# Ultraviolet mutagenesis and inducible DNA repair in *Caulobacter crescentus*

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**Summary.** The ability to reactivate ultraviolet (UV) damaged phage $\Phi$CbK (W-reactivation) is induced by UV irradiation of *Caulobacter crescentus* cells. Induction of W-reactivation potential is specific for phage $\Phi$CbK, requires protein synthesis, and is greatly reduced in the presence of the *rec-526* mutation. The induction signal generated by UV irradiation is transient, lasting about $1^1/_2$–2 h at 30° C; if chloramphenicol is present during early times after UV irradiation, induction of W-reactivation does not occur. Induction is maximal when cells are exposed to 5–10 J/m$^2$ of UV, a dose that also results in considerable mutagenesis of the cells. Taken together, these observations demonstrate the existence of a UV inducible, protein synthesis requiring, transiently signalled, *rec*-requiring DNA repair system analogous to W-reactivation in *Escherichia coli*. In addition, *C. crescentus* also has an efficient photoreactivation system that reverses UV damage in the presence of strong visible light.

## Introduction

*Caulobacter crescentus* are unusual bacteria that have been widely studied because of their novel program of cellular differentiation during exponential growth (see Shapiro 1976; Bender et al. 1980 for reviews). The *caulobacters* are ubiquitous, but are particularly in evidence in the surface films of clear water (Poindexter 1981). Thus it is likely that *Caulobacter* would be exposed to considerable doses of ultraviolet (UV) irradiation from sunlight. In *Escherichia coli*, several mechanisms for the repair of UV-induced DNA damage have been described including photoreactivation in the presence of visible light, excision repair, and a repair synthesis (called Weigle Reactivation) induced as part of the SOS response (Witkin 1976).

As part of our studies of DNA metabolism, we began an investigation of the ways *C. crescentus* deals with UV-induced DNA damage. Our studies show that *C. crescentus* has at least three distinguishable mechanisms for dealing with UV-induced DNA damage, two of which are described here. Although the molecular basis for these repair mechanisms is unknown, their phenomenological similarities to the well-studied *E. coli* systems of W-reactivation and Photoreactivation prompted us to adopt these terms to describe the *C. crescentus* phenomena described here.

## Materials and methods

*Bacterial and phage strains.* Strain CM5000 is CB15 (Poindexter 1964) carried for several years in our laboratory. Strain CM5256 is a recombination deficient mutant of CM5000 detected as an extremely UV-sensitive strain following mutagenesis with ultraviolet light (manuscript in preparation). Except for the extreme UV-sensitivity and lack of recombination activity, strain CM5256 appears quite normal: the growth rate of CM5256 matches its parent strain, CM5000, in broth and minimal glucose medium; the plating efficiency of phages $\Phi$Cbk, $\Phi$Cr40, $\Phi$Cr30, and $\Phi$Cb5 is 1.0 relative to the wild-type strain; and the morphology of the cells is normal when viewed in the electron microscope. Phage $\Phi$CbK (Agabian-Keshishian and Shapiro 1970) was obtained from L. Shapiro. Phage $\Phi$Cb5 (Bendis and Shapiro 1970), $\Phi$Cr30, and $\Phi$Cr40 (Johnson et al. 1977) were obtained from B. Ely. Phage were propagated as plate stocks on fresh PYE plates.

*Media.* Cells were grown in PYE broth medium (Poindexter 1964) supplemented with 0.5 m$M$ CaCl (Johnson and Ely 1977). Chloramphenicol (Sigma) was added to a final concentration of 5 µg/ml (Osley and Newton 1978). Minimal medium was M2 glucose (Johnson and Ely 1977). Solid media contained 1% Agar (Difco).

