Rev. Brasil. Genet. 16, 4, 893-902 (1993)
(Brazil. J. Genetics)

EFFECTS OF UmuDC AND MucAB OVERPRODUCTION ON RESISTANCE TO UV INDUCED MORTALITY, ON W-REACTIVATION AND ON THE KINETICS OF UV-LYSOGENIC INDUCTION OF LAMBDA PHAGE IN *Escherichia coli*

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ABSTRACT

We analysed the effect of overproduction of UmuDC and MucAB proteins on phage survival, W-reactivation, cell survival and the kinetics of phage production in *Escherichia coli*. We could not detect W-reactivation in *uvrA6* mutants (AB1886) even with an excess of UmuDC or MucAB proteins. We also observed a strong inhibition of lysogenic induction and of spontaneous phage production in *uvrA6* mutants carrying a multicopy plasmid containing *umuDC* genes. However this effect was weaker in strains carrying a multicopy plasmid containing *mucAB* genes.

INTRODUCTION

In *Escherichia coli* the *umuDC* genes encode mutagenic repair activity (translesion synthesis) which can also be provided by *mucAB* genes, an analog of *umuDC* carried on plasmid pKM101 (Walker, 1984). The existence of this repair system has been demonstrated by the nonmutability and sensitivity of *umuDC* strains of *E. coli* after DNA-damaging treatments (Kato and Shinoura, 1977; Steinborn, 1978). Furthermore, the low mutability of *umuDC* mutants, as well as the poor W-reactivation of lambda phage, can be restored by the introduction of plasmids carrying the *mucAB* operon (Walker and Dobson, 1979).

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The expression of *umuDC* and *mucAB* operons is repressed by LexA protein and is regulated as a part of the SOS response, in which an activated form of RecA protein mediates the cleavage of LexA protein (Bagg *et al.*, 1981). The cleavage of UmuD is necessary for its role in the UV-mutagenesis and is also mediated by the activated form of RecA. On the other hand, there is evidence indicating that the proteolysis of MucA is not required for its role in mutagenesis (Shiba *et al.*, 1990). Although MucA and UmuD are functionally and structurally similar, MucAB enhances UV-protective and mutagenic activities more than UmuDC (Perry *et al.*, 1985).

It has been reported that overproduction of UmuDC proteins interferes with some aspects of cellular physiology, causing an increase in UV-lethality in *uvrB5* strains (Blanco *et al.*, 1986). It was found that cells carrying a high-copy-number plasmid containing *mucAB* genes have an inhibited expression of the *umuD*::*lacZ* fusion, probably due to competition between MucA and LexA proteins in their interaction with RecA protein (Marsh and Walker, 1987). Another observation was that the overproduction of UmuDC in *E. coli* results in cold-sensitive growth with reversible inhibition of DNA synthesis at the nonpermissive temperature (Marsh and Walker, 1985). We studied UV-related lethality and inhibition of UV-lysogenic induction in *uvrA6* strains carrying multicopy plasmids containing *umuDC*.

MATERIAL AND METHODS

Bacterial strains, phage and plasmids

The *E. coli* K-12 strains and the plasmids used are listed in Table I. The strains were transformed with plasmids pBR322 (vector), pICV80, which contains the *mucAB* operon (Blanco *et al.*, 1986) and pSE117, which contains the *umuDC* operon (Marsh and Walker, 1985). Strains carrying the vector plasmid (pBR322) and strains without plasmids were used as controls. AB1157 and AB1886 strains were lysogenised with lambda phage. AB2480 strain was used as the indicator for plaque forming units (pfu) in the kinetics of phage induction and in the W-reactivation experiments. The phage used was the lambda wild type.

Media, growth of bacteria and UV-irradiation

Cells were grown overnight in LB medium (Miller, 1972), diluted (1:50) and incubated with shaking at 37°C to yield suspensions of about 2 x 10^8 cells per ml. Cultures were then centrifuged at 8000 g for 10 min and were resuspended in M9 buffer (Miller, 1972), giving a similar titer. Ten ml samples were irradiated in glass Petri dishes, using a General Eletric UV-lamp (G15T8, 15W). The dose rate was measured by a Latarjet

Table I - Strains and plasmids.

