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RESEARCH ARTICLE

Survival and SOS response induction in ultraviolet B irradiated *Escherichia coli* cells with defective repair mechanisms

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ABSTRACT

Purpose In this paper, the contribution of different genes involved in DNA repair for both survival and SOS induction in *Escherichia coli* mutants exposed to ultraviolet B radiation (UVB, [wavelength range 280–315 nm]) was evaluated.

Materials and methods *E. coli* strains defective in *uvrA*, *oxyR*, *recO*, *recN*, *recJ*, *exoX*, *recB*, *recD* or *xonA* genes were used to determine cell survival. All strains also had the genetic *sulA::lacZ* fusion, which allowed for the quantification of SOS induction through the SOS Chromotest.

Results Five gene products were particularly important for survival, as follows: UvrA > RecB > RecO > RecJ > XonA. Strains defective in *uvrA* and *recJ* genes showed elevated SOS induction compared with the wild type, which remained stable for up to 240 min after UVB-irradiation. In addition, *E. coli* strains carrying the *recO* or *recN* mutation showed no SOS induction.

Conclusions The nucleotide excision and DNA recombination pathways were equally used to repair UVB-induced DNA damage in *E. coli* cells. The *sulA* gene was not turned off in strains defective in UvrA and RecJ. RecO protein was essential for processing DNA damage prior to SOS induction. In this study, the roles of DNA repair proteins and their contributions to the mechanisms that induce SOS genes in *E. coli* are proposed.

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Escherichia coli; SOS induction; UVB radiation; DNA repair genes

Introduction

Currently, research regarding the biological effects of ultraviolet radiation (UV) has become increasingly important due to the adverse effects of over-exposure to solar radiation in living organisms (Matsumura and Ananthaswamy 2004). UVB [wavelength range 280–315 nm] is considered an agent of biological relevance because it induces DNA damage, such as cyclobutane pyrimidine dimers (CPD), 6-4-pyrimidine pyrimidone photoproducts (6-4PP) and 8-hydroxydeoxyguanosine (8-OHdG), which in turn trigger the appearance of skin cancer (Beehler et al. 1992; Mitchell et al. 1999). In this context, research aimed to elucidate the pathways of UVB-induced DNA damage represents the basis for an effective photoprotection strategy (Lea et al. 2007).

In *Escherichia coli,* exposure to UV activates a cellular response to genotoxic stress known as the SOS response (Witkin 1976). During this response, approximately 40 genes involved in different cellular functions, such as DNA repair, cell filamentation, mutagenesis and replication restart, are induced (Bagg et al. 1981; Fogliano and Schendel 1981; Huisman and D'Ari 1981; Kenyon and Walker 1981; Courcelle and Hanawalt 2001; Quillardet et al. 2003). The LexA protein is a common repressor of this pathway, binding to a consensus sequence known as the 'SOS box', which is present in the operator of the SOS genes and limits their expression (Little and Mount 1982; Lewis et al. 1994). The RecA protein is a positive regulator of the SOS response and shows high single-stranded DNA (ssDNA) region affinity, which serves as a primer SOS induction signal (Higashitani et al. 1995). When DNA damage occurs, the RecA protein binds to ssDNA regions that result from RecFOR, RecBCD and RecJ/Q activities (Anderson and Kowalczykowski 1998; Hishida et al. 2004; Chow and Courcelle 2007; Vlašić et al. 2011), constructing RecA-ssDNA nucleoprotein filaments that promote self-digestion of the LexA repressor and allow transcription of the SOS genes (Courcelle and Hanawalt 2003).

In *E. coli*, UV-induced DNA lesions (i.e., CPD and 6-4PP) are mainly removed by nucleotide excision repair and photoreactivation (Sancar and Rupp 1983; Kneuttinger et al. 2014). Exonucleases are also important in helping proteins from the RecBCD-dependent homologous recombination pathway in UV-treated *E. coli* cells (Viswanathan and Lovett 1998; Đermić 2006). The exonucleases I, SbcCD and RecJ participate in processing protruding ends in fragmented DNA to allow RecA/RecBCD-mediated homologous recombination (Thoms and Wackernagel 1998; Thoms et al. 2008). The photoproducts are also an obstacle for *E. coli* chromosome replication. The RecQ/RecJ helicase/nuclease with the RecA/RecFOR recombination pathway appears to also be essential for the recovery of DNA replication in *E. coli*

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bacteria exposed to UV (Courcelle and Hanawalt 1999, 2001; Courcelle et al. 1999, 2003, 2006; Chow and Courcelle 2004).

