

## Inducible Repair System in *Haemophilus influenzae* Unaccompanied by Mutation

NIHAL K. NOTANI† AND JANE K. SETLOW\*

Biology Department, Brookhaven National Laboratory, Upton, New York 11973

Weigle reactivation of ultraviolet-irradiated HPlc1 phage was observed after ultraviolet or mitomycin C treatment of *Haemophilus influenzae* cells. The amount of reactivation was considerably increased when the treated cells were incubated in growth medium before infection. The presence of chloramphenicol during this incubation abolished the reactivation. No mutation of this phage accompanied the reactivation. When cells were treated so as to produce a maximal reactivation of phage, neither reactivation nor mutation of cells was observed. It is concluded that *H. influenzae* has an inducible repair system that is not accompanied by mutation.

Weigle (24) observed that UV-irradiated lambda phage produced more plaques when plated on lightly irradiated rather than unirradiated *Escherichia coli* host cells and that phage mutations accompanied the reactivation. X-irradiation and nitrogen mustard, but not hydrogen peroxide, treatment of the cells had a similar effect. Subsequently, it was noted that chloramphenicol interfered with such Weigle (W) reactivation of phage R (14) and that this phenomenon for lambda required *recA* and *lexA* gene expression (8). Radman (15, 16) and Witkin (25) unified a variety of observations by proposing the SOS hypothesis, in which UV and possibly other agents induced a set of coordinately regulated functions, among which was an inducible error-prone repair, resulting in mutation. *Haemophilus influenzae* is notable in that although UV irradiation causes some of the events considered part of the SOS system (such as induction of prophage and filamentation of the cells), the cells are not mutable by UV (12). An investigation of W reactivation in this microorganism was undertaken in an attempt to understand these phenomena.

Microorganisms were the *H. influenzae* phage HPlc1 (10) and its temperature-sensitive mutants, *ts2* and *ts3* (4) and *H. influenzae* strains Rd, *rec-1* (13), and BC200 (1). Media were as described before (21), except that 3% Eugonbroth was used to dilute cells and phage before plating. Phage assays were performed as described previously (10). Stocks were made by inducing lysogens with mitomycin C (MMC) (5). UV irradiation was under a germicidal lamp at a dose rate of 0.5 J/m<sup>2</sup> per s. Exponentially

growing cells were irradiated in buffer (21) at a concentration of ca.  $5 \times 10^8$ /ml (3.5 ml in a 14-cm petri dish). Phage were diluted  $\times 10^2$  in buffer immediately before irradiation (2.5 ml in a 9-cm petri dish). The survival after 240 J/m<sup>2</sup> was between 0.1 and 0.01%. Cell suspensions in growth medium (ca.  $5 \times 10^8$ /ml) were also treated with MMC at 37°C for various times, usually with 0.5 µg/ml. Samples were immediately chilled by adding cold dilution medium and then centrifuging, washing, and finally resuspending the samples in growth medium. Phage were also treated with MMC in growth medium, and samples were diluted immediately in cold medium for assay (even 17 µg of MMC per ml for 16 min did not have an appreciable effect on phage survival).

After the cells were treated with UV or MMC, they were resuspended in growth medium at ca.  $5 \times 10^8$ /ml and exposed to UV-irradiated or unirradiated phage at ca.  $10^7$ /ml at 37°C. After 10 min for attachment, the mixture was chilled, centrifuged, and washed to eliminate free phage and then resuspended in growth medium at half the original concentration. After 2 h of incubation with vigorous shaking at 37°C, the samples were centrifuged and the supernatants were removed for progeny phage assay. In some experiments before the exposure to phage, the cells were incubated in growth medium at 37°C for various times with or without 100 µg of chloramphenicol per ml. In other experiments the final incubation was omitted and the mixtures were assayed for infective centers.

Mutations to novobiocin resistance and to the ability to utilize protoporphyrin in place of hemin were measured as described before (6). Phage mutations were measured as reversions of progeny of two temperature-sensitive mutants,

† On leave of absence from the Biology and Agriculture Division, Bhabha Atomic Research Centre, Bombay 400085, India.