Strain or plasmid	Relevant genotype	Reference
AB1157	Wild type	Howard-Flanders and Theriot (1962)
AB1157 (λ)	Wild type (λ_{ref})	This study
GW2100	*umuC122*::Tn5	Elledge and Walker (1983)
AB1886	*uvrA6*	Howard-Flanders and Theriot (1966)
AB1886 (λ)	*uvrA6* (λ_{ref})	This study
AB2480	*uvrA6 recA13*	Howard-Flanders *et al.* (1969)
AB2463	*recA13*	Howard-Flanders and Theriot (1966)
pBR322	vector	Bolivar *et al.* (1977)
pSE117	*umuDC*	Marsh and Walker (1985)
pICV80	*mucAB*	Blanco *et al.* (1986)

dosimeter. The cell dilutions were made in M9 buffer. After dilution the cells were plated on LB-plates (cells without plasmids) or LB-Amp plates (cells with plasmids) for colony forming unit (cfu) determination. Incubation temperature was 37°C.

In order to determine phage survival and W-reactivation or free-phages (kinetics of induction), after UV-irradiation, samples were collected, diluted, and 0.1 ml aliquots were added to melted agar containing indicator bacteria, and were poured on GT plates (Devoret *et al.*, 1983). The free-phage production was determined by mixing each sample with 50 μl chloroform before dilution and plating.

Phage survival

Unirradiated bacterial hosts were used in the stationary phase to measure the pfu of lambda phages that were irradiated with different doses. A stock of lambda phage was diluted to 10^7 pfu/ml in 10 mM $MgSO_4$, irradiated and diluted. Infection mixtures (0.1 ml of phage dilution plus 0.3 ml of host culture) were incubated for 20 min, plated and incubated overnight.

W-reactivation

Phage lambda suspensions were diluted to about 10^7 pfu/ml in 10 mM $MgSO_4$ and irradiated to 0.1% - 0.01% survival. The host cells were harvested in the exponential phase, resuspended in 10 mM $MgSO_4$ and irradiated with different doses. Aliquots of the

irradiated bacteria were incubated in LB for 30 min to allow maximal SOS expression (Defais *et al.*, 1976). Aliquots of the irradiated bacteria were used for the infection with the phage suspension (irradiated and unirradiated) (m.o.i. < 0.1). The mixture was incubated for 20 min in order to permit adsorption and was plated as described before. The reactivation efficiency was calculated according to Devoret *et al.* (1975) in order to minimize the effect of different phage survival levels.

Kinetics of phage induction

Lysogenic cells were submitted to UV-radiation doses which resulted in about 10% cell survival. Then they were diluted (1:10) in pre-warmed LB medium and incubated with shaking. Samples were collected at different periods of time and the pfu/ml was determined as free-phages. Spontaneous induction and bacterial growth were also assayed.

RESULTS

Phage survival and W-reactivation in cells overproducing UmuDC or MucAB proteins

In order to analyse the effects of overproduction of UmuDC and MucAB on phage repair in *E. coli* K12 cells, the survival of UV-irradiated lambda phage was determined (Figure 1). The various unirradiated host cells containing *mucAB*$^+$ plasmid

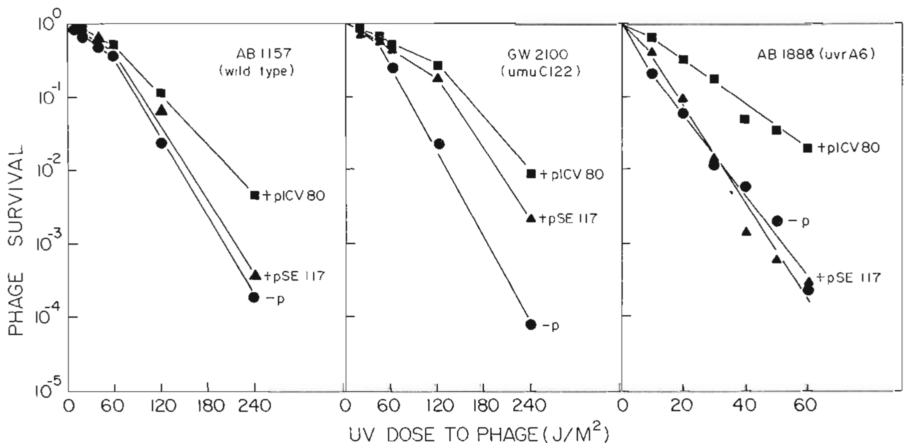

Figure 1 - Phage survival after UV irradiation in *Escherichia coli* strains containing pICV80 (*mucAB*) or pSE117 (*umuDC*) plasmids.