*UV Irradiation.* The source of UV light was a General Electric ASU5T8G lamp. Fluence rate (0.5 J/m$^2$·s) was measured with a Black-ray Ultraviolet Intensity Meter (Ultraviolet Products, Inc.). Phage were irradiated in Caulobacter Diluent (CD; 1 g of Bactotryptone per 1 of water) at concentrations of about 10$^9$ p.f.u. per ml with constant agitation. Bacteria were collected from broth cultures in mid-log phase (5 × 10$^8$ cells/ml) by centrifugation and were resuspended in BMO (M2 medium lacking a carbon source) at the same concentration. Irradiation volumes were less than 10 ml in 9 cm diameter glass petri dishes resulting in a depth of less than 1.5 mm.

*Experimental procedure.* *C. crescentus* host cells were grown in broth to a density of 5 × 10$^8$/ml, collected by centrifugation, and resuspended in BMO. Following UV irradiation, cells were collected by centrifugation, resuspended in broth, and incubated at 30° C with aeration for 30 min unless otherwise stated. These cells were infected with an appropriate dilution of phage $\Phi$CbK which had been irradiated with

UV light to a survival of $10^{-3}$. After adsorption (15 min at 33° C) the mixture was plated in 2 ml of PYE soft agar either with or without added indicator cells (CM5000 unirradiated). The presence of indicator did not alter the titer obtained but was necessary when the UV dose to the cells prevented formation of a lawn, especially when the UV sensitive strain CM5256 was used. In some experiments, CM5256 was used as indicator lawn and again no effect was seen in the titers.

When chloramphenicol was used it was added at 5 µg/ml 15 min before UV irradiation of the cells. The cells were resuspended, irradiated, and reincubated in the presence of chloramphenicol. Before phage adsorption, cells were collected by centrifugation and resuspended in chloramphenicol-free broth.

Defais et al. (1976) observed that although the qualitative patterns of SOS effects are readily reproducible in *E. coli*, the extent of induced repair and mutagenesis varies from one experiment to another. A close comparison of the experiments reported here shows a similar phenomenon for *C. crescentus* and the data presented are representative experiments rather than averages.

*Photoreactivation.* After UV irradiation of strain CM5256, the cells were immediately diluted in CD (the reincubation in broth being omitted). The dilution tubes were placed about 2 cm from a pair of fluorescent lamp bulbs for varying lengths of time and samples were plated to determine the viable titer. All the experiments reported in this paper were performed in very dim light from a time before irradiation of the cells in order to prevent photoreactivation.

## Results

### W-reactivation of bacteriophage ΦCbK

The survival of UV-irradiated bacteriophage is greatly increased if the host cells are irradiated as well (Weigle 1953). In *E. coli* this phenomenon is known as Weigle Reactivation or W-Reactivation (Radman 1974). To test for W-Reactivation in *C. crescentus*, UV-killed bacteriophage ΦCbK (irradiated to a survival of 0.5%) were plated on *C. crescentus* cells which had been irradiated with 10 J/m² of UV light (20% survival) and on cells which had not been irradiated. As shown in Table 1, the titer of UV-treated ΦCbK was 22-fold higher when irradiated cells were used than when

**Table 1.** Weigle reactivation of *Caulobacter phage*

| Phage | Plaques on strain CM5000 | | W-reactivation factor |
| --- | --- | --- | --- |
| | −UV | +UV | |
| Irradiated ΦCbK | 40 | 900 | 22.5 |
| Irradiated ΦCr30 | 128 | 91 | 0.7 |
| Irradiated ΦCr40 | 520 | 460 | 0.9 |
| Unirradiated ΦCbK | 85 | 91 | 1.1 |

Irradiated phage were exposed to 30 J/m² of UV light resulting in a survival of $10^{-2}$ (ΦCr30), $6 \times 10^{-3}$ (ΦCbK), or $3 \times 10^{-3}$ (ΦCr40). Wild type *C. crescentus* were irradiated with 10 J/m² of UV light and incubated in broth for 30 min before adsorption of phage. W-factor is the ratio of the phage titer measured with irradiated cells to that measured with unirradiated cells. The m.o.i. was less than $10^{-2}$ in each case (where phage titre is measured before UV inactivation)
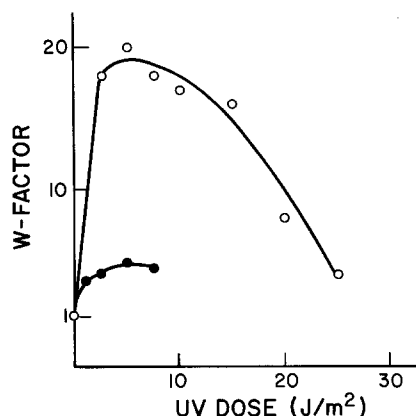


