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Repair of phage λ DNA damaged by near ultraviolet light plus 8-methoxypsoralen

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Treatment of phage λ with 8-methoxypsoralen plus near ultraviolet light (PUVA) and its subsequent infection and growth on various mutant and non-mutant hosts were investigated. A number of *Escherichia coli* DNA repair-deficient mutants, particularly those deficient in genes producing proteins known to participate in interstrand crosslink repair, were used as hosts to assess the roles of these gene products in the activation of phage affected by PUVA. Results show that *puvA*, *uvrA*, *uvrD*, *recA*, *recO*, *sulA* and *recN* of *E. coli* are involved in the repair process. Based on the data presented it is proposed that phage λ DNA is repaired, following PUVA damage, using the recombinational repair process. This may be in agreement with the recombinational model of the repair of *E. coli* DNA.

Near-ultraviolet light (300–400 nm) plus 8-methoxypsoralen (PUVA) interacts with DNA and has the ability to arrest its replication in dividing cells. This feature has led to its use in the treatment of human diseases such as psoriasis (characterized by the proliferation of certain epithelial cells in the skin), mycoses and vitiligo (Pathak *et al.*, 1974). The reaction between 8-methoxypsoralen (MOP) and DNA takes place in three stages. In the first stage MOP binds non-covalently between two bases in the complementary DNA strands. Next, in the presence of a near ultraviolet (NUV) photon, a covalent monoadduct forms between MOP and a DNA base. This base is usually thymine although photoadducts have been reported with cytosine and purines (Davies, 1980; Calvin & Hanawalt, 1987). Exposure of the monoadduct to a second photon of NUV produces a covalently bound adduct with another base in the complementary DNA strand. Thus the reaction between PUVA and DNA results in the crosslinking (CL) of complementary strands of DNA (Cimino *et al.*, 1985; Smith, 1988).

The repair of PUVA-damaged DNA has been studied in T3 and T4 phages (Strikes *et al.*, 1981; Belogurove & Zavilgelsky, 1981), in *Escherichia coli* (Sinden & Cole, 1978), in *Deinococcus radiodurans* (Kitayama *et al.*, 1983), in yeast (Chanet *et al.*, 1985) and in mammalian cells (Zolen *et al.*, 1984). Despite extensive studies on repair of PUVA-damaged DNA, particularly in *E. coli* and in yeast, its exact mechanism is not yet clear (Demple & Harrison, 1994; Smith, 1988). In *E. coli* the *uvrA*, *B*, *C*, *uvrD*, *recA* and *polA* gene products have been implicated in the repair process. More importantly recombination repair has been implicated, as blockage of the recombination process (mutation in *recA*, *recBC sbcB recF*) has been found to lead to inefficient repair (Sinden & Cole, 1978).

In our earlier studies on PUVA we isolated two novel PUVA mutants of *E. coli*: the *puvA* mutant (JH110) is hyper-sensitive to PUVA and the *puvR* mutant (SA270) is hyper-resistant to these agents (Holland *et al.*, 1991; Ahmad & Holland, 1985). Genetic mapping showed that both the *puvR* and *puvA* genes were located near 57·2 min region of the linkage map of *E. coli* (Holland *et al.*, 1991). Further studies showed that *puvR* is a regulatory gene for the structural gene, *puvA*. The gene product of *puvA* (a 55 kDa protein) was postulated to be a PUVA-specific endonuclease. The PUVA sensitivity in JH110 and PUVA hyper-resistance in SA270 were explained as follows: in wild-type *E. coli* the synthesis of the 55 kDa protein is inducible and the basal level synthesis of this protein limits the ability of the cell to incise and subsequently repair the crosslinks from the DNA. The *puvR* gene encodes a regulatory repressor protein which binds to a putative operator site (*puvO*) of the PUV operon. Basal level expression of the operon ensues. In the *puvR* strain (SA270) the synthesis of this repressor is eliminated (or reduced) and hence the 55 kDa protein (the *puvA* product) is synthesized constitutively and cells show enhanced resistance to PUVA. The PUVA sensitivity of JH110 is associated with the lack of active

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Table 1. *Genotype/phenotype of bacterial strains used in this study*