Previous studies have shown the importance of DNA repair proteins for survival and SOS induction in γ-irradiated *E. coli* cells (Breña-Valle and Serment-Guerrero 1998; Serment-Guerrero et al. 2008). These findings suggest that while UvrA, RecN and OxyR proteins take part in different repair or protective pathways, RecB, RecJ, RecO and ExoI participate in processing DNA damage by generating single-strand gaps (ssg) in DNA that finally leads to SOS induction and recombination. This study aimed to determine the influence of *uvrA*, *oxyR*, *recO*, *recN*, *recJ*, *exoX*, *recB*, *recD* and *xonA* mutations on both survival and SOS induction in *E. coli* exposed to UVB.

Materials and methods

Chemicals and media

Luria-Bertani (LB) media (10 g/l tryptone, 5 g/l yeast extract, 10 g/l sodium chloride, pH 7.4) and substrates for β-galactosidase (*o*-nitrophenyl-β-D-galactopyranoside or ONPG) and alkaline phosphatase (*p*-nitrophenylphosphate or PNPP) were purchased from AMRESCO, Solon, OH, USA. Antibiotics (ampicillin and tetracycline) and mutagens (mitomycin C [MMC] and 4-nitroquinoline-1-oxide [4-NQO]) were obtained from Sigma, St. Louis, MO, USA. The remaining chemicals used in this study were purchased from Merck, Darmstadt, Hessen, Germany.

Bacterial strains and culture

The *E. coli* strains used and their corresponding genotypes are indicated in Table 1. The PQ30, PQ37 and OG100 strains were developed and generously supplied by Dr Phillipe Quillardet from Pasteur Institute, France. The strains with the suffix IN were developed at the National Institute of Nuclear Research, Mexico (Breña-Valle and Serment-Guerrero 1998; Serment-Guerrero et al. 2008), from a spontaneous PQ30 mutant sensitive to tetracycline (see method developed by Bochner et al. 1980). All strains carried the *sulA::lacZ* gene fusion, and β-galactosidase expression was regulated by the SOS response. In addition, alkaline phosphatase was constitutively expressed to certify that transcription was unaffected.

Cell irradiation

Liquid cultures supplemented with ampicillin (50 μg/ml) and/or tetracycline (17 μg/ml) were grown overnight, diluted 50-fold in fresh LB broth and incubated in a water bath shaker at 37 °C until reaching mid-logarithmic growth ($\sim 2 \times 10^8$ cells/ml). Then, 7 ml of each culture was distributed into Petri dishes with a 9 cm diameter and irradiated in darkness using a UVA (320–400 nm)/UVB (280–315 nm) irradiation chamber BS-02 equipped with a radiation controller UV-MAT obtained from Opsytec Dr. Groebel, Ettlingen, Baden-Würtemberg, Germany. This radiation controller continuously measured the irradiance, calculated the irradiation dose and switched the lamps off after reaching the target dose. The UVB dose range used depended on each strain with the widest between 10 and 3000 J/m^2.

Cell survival

Irradiated and non-irradiated cells were diluted in a sodium chloride solution (7 g/l) and plated on LB agar media. After 48 h of incubation at 37 °C in the dark, colony forming units (CFU) were counted and bacteria concentrations were determined. The results are expressed as survival percentages.