pICV80 were more efficient in the recovery of the lambda phage than the cells without plasmids. The *umuDC*$^+$ plasmid pSE117 was only effective in complementing the *umuC122* mutation in *umuC* mutant strain GW2100. This plasmid did not influence lambda UV survival in the wild-type or *uvrA6* mutant strain AB1886. Neither MucAB nor UmuDC overproduction could promote additional repair in the absence of wild type RecA protein (data not shown).

In the W-reactivation experiments only the host cells were exposed to different doses, while the phage dose was held constant for each strain. The maximum W-reactivation was obtained at 80 J/m^2 in wild-type strain AB1157 with or without plasmids and in *umuC* mutant strain GW2100 only when carrying the plasmids (Figure 2). Therefore, W-reactivation efficiency was measured when 80 J/m^2 was applied to the hosts, and corresponded to 54% in AB1157/pICV80, 45% in AB1157/pSE117 and 53% in AB1157 without plasmids. The *umuC* mutant strain GW2100 recovered the capacity for W-reactivation when transformed by *umuDC*$^+$ or *mucAB*$^+$ plasmids. In the GW2100 strain the plasmids pICV80 (*mucAB*$^+$) and pSE117 (*umuDC*$^+$) restored the W-reactivation efficiency to proportions of 34% and 32% respectively.

The introduction of multicopy plasmids did not restore the W-reactivation in the *uvrA6* mutant strain (AB1886) (Figure 2).

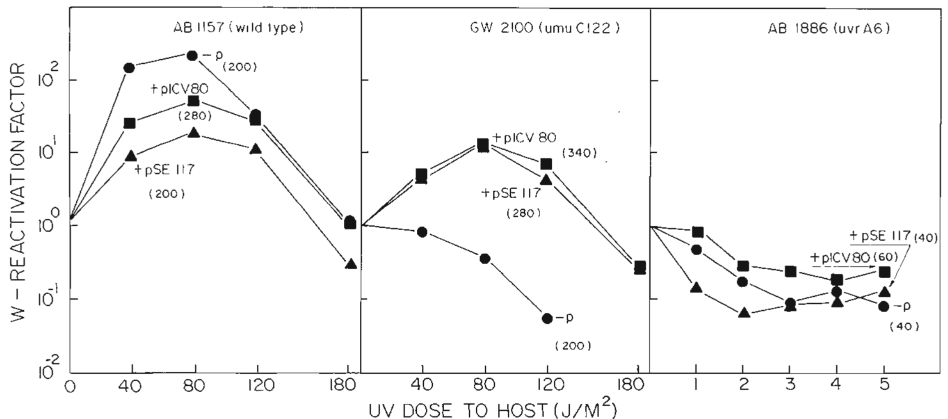

Figure 2 - W-reactivation of UV-irradiated lambda phage in strains carrying pICV80 (*mucAB*), pSE117 (*umuDC*) and without plasmids. In this experiment, the dose to the phage remained constant while the dose to the host varied. The dose applied to the phage (J/m^2) is indicated in brackets.

Survival and kinetics of lysogenic induction in bacteria overproducing MucAB or UmuDC proteins

The *mucAB*$^+$ plasmid pICV80 greatly increased resistance to UV-lethality in all strains, except in *recA* mutants (Figure 3). We demonstrated that pSE117 (*umuDC*) has a limited capacity for translesion synthesis when compared with pICV80 (*mucAB*) as did Blanco *et al.* (1986) (Figure 3). However, a decreased survival of the *uvrA6* mutant was detected in the presence of the *umuDC*$^+$ plasmid pSE117. The pSE117 and pICV80 plasmids did not modify cell survival in *recA* strains.