Bacterial strain	Genotype/phenotype	Derivation/reference
SA235	Hfr KL16	Bachmann (1972)
SA270	*puvR*	From SA235 (Ahmad & Holland, 1985)
AB2463	*thr-1 leu-6 thi-1 lacY1 galK2 proA2 his-4 argE3 tsx-33 recA13* and A*	Bachmann (1972)
SA162	Hfr *P4X uvrA*	Our laboratory
N1412	F⁻ *uvrD210 lacI3 lacZ118 proB48 trpA9605 nalA str-r*	From R. G. Lloyd
N2454	F⁻ *recN262 tyrA16*::Tn*10 thyA lacZ118 metE proC leu* Nal^r *and A**	From R. G. Lloyd
N2451	F⁻ *recO1504*::Tn*5 lacI3 lacZ118 metE proC leuB6 thyA54 spc-15 nalA* and A*	From R. G. Lloyd
JH110	*puvR puvA*::*lacZ* Tn*5* Kan^r	From SA235 (Holland *et al.*, 1991)
DM4000	Δ*lac-proXIII hisG4 argE3 sulA*::*Mud1* (*bla lac*)*X Cam thr-1 tsx-33 ilvTS* and A*	From M. Volkert

* A: *ara xyl mtl* Str^r *sup.*

55 kDa protein owing to a mutation in the structural gene *puvA*.

A model for the repair of PUVA-damaged DNA has been proposed for *E. coli* in which firstly the *uvr* excision nuclease (*uvrA,B,C*) makes cuts in the DNA strand, one on each side of the damage. Next a gap is created which, in the third step, is filled by a recombination event mediated by the RecA protein. Subsequently a second round of ABC excision nuclease activity creates incisions in the complementary strands and finally the ABC nuclease and the cross-link dissociate and the gap is filled by the combined action of DNA polymerase I and DNA helicase II (Van Houten *et al.*, 1986).

Unlike phage T4 which is known to have specific enzymes to repair UV-induced DNA damage (Walker, 1984) phage λ normally depends on its host for repair. Devoret *et al.* (1975) classified repair into 3 types: (i) host cell reactivation (ii) prophage reactivation and (iii) UV reactivation. All three processes are applicable to forms of UVC-induced DNA damage which principally involve formation of pyrimidine dimers. Little is known about the repair of DNA in phage λ affected by PUVA. Hence it was of interest to determine what mechanism(s) are used to repair λ DNA damaged by PUVA. In particular we have been interested in determining the contribution of the *puvA* and *puvR* genes to the repair process.

In this short communication we provide data suggesting that phage λ makes use of its host's repair system(s) to rectify PUVA-damaged DNA. *E. coli* genes have been identified participating in the phage DNA repair process. Along with the genes proposed by Van Houten *et al.* (1986) for *E. coli* DNA repair we show that several other *E. coli* genes (*puvA*, *recO*, *sulA* and *recN*) are involved in λ DNA repair.

The study of phage λ DNA repair (using phage from our own laboratory with no known mutation) involved addition of 8-methoxypsoralen (Sigma; final concentration of 25 μg/ml), to suitable phage lysate dilutions in phosphate buffer (pH 7·0) followed by irradiation under a Philips lamp (Model HP 3148/A) for varying doses (UVX digital radiometer with UVX-36 sensor assembly, 365 nm; from UVP, Cambridge, UK was used to measure the light intensity). At intervals, samples were removed, diluted in phosphate buffer and the number of phages was determined using relevant hosts by the standard soft layer agar method (Miller, 1972). Each experiment was repeated at least three times and results presented are the mean values of the set of experiments. The number of plaques produced on various hosts were considered as indicative of the repair efficiency of phage DNA by the host repair system(s).

Results for phage λ growth on wild-type *E. coli* (Fig. 1) show that as the dose of UV is increased the amount of infectious virus is proportionately decreased. This is taken to represent the basal level of repair against which results obtained with mutant bacteria are compared.