SOS induction

The SOS induction in *E. coli* strains was measured as previously described by Quillardet and Hofnung (1985). Briefly, 200 μl of the treated cell suspensions (UVB, 10–120 J/m^2; MMC, 0.187 μM; 4-NQO, 2.34 μM; untreated control) were diluted in 2 ml of LB media and then incubated in the dark at 37 °C for 2 h in a Thermomixer Comfort apparatus from the Eppendorf Co., Hamburg, Germany. Both the β-galactosidase and alkaline phosphatase assays were performed in 96-microwell plates from Brand GmbH, Wertheim, Baden-Württemberg, Germany. For the former, bacteria were disrupted by mixing 135 μl of Z buffer (60 mM Na_2HPO_4, 40 mM NaH_2PO_4, 10 mM KCl, 1 mM Mg_2SO_4, 0.1% sodium dodecyl sulfate (SDS) and 40 mM β-mercaptoethanol, pH 7.0) with 15 μl of the cell suspension for 20 min at room temperature. The reaction was initiated by adding 30 μl of ONPG (4 mg/ml in 0.1 M phosphate buffer, pH 7.0). After 40 min, the enzymatic reaction was stopped by adding 100 μl of 1 M Na_2CO_3. For the alkaline phosphatase activity, cell membranes were disrupted by mixing 135 μl of T buffer (1 M Tris HCl, 0.1% SDS, pH 8.8) with 15 μl of the cell suspension. The enzyme reaction was started by adding 30 μl of the PNPP solution (4 mg/ml in T buffer). After 40 min, the reaction was stopped by adding 50 μl of 2 M HCl, and 5 min later, 50 μl of 2 M Tris was added to restore the color. The final absorbance of the β-galactosidase and alkaline phosphatase assays were measured at 420 nm using a Multiskan GO microplate reader from Thermo Fisher Scientific, Waltham, MA, USA. The β-galactosidase and alkaline phosphatase activities were calculated according to Miller's equation, enzyme units $=$ ($A420/t \times 1000$), where *A420* is the optical density at 420 nm and *t* is the incubation time in minutes.

Next, the ratios of both enzymatic units were determined ($R =$ β-galactosidase units/alkaline phosphatase units). This *R* value at UVB dose *x* was normalized to the *R* value for control cells. Therefore, the induction factor ($I_x = R_x/R_0$) represents the normalized induction data for the *sulA* gene in each treatment and is proportional to the specific activity of β-galactosidase (Quillardet and Hofnung 1984).

SOS induction kinetics

The post-irradiation induction kinetic of the *sulA::lacZ* fusion gene was studied in *E. coli* strains defective in *uvrA*, *recJ*, *recO* and *recN* genes and in the wild-type (*wt*) strain. These strains used the expression of β-galactosidase to assay the functional transcription of the *sulA* gene (Casadaban and Cohen 1979),

Table 1. *E. coli* strains used in this study.

Strains	Genotypes	Phenotypes	Sources
PQ30	F-, *thr*, *leu*, *his*, *pyrD*, *thi*, *trp*::*MuC*+, *srl300*::*Tn10*, *rpoB*, *lac*Δ*U169*, *galE*, *galY*, *phoC*, *sulA*::*Mu d*(*Ap lacZ*)*cts*	Wild type	(Quillardet and Hofnung 1985)
PQ37	As PQ30 but *uvrA*	Defective UvrABC excinuclease for photoproduct detection activity	(Quillardet and Hofnung 1985)
OG100	As PQ30 but Δ*oxyR4*	Defective OxyR positive regulator	(Goerlich et al. 1989)
IN237	As PQ30 but *recO1504*::*Tn5*	Defective RecFOR recombination pathway for ssDNA binding activity	(Breña-Valle and Serment-Guerrero 1998)
IN250	As PQ30 but *tyrA*::*Tn10 recN*	Defective RecFOR recombination pathway for DSB detection activity	(Breña-Valle and Serment-Guerrero 1998)
IN400	As PQ30 but *recJ284*::*Tn10*	Defective RecQ/RecJ helicase/nuclease for 5′→3′ exonuclease activity	(Breña-Valle and Serment-Guerrero 1998)
IN520	As PQ30 but Δ*exoX*::*kan*	Defective ExoX protein for 3′→5′ exonuclease activity	(Serment-Guerrero et al. 2008)
IN602	As PQ30 but *recB21 zga*::*Tn10*	Defective RecBCD helicase/nuclease for 5′→3′ helicase activity	(Breña-Valle and Serment-Guerrero 1998)
IN650	As PQ30 but *recD1013*	Defective RecBCD helicase/nuclease for 5′→3′ helicase activity	(Serment-Guerrero et al. 2008)
IN900	As PQ30 but Δ*xonA300*::*cat*	Defective ExoI (XonA) protein for 3′→5′ exonuclease activity	(Serment-Guerrero et al. 2008)

ssDNA, single-strand DNA; DSB, DNA double-strand break.