*ts2* and *ts3*, to wild type, which is able to form plaques at 40°C, a temperature restrictive for the mutants.

Figure 1 shows results of treating cells with various doses of UV or MMC on the number of viable progeny phage obtained from subsequent infection with irradiated phage. In the case of both inactivating agents, the number of progeny increased more than a factor of 10 and then decreased with larger doses. The effect of UV and MMC on the number of viable infective centers from irradiated phage was much smaller than on the number of progeny phage, and the peak was much broader (data not shown), indicating that changes in burst size are responsible for much of the effect seen in Fig. 1. Similar results were obtained when the host cell was BC200, a strain that is not inducible for defective phage (2, 20), indicating that W reactivation in *H. influenzae* does not involve induction of defective phage. However, the *rec-1* mutation eliminated the increase in survival of irradiated phage (data not shown). This latter result was also obtained by Bamji (Manijeh S. Bamji, M. S. thesis, University of Bombay, Bombay, India, 1978).

In contrast to the results of Fig. 1, UV and MMC only decreased, but did not increase, the number of progeny from unirradiated phage (data not shown). Again most of the observed decrease presumably resulted from a decrease in

burst size caused by these agents, since capacity, measured as ability of infected cells to produce at least one viable phage, is relatively insensitive to UV (19). The dose equivalence of UV and MMC appeared to be the same for irradiated and unirradiated phage (2.5 J/m<sup>2</sup> had the same effect as 4 min × micrograms of MMC per milliliter).

A further increase in the number of phage progeny was observed when UV-irradiated cells were incubated in growth medium before infection (Fig. 2), provided the incubation time was not too long. Similar results were previously obtained with lambda phage (7). Incubation of MMC-treated cells before infection also caused an increase in survival of UV-irradiated phage, but there was little or no effect on unirradiated phage (Fig. 3). If the UV-irradiated cells were incubated in the presence of chloramphenicol sufficient to eliminate almost all protein synthesis (12), there was only a decrease in the number of progeny as a function of incubation time (Fig.

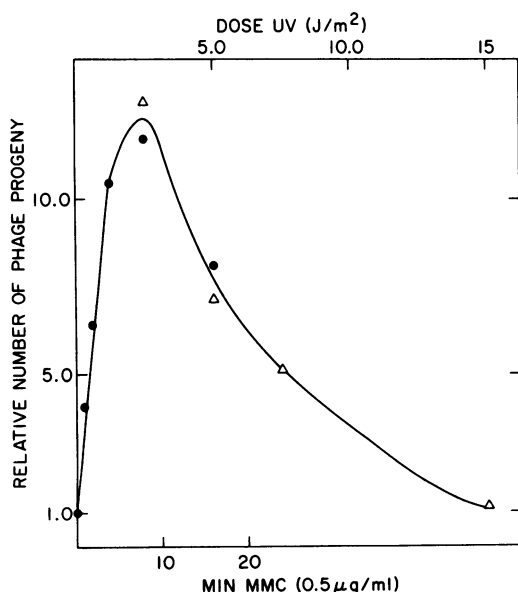


FIG. 1. UV and MMC reactivation of phage (irradiated with a UV dose of 240 J/m<sup>2</sup>) as a function of UV (Δ) or MMC (●) dose to the wild-type cells before infection.

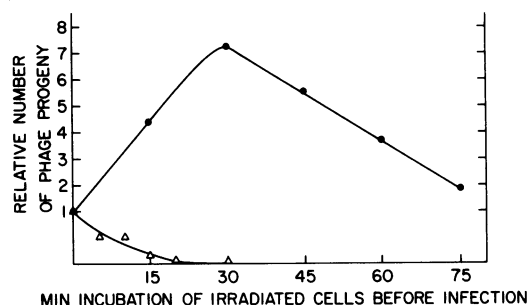


FIG. 2. Effect of incubation of wild-type cells after UV (2.5 J/m<sup>2</sup>) and before infection with irradiated phage (240 J/m<sup>2</sup>). Incubation was with (Δ) or without (●) 100 µg of chloramphenicol per ml.