**Fig. 1.** Dose response of Weigle Reactivation to UV irradiation. Strains CM5000 (*rec*⁺) and CM5256 (*rec*⁻) were irradiated for varying times and the Weigle reactivation factor for irradiated ΦCbK (see Table 1) was measured. W-factor is defined in Table 1. o——o, CM5000; ●——● CM5256. The ordinate, which does not begin at 0, in Fig. 1 and 2 is a linear scale with values less than 1 being meaningless

unirradiated cells were used. This increase in titer represents a reactivation phenomenon since no increase in the plating efficiency of unirradiated ΦCbK was seen on irradiated cells. Thus there appears to be a W-Reactivation of ΦCbK in irradiated *C. crescentus*. In contrast no W-Reactivation of irradiated ΦCr 30 or ΦCr40 was detected (Table 1).

To determine the optimum UV dose for inducing W-Reactivation of ΦCbK, cells were treated with increasing doses of UV light and the degree to which these cells would reactivate UV-killed ΦCbK was measured. The absolute magnitude of the effect is somewhat variable (typical results are shown in Fig. 1) but the maximal response occurs when the cells have been irradiated with 5–10 J/m² of UV light (Fig. 1).

### Dependence of W-Reactivation on rec⁺ product

In *E. coli*, W-Reactivation requires an active *recA* gene product (Little and Mount 1982). A *C. crescentus* mutant strain, CM5256, is severely defective in recombination and very sensitive to UV irradiation (manuscript in preparation). Since the *rec-526* allele of strain CM5256 may be analogous to *recA* mutations of *E. coli*, we tested the effect of *rec-526* on W-Reactivation. The data in Fig. 1 show that *rec-526* severely reduced W-Reactivation in strain CM5256 but did not eliminate it. Since *rec-526* is the only *rec* mutant of *C. crescentus* isolated to date, we cannot distinguish whether the residual W-Reactivation in strain CM5256 (never more than 5-fold) represents W-Reactivation in the absence of *rec* product or residual *rec* function in the *rec-526* strain. In any event, *rec-526* severely reduces the potential of *C. crescentus* for W-Reactivation.

### Induction of W-reactivation

In the standard W-Reactivation assay, the irradiated cells were incubated under growth conditions for 30 min to allow them to express any functions necessary for W-Reactivation. When this incubation time was varied, the *C. crescentus* cells were found to continue increasing their W-Reactivation potential for $1^{1}/_{2}$ h after irradiation, after which time the amount of W-Reactivation potential shown by the cells
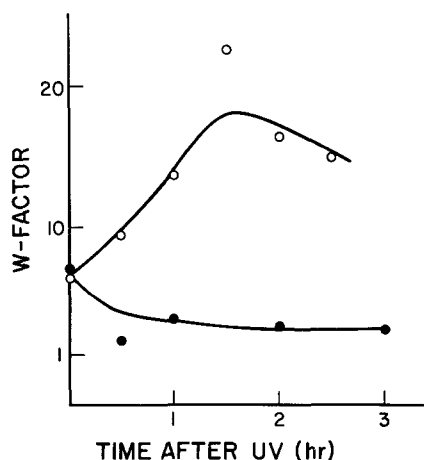
**Fig. 2.** Kinetics of induction of W-Reactivation in *C. crescentus* assayed with phage *Φ*CbK. Strain CM5000 was irradiated with 7.5 J/m² of UV light in the presence or absence of chloramphenicol (5 µg/ml). After irradiation cells were resuspended in broth with or without chloramphenicol for varying times. Samples were removed and incubated with irradiated *Φ*CbK for 15 min to allow adsorption and then plated as described in Materials and methods. Chloramphenicol-treated cells were removed from chloramphenicol by centrifugation and resuspension in chloramphenicol-free broth immediately prior to addition of irradiated *Φ*CbK. o——o, no chloramphenicol present; ●——●, chloramphenicol present from before UV irradiation until the time indicated