Results (Fig. 1) show that all the DNA repair-deficient mutants of *E. coli* tested in these experiments show various degrees of reduction in their ability to support the growth of phage λ. The highest reduction in the activity is observed for the *puvA* mutant, JH110. The recovery of phage on SA270 (*puvR*), on the other hand, is not increased over the wild-type recovery rate. This is unexpected but a likely explanation is that in wild-type *E. coli* the endogenous synthesis of the 55 kDa protein is high enough to carry out the repair of phage λ DNA, but not high enough for its own efficient repair. Thus the increased concentration of this protein in the mutant bacteria SA270 has no effect on the recovery of phage λ whereas lack of this protein (as in JH110) produces a significant effect.

Other important gene products for the activation of phage λ appear to be UV excision nuclease (the *uvrA* product), DNA helicase II (the *uvrD* product) and the Rec*A* protein (Fig. 1). These results are not unexpected as these genes have been implicated in the repair of PUVA-damaged *E. coli* DNA (Van Houten *et al.*, 1986).

From the results for the *recN* mutant it is clear that this mutant shows a moderate effect on the recovery of phage λ. Earlier studies have implicated *recN* in the repair of interstrand cross-links affected by mitomycin C (Picksley *et al.*, 1985). Data presented in this communication imply that the *recN* gene may also have roles (albeit limited) in the repair of PUVA-damaged DNA.

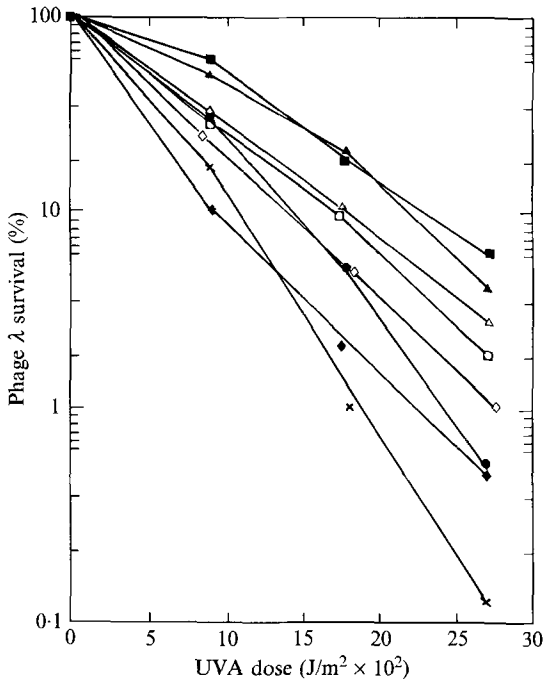

Fig. 1. Growth of PUVA-treated bacteriophage λ on various mutant and non-mutant host strains of *E. coli*. Symbols: (■) SA235, wild-type *E. coli*; (×) JH110, *puvA* mutant; (▲) SA270, *puvR* mutant; (●) N1412, *uvrD* mutant; (◆) SA162, *uvrA* mutant; (◇) AB2463, *recA* mutant; (□) N2451, *recO* mutant; (○) N2454, *recN* mutant and (△) DM4000, *sulA* mutant.

The lack of efficient recovery of λ on a *rec*O mutant of *E. coli* implies a limited role for this gene in λ DNA repair. The *recO* gene of *E. coli* participates in the recF pathway of DNA repair and the gene product has been shown to promote renaturation of complementary ssDNA (unpublished work of Luisi-DeLuca reported by Umezu *et al.*, 1993). Genetic studies suggest that the *recO* gene product functions along with the *recF* and *recR* gene products at the same step of recombination and interacts with Rec*A*. Mutation in *sulA* (previously known as *sfiA*; Johnson & Greenberg, 1975) shows a small effect on the recovery of phage λ implying certain minor roles of this gene in the process.

From the data presented it can be concluded that *puvA*, *uvrA*, *uvrD*, *recA* and to some extent *sulA*, *recO* and *recN* of *E. coli* are involved in the repair of phage λ DNA damaged by PUVA. Out of the several repair systems in *E. coli* (Hanawalt *et al.*, 1979), it can be postulated that phage λ makes use of the host's recombinational repair system and we propose that the model presented by van Houten *et al.* (1986) for *E. coli* may also be applicable to phage. In the model the roles of *uvrA*, *uvrD* and *recA* have been assigned and the roles of *recO*, *recN* and *sulA* gene products in the repair process have yet to be elucidated.

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