62	0.38
0.005	0.64	0.36

[a] Calculated from data in Fig. 1.

[b] The dose reduction factor equals the UV dose for a given survival with acriflavine divided by the UV dose for the same fraction survival without acriflavine, similar to the definition of dose reduction factor for photoreactivation (13).

[c] The reactivation efficiency equals one minus the dose reduction factor (5) for each survival fraction.

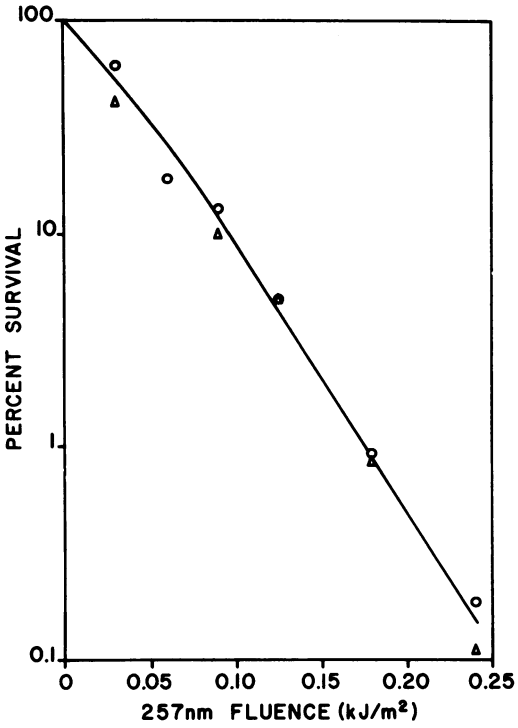

FIG. 2. *Survival curves for UV-irradiated MVL51, a single-stranded DNA mycoplasmavirus, plated on untreated* (○) *or acriflavine-treated* (△) *cells.*

single-stranded DNA mycoplasmavirus was consistent with the presumed action of acriflavine as an inhibitor of excision repair.

UVR. UVR of the double-stranded DNA virus MVL2 was examined by comparing the survival of UV-irradiated virus plated on untreated cells with virus plated on cells that had been lightly irradiated (14 J/m²) just prior to plating. This particular UV dose was found optimal for these studies: it gave the maximum amount of UV reactivation without affecting plaque visibility. Survival curves of UV-irradiated MVL2 plated on unirradiated and UV-irradiated cells are depicted in Fig. 3. The slope of the survival curve decreased from 11.0 m²/kJ for virus plated on unirradiated cells to 8.8 m²/kJ on the UV-irradiated cells. A similar experiment with MVL51, a single-stranded DNA virus, showed a small survival enhancement of the UV-irradiated virus when it was plated on lightly UV-irradiated host cells, as compared with its survival on unirradiated cells. This UVR effect is being investigated further.

DISCUSSION

These studies have shown that *A. laidlawii*, one of the smallest self-reproducing cells, has both HCR and UVR mechanisms similar to those found in more complex cellular systems such as bacteria and mammalian cells. Since there is indirect evidence that mycoplasmas have an excision repair capability (4, 22), the finding of HCR was probably predictable. Preliminary studies on the effect of acriflavine on *A. laidlawii* indicated that the dye inhibits cellular excision repair, although we have not eliminated the possibility of other effects of acriflavine on the cells. Since no excision repair-deficient mycoplasma mutants have been isolated, HCR was investigated by using cells

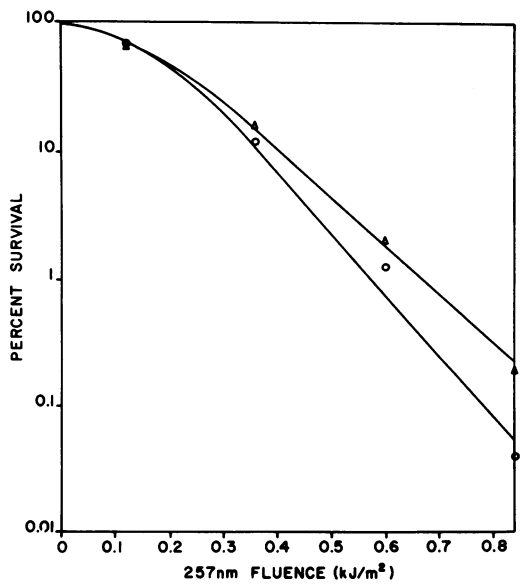

FIG. 3. *Survival curves for UV-irradiated MVL2, a double-stranded DNA mycoplasmavirus, plated on unirradiated* (○) *or lightly UV-irradiated (14 J/m²) cells* (△).

treated with acriflavine. The finding of HCR of a double-stranded, but not a single-stranded, DNA mycoplasmavirus was consistent with excision repair being involved in HCR. The efficiency of this process was dependent on the virus survival fraction (Table 1), and these efficiency values were comparable to those for bacteriophages (12).

UVR was observed using both single- and double-stranded DNA mycoplasmaviruses. The efficiency of this repair process (calculated from the dose reduction factor at 1% virus survival as described in Table 1) was 11% for the single-stranded DNA virus and 14% for the double-stranded DNA virus. In the presence of an excision repair system, the efficiencies were comparable to those reported for bacteriophages (5).

UVR has been shown to involve a DNA repair mechanism different from either excision or recombinational repair (2, 3). Radman (20) has proposed that the UVR mechanism may be due to an inducible error-prone repair system. The finding of UVR by *A. laidlawii*, therefore, may indicate that such an inducible repair system is present in mycoplasmas.

The data presented here are the first reported on the UV survival of group 2 mycoplasmaviruses and allow the comparison of the UV survival of group 1 and 2 viruses. The D_{37} (assuming fluence and dose are the same) evaluated from Fig. 1 and 2 must be multiplied by 0.625, which is the modification factor for the amount of medium in the buffer during irradiation. With this correction, the D_{37} for the group 1 virus MVL51 is 28 J/m^2, and for the group 2 virus MVL2 it is 109 J/m^2. The inactivation cross sections (calculated from the slope of the linear part of each survival curve) are 29 m^2/kJ for MVL51 and 15 m^2/kJ for MVL2. Hence, the double-stranded DNA group 2 mycoplasmavirus is significantly less sensitive to UV inactivation than the single-stranded DNA group 1 virus. Part of this decreased sensitivity is due to the HCR of group 2 viruses described here.

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