**Fig. 3.** UV induced mutagenesis of *C. crescentus*. Strains SC146 (*argG105*) and SC451 (*proC104*) (Barrett et al. 1982) were irradiated with increasing doses of UV light. Irradiated samples were plated on broth plates to determine total viable count and on M2-glucose plates to determine the number of prototrophic mutants. Reversion frequency is the ratio of prototrophic cells to the total cells. o——o, SC451; ●——●, SC146
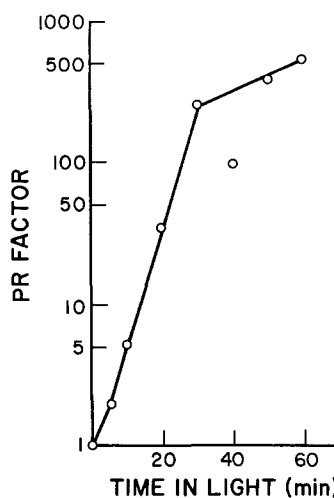


**Fig. 4.** Photoreactivation of UV irradiated *C. crescentus*. Strain CM5256 was irradiated with $7^1/_2$ J/m² of UV light (survival approx. $10^{-3}$) and immediately diluted in CD. The dilution tubes were exposed to fluorescent lamps and the viable count was determined after increasing times of illumination. The photoreactivation (PR) factor is the ratio of the titer after illumination to the titer before illumination

stopped increasing (Fig. 2). These data suggest that UV irradiation generates a signal that causes induction of some element (s) responsible for W-reactivation and that this induction signal is no longer effective after about 90 min in active cells.
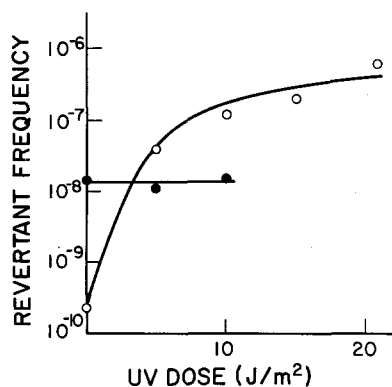
When chloramphenicol was present from the time of irradiation to the time of infection, the potential to repress W-reactivation was detected if the chloramphenicol was removed immediately after irradiation. However, the induction seen in chloramphenicol-free cultures fails to occur, if chloramphenicol is present for 30 min or longer. This result suggests that induction of W-reactivation may require protein synthesis.

*UV-inducible mutagenesis*

W-Reactivation of phage is a manifestation of UV-induced DNA repair. In *E. coli*, this DNA repair synthesis is accompanied by mutagenesis (Weigle 1953). *C. crescentus* is known to be susceptible to UV-induced mutagenesis and the data in Fig. 3 show that the rate of increase of UV-inducible mutagenesis is slower after cells have received UV doses greater than 5–10 J/m². As expected not all mutant alleles can be reverted by UV treatment. Mutation of *argG105* to *argG*⁺ is not increased in UV-irradiated cells while mutation of *proC104* to *proC*⁺ is increased about 1000-fold under our conditions. No mutants of *Φ*CbK have been described so we have not yet been able to show W-Reactivation and UV-induced mutagenesis of the same DNA. Thus the evidence for an error-prone character of the DNA repair synthesis in *C. crescentus* remains at best indirect.

*Photoreactivation*

The data presented so far illustrate the existence of a UV-inducible, *rec*-dependent, DNA repair system in *C. crescentus*, perhaps analogous to that of *E. coli* and other organ-

isms (Witkin 1976). Many organisms also have a "photoreactivation enzyme" capable of cleaving pyrimidine dimers *in situ* in the presence of visible light (Witkin 1976). The *rec-526* carrying strain CM5256 is particularly sensitive to UV damage and was tested to determine whether irradiation with visible light could overcome the lethal effects of prior irradiation with UV light. As seen in Fig. 4, exposure of UV-irradiated CM5256 to visible light resulted in a reversal of the UV-induced killing. A 300–500 fold increase in viability, raising survival from less than 0.1% to about 30% under the conditions used, is routinely seen. Thus *C. crescentus*, like *E. coli*, very likely has a *rec*-independent photoreactivation system for repairing UV-induced DNA damage.