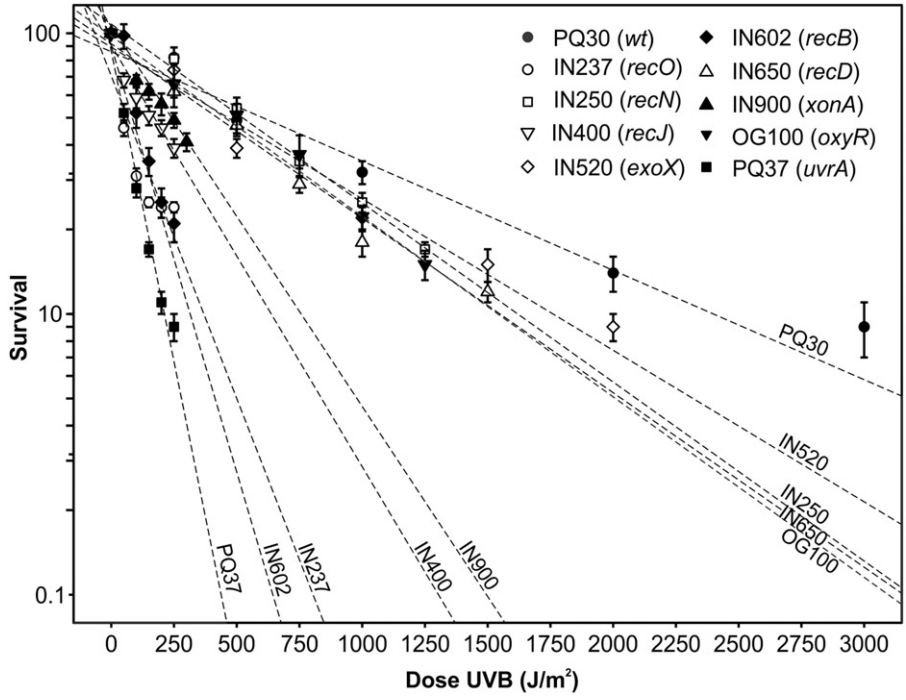

Figure 1. Effects of UVB on cell survival for specific *E. coli* strains. Error bars indicate the standard error of the mean ($n = 27$).

and the *sulA* gene product is responsible for cellular division arrest in *E. coli* (Huisman et al. 1984); therefore, we assumed that a post-irradiation reduction of *sulA* gene expression can be considered indicative of cellular division recovery. For this purpose, the strains (not including the *wt*) were exposed to a dose of 10 J/m^2, which considerably induced the *sulA* gene. Because this dose barely doubles the basal level of *sulA* gene expression in the *wt* strain (PQ30), cells were exposed to a higher dose (120 J/m^2), which raised the SOS induction to a level similar to those observed in the PQ37 (uvrA) strain. Cells were incubated at 37 °C in the dark and aliquots were taken at different incubation times between zero and 240 min. In this case, the induction factor (I_x) indicates the normalized post-irradiation induction of *sulA*, and the ratios R_x and R_o were defined as the post-irradiation incubation time x and *zero*, respectively.

Statistical analyses

All results are the mean of at least three independent experiments with at least three replica plates *per* dose. The mean I values of each strain were compared with the wild type using the Tukey test from the R program (R Core Team 2013). Similarly, I values obtained from the kinetics experiments were compared with those from non-irradiated controls using the same test. The survival data and I average values and their confidence limits were plotted using the *ggplot2* package from the R program.