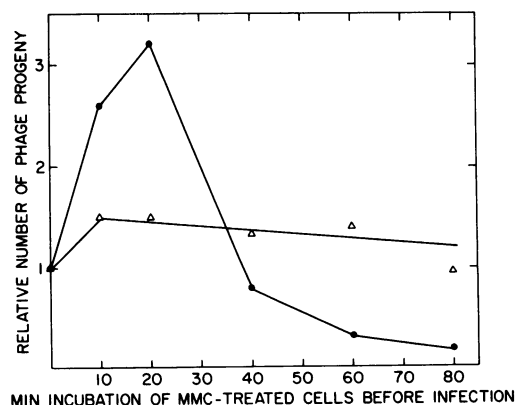


FIG. 3. Effect of incubation of wild-type cells after 4 min of MMC at 5 µg/ml and before infection with irradiated (●, 240 J/m<sup>2</sup>) or unirradiated (Δ) phage.

2). It is improbable that this inhibition of the reactivation by chloramphenicol is the result of a general toxic effect of the drug, since plasmid DNA continues to be synthesized in plasmid-bearing strains of *H. influenzae* for a long time in the presence of 100  $\mu\text{g}$  of the compound per ml (unpublished data by D. M. McCarthy of this laboratory). Thus it appears that new protein synthesis is required for reactivation, and therefore reactivation must be an inducible phenomenon. Since there was no comparable effect on unirradiated phage, a repair system was apparently induced.

A small UV dose to cells followed by 30 min of incubation (the time to produce maximum reactivation of UV-irradiated phage) might be expected to decrease the sensitivity of the cells to subsequent UV doses, by analogy with the reactivation of phage. However, this was not the case in that the previous UV dose caused the cells to be somewhat more sensitive. Furthermore, there was no induction of mutations under these conditions (data not shown). Table 1 also shows that under conditions giving maximal reactivation of phage, there was no significant increase in mutations of UV-irradiated *ts2* phage as a function of dose. Similar results were obtained with *ts3* phage. The reversion frequencies were well within the previously measured spontaneous reversions for these phages (3). The lack of mutation accompanying W reactivation sets *H. influenzae* apart from other bacteria, as does its apparent immutability by UV (12).

There are several gross common features between W reactivation in *E. coli* and in *H. influenzae*. (i) They both show reactivation of UV-irradiated phage under cellular genetic control (by the somewhat analogous *recA* and *rec-1* genes of *E. coli* and *H. influenzae*, respectively), and the phenomenon in both species appears to involve an inducible repair system (7). (ii) Other agents besides UV can induce the phenomenon (24). However, there are a number of ways in which *E. coli* responds to UV radiation very differently from *H. influenzae* that could have relevance for the lack of mutation in the latter. First, there is UV stimulation of recombination

in irradiated phage of *E. coli* (11), whereas UV irradiation does not increase the genetic recombination of *H. influenzae* phage or transforming DNA (4). Second, chloramphenicol decreases the rate of filling the single-strand gaps in *E. coli* DNA synthesized after UV (18), whereas there is only a small or no effect observable in *H. influenzae* (12). Third, exchange between sister strands of DNA takes place in *E. coli* after UV by a process that at least in part results in gap filling (17) and also causes some of the UV-induced pyrimidine dimers to go from parental DNA into DNA synthesized after irradiation (9). There is also UV-induced exchange in *H. influenzae*, but it occurs to a much greater extent than in *E. coli*, and apparently there are considerably more exchanges than pyrimidine dimers (22), unlike the situation in *E. coli* (17). The result of these exchanges is that the dimers become equally distributed between daughter and parental strands of *H. influenzae* DNA very rapidly (23), whereas relatively long times are required in *E. coli* (9).

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TABLE 1. Reversion of *ts2* phage to wild type as a function of UV dose to the phage<sup>a</sup>

UV dose to phage (J/m <sup>2</sup> )	Reversion frequency
0	$4 \times 10^{-7}$
5	$6 \times 10^{-7}$
10	$4 \times 10^{-7}$
20	$2 \times 10^{-7}$

<sup>a</sup> Cells were irradiated at 2.5 J/m<sup>2</sup> and then incubated 30 min before infection.

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