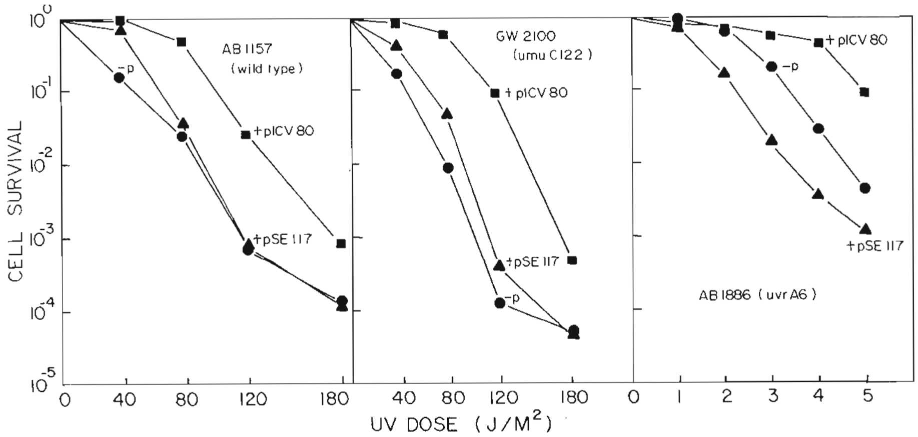

Figure 3 - Influence of pICV80 (*mucAB*) and pSE117 (*umuDC*) on cell survival after UV irradiation.

The kinetics of lysogenic induction was studied in the *uvrA6* mutant carrying the vector (pBR322), pSE117 and pICV80 plasmids, since overproduction of UmuDC proteins increased the UV lethality of this strain. In the wild type strain, free-phages were first observed 60 min after UV-irradiation and within 90 min all infective centers had burst and had liberated mature virus (Figure 4). In the *uvrA6* mutant, carrying pBR322, pICV80 (*mucAB*$^+$) or pSE117 (*umuDC*$^+$), we observed that only about 120 min after UV-irradiation the phages began to burst the cells and at from 180-240 min maximum induction had occurred. However, in the *uvrA6* mutant strain AB1886 carrying the *umuDC*$^+$ plasmid pSE117 the level of phages produced was lower than with either pBR322 or pICV80 (*mucAB*$^+$). Maximum induction occurred after 270 min and was 92.5% less than with AB1886 carrying pBR322 (Table II). The percentage of maximum induction demonstrated that pICV80 and pSE117 caused a decrease in phage induction in both strains (wild type and *uvrA6* mutant), however the reduction was more evident

in the excision repair deficient strain. In addition, pICV80 and pSE117 plasmids also interfered with spontaneous induction (Figures 4 and 5).

Table II - Lysogenic induction.

Strains	UV dose (J/m^2)	Time (min)	Maximal induction[a] (pfu/ml)
AB1157 (λ)			
+pBR322	20	90	4.4×10^8 (100%)
+pICV80	40	90	3.2×10^8 (72%)
+pSE117	30	90	2.4×10^8 (54%)
AB1886 (λ)			
+pBR322	2	180	5.6×10^7 (100%)
+pICV80	3	180	1.6×10^7 (29%)
+pSE117	2	270	4.2×10^6 (7.5%)

The numbers obtained are the average of three experiments.

[a]Phage production was considered 100% in strains carrying pBR322.

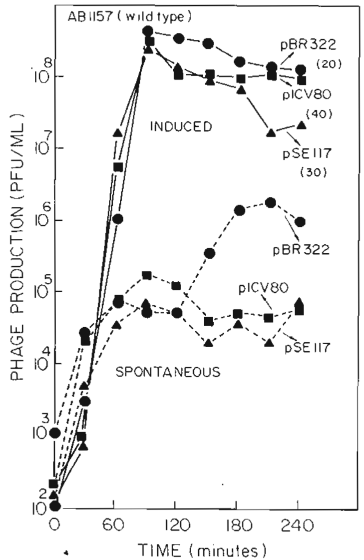

Figure 4 - Kinetics of lambda phage production following UV-induction in AB1157 (wild type) lysogenic strain with pICV80 (*mucAB*), pSE117 (*umuDC*) and pBR322 (vector). The dose applied to the lysogenic cells (J/m^2) as indicated in brackets. The broken lines represent spontaneous induction.