**Discussion**

The data presented here demonstrate a UV inducible DNA repair system in *C. crescentus* at least superficially analo-

gous to that found in *E. coli* (Radman 1974). The best characterized aspect of this system is the W-Reactivation of UV-irradiated phage ΦCbK by *C. crescentus*. The failure to detect W-Reactivation of other *C. crescentus* phages was unexpected (particularly for the generalized transducing phage ΦCr30) but may have been an artifact of their adsorption kinetics. Both ΦCr30 and ΦCr40 adsorb very slowly to strain CM5000 (data not shown) and it is possible that the transient induction signal for W-reactivation may have passed before a significant number of particles had adsorbed. The failure to observe W-Reactivation in ΦCr30 and ΦCr40 was not studied further.

One obvious difference between the *E. coli* and *C. crescentus* W-reactivation responses is in the time required for maximum induction. *E. coli* growing in broth at 37° C show maximal W-Reactivation potential about 30 min after irradiation (Defais et al. 1976); *C. crescentus* growing in broth at 30° C, about 90 min after irradiation. However, this difference correlates with the different generation times for the organisms (20–30 min for *E. coli* and 90–100 min for *C. crescentus*), extending the similarity between the two systems.

Among the features characteristic of W-reactivation in *E. coli*, *recA* dependence could not be clearly demonstrated in *C. crescentus*. The *rec-526* strain CM5256 is clearly defective in its induction of W-reactivation potential. Whereas wild type displayed W-reactivation factors as high as 35–40, strain CM5256 never displayed a W-reactivation factor greater than about 5. If the *rec-526* mutation identifies the *C. crescentus* analog of the *E. coli recA*, then CM5256 should be devoid of functions such as W-reactivation. However it is also possible that *rec-526* does not completely inactivate the inducing activity of the putative *recA* protein of *C. crescentus*. Strain CM5256 is very sensitive to UV irradiation and shows no detectable recombination activity (at least 5000-fold less than wild type). The fact that this strain is also at least partially defective in its W-reactivation induction lends some support to the suggestion that *rec-526* may define the *C. crescentus* analog of the *E. coli recA* gene. A third possibility is that the effect of the *rec-526* mutation is different from that of *E. coli recA* mutations. As more *rec* alleles become available, it should be possible to establish the role (if any) of the gene defined by *rec-526* in the *C. crescentus* W-reactivation.

Implicit in this entire discussion is the obvious analogy to the SOS-response of *E. coli*. At the phenomonological level, the W-reactivation and UV mutagenesis described here are quite similar to elements originally lumped together under the term SOS-response (Witkin 1976). The molecular basis of the SOS-response has become clear in recent years and both W-reactivation and UV-induced mutagenesis are known to be rather indirect consequences of the central feature of the SOS-system: the *recA*-facilitated cleavage of the *lexA* product (Litle and Mount 1982; Walker 1984). There are no data to suggest whether the molecular basis of the W-reactivation and UV mutagenesis we see in *C. crescentus* bears any similarity whatsoever to the *lexA*-repressible (SOS) system of *E. coli*. In particular we have not yet tested whether the gene identified by the *rec-526* mutation encodes a protein analogous or homologous to *recA* of *E. coli*.

We have shown here that C. crescentus has at least two

of the well-characterized systems used by other microorganisms to repair UV-induced DNA damage: W-reactivation and photoreactivation. This does not imply that a third well-characterized system, excision repair, is absent. In fact, we have isolated a number of UV sensitive *C. crescentus* mutants that retain full recombination activity and are likely candidates for analogs of the *uvr* genes that define the excision repair system of *E. coli*.

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