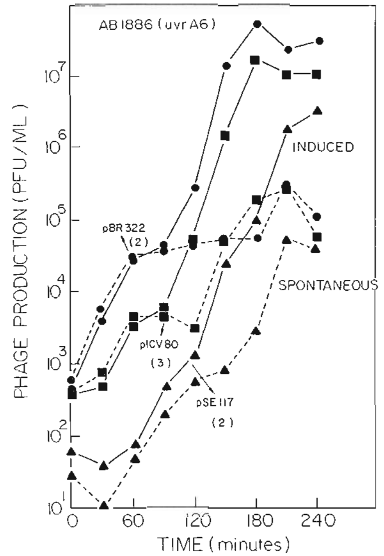

Figure 5 - Kinetics of lambda phage production following UV-induction in AB1886 (*uvrA6*) lysogenic strain with pICV80 (*mucAB*), pSE117 (*umuDC*) and pBR322 (vector). The dose to the lysogenic cells (J/m^2) is indicated in brackets. The broken lines represent spontaneous induction.

DISCUSSION

A significant enhancement of phage survival was observed when pICV80 plasmid was introduced into unirradiated hosts (wild type, *uvrA6* and *umuC122*), however, the introduction of pSE117 in the same strains had no effect on phage survival. Furthermore, the introduction of such plasmids in the *umuC122* mutant restored their W-reactivation capacity and the presence of an excess of MucAB or UmuDC proteins did not interfere with W-reactivation in the wild type strain. On the other hand, in the *uvrA6* mutant, such plasmids were unable to restore the W-reactivation capacity.

It has been proposed that translesion synthesis is the main mechanism of SOS repair in lambda DNA and is probably *umuDC*-dependent (Defais *et al.*, 1989). However, our experiments could not detect any W-reactivation in the *uvrA* mutant, even when overproduction of UmuDC or MucAB proteins occurred, suggesting that the *uvrA* alele effectively prevents the expression of W-reactivation. The activity of MucAB (pICV80) to repair lambda DNA is limited in induced cells, when wild type UvrA protein is absent.

Introduction of pSE117, a multicopy plasmid carrying the *umuDC* genes into *E. coli* K-12 strains which are *uvrA6* mutants resulted in an increased lethality after UV irradiation and a strong inhibition of lysogenic induction and spontaneous phage induction. The UV-sensitivity may be due to a SOS inhibition, detected by lysogenic induction, resulting in an inhibition of other paths of SOS repair that could be acting to compensate the absence of excision repair. Additionally, SOS inhibition is involved with

an interference in recA production, which is necessary for the repair mediated by UmuDC proteins. Therefore, overproduction of UmuDC can cause a decrease in the RecA level, probably by competition with repressor proteins (LexA and cI) as Marsh and Walker (1987) have proposed.

The phage and bacterial survival and lysogenic induction data confirm the difference between UmuDC and MucAB proteins (Blanco *et al.*, 1986). UmuDC protein seems to be more dependent on RecA (Shiba *et al.*, 1990) and may have more affinity for RecA protein; therefore it captures RecA with greater intensity, producing a strong SOS inhibition, which is more evident in the *uvrA6* mutant. However, UmuDC overproduction causes deep changes in cellular physiology (SOS induction), especially in the *uvrA6* mutant, and the effect of MucAB overproduction is weaker in this situation.

ACKNOWLEDGMENTS

We are grateful to B.W. Glickman, P.L. Moreau, L.R. Asad and R. Devoret for useful discussions of this work, and to M. Blanco for the gift of strains and plasmids. This work was supported by grants from CNPq, CEPG-UFRJ and FINEP.

RESUMO

Nós analisamos o efeito da superprodução de proteínas UmuDC e MucAB na sobrevivência de fagos, reativação Weigle, sobrevivência celular e na cinética de produção de fagos em *Escherichia coli*. Nós não detectamos reativação Weigle nos mutantes *uvrA6* (AB1886) mesmo com excesso de proteínas UmuDC ou MucAB. Nós observamos uma forte inibição da indução lisogênica e da produção espontânea de fagos nos mutantes *uvrA6* contendo o plasmídeo multicópia com os genes *umuDC*. Entretanto, este efeito não foi tão acentuado nas cepas contendo plasmídeo multicópia com os genes mucAB.

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(Received September 28, 1992)