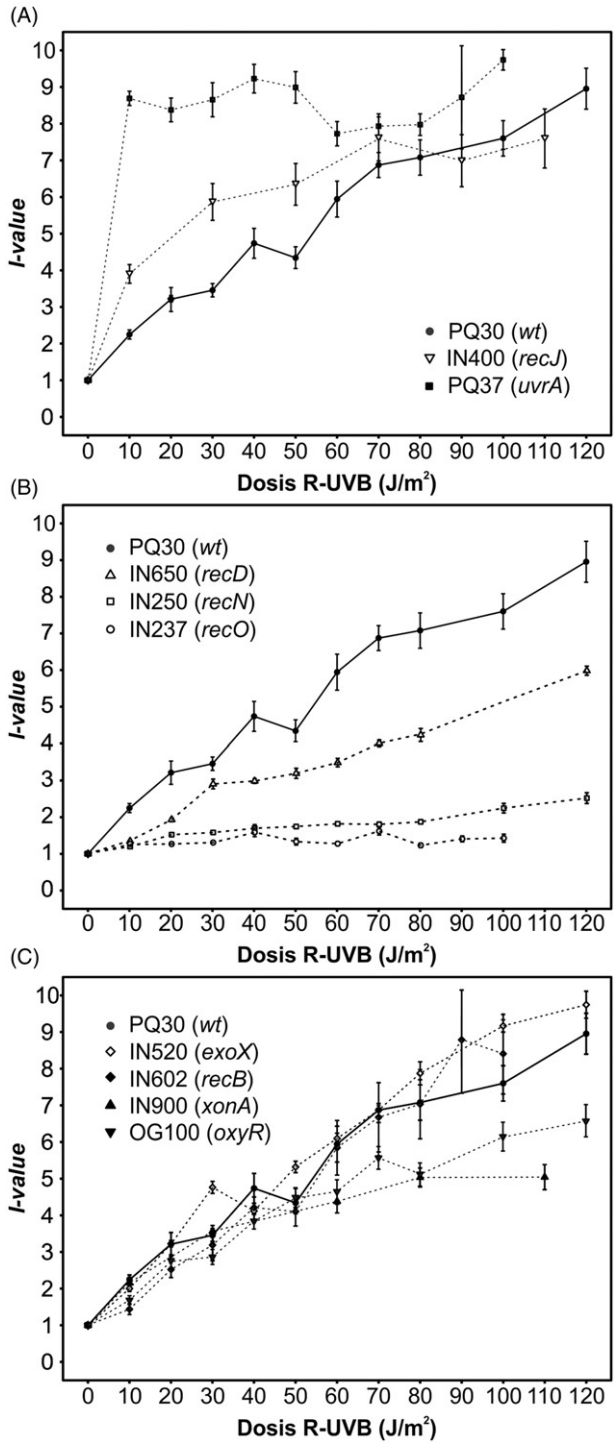

Figure 2. SOS induction (*I* values) responses to UVB radiation doses in *E. coli* strains. Error bars indicate the standard error of the mean ($n = 12$).

Results

The *E. coli* strain sensitivities to UVB (Figure 1) were as follows: PQ37 (*uvrA*) > IN602 (*recB*) > IN237 (*recO*) > IN400 (*recJ*) > IN900 (*xonA*) > OG100 (*oxyR*) > IN650 (*recD*) > IN250 (*recN*) > IN520 (*exoX*) > PQ30 (*wt*).

Figure 2 shows the *I* values of *E. coli* strains exposed to several radiation doses. A dose of 30 J/m^2 doubled the *I* value in respect to the control cells in all strains, and a clear dose-response relationship was observed; the exceptions were the IN237 and IN250 strains in which SOS induction was limited.

Table 2. SOS induction factor (*I*) values for the PQ37, IN237 and IN250 strains treated with the standard mutagens MMC and 4-NQO.

E. coli strains (phenotypes)	*I* values†		
	NT	MMC	4-NQO
PQ37 (*uvrA*)	1.00 ± 0.04	4.57 ± 0.22*	11.63 ± 0.96*
IN237 (*recO*)	1.00 ± 0.29	2.34 ± 0.31 n.s.	2.52 ± 0.95 n.s.
IN250 (*recN*)	1.00 ± 0.06	12.80 ± 5.09*	2.84 ± 0.56 n.s.

†Errors indicate the standard error of the mean for $n = 12$. MMC and 4-NQO were assayed at concentrations of 0.187 μM and 2.34 μM, respectively, as previously described (Quintero et al. 2012). *Significant differences for *I* values ($p < 0.05$) when compared with no treated (NT) cells using the Tukey test. n.s., no significant difference was found.

To evaluate SOS induction, the IN237 and IN250 strains were treated with the genotoxic agents MMC or 4-NQO, which generated relatively high *I* values in the standard *E. coli* PQ37 strain (Quintero et al. 2012). The *I* values of IN237 (*recO*) did not increase when treated with these agents (Table 2). In contrast, MMC considerably elevated the SOS induction of the IN250 (*recN*) strain; however, 4-NQO did not.

In this study, we hypothesized three possible scenarios for SOS induction by UVB treatments: (i) The SOS induction in defective strains would be higher compared with the *wt*, (ii) the SOS induction in defective strains would be lower compared with the *wt* and, (iii) the induction in defective strains would be similar to the *wt*. The data presented in Figure 2 showed three possibilities. First, IN400 and PQ37 strains exhibited *I* values considerably higher compared with the *wt* when exposed to a dose range between 10 and 60 J/m^2, indicating that RecJ and UvrA proteins are key factors in processing UVB-induced DNA damage in *E. coli*. Second, in contrast to the first possibility, IN237, IN250, and to a lesser extent IN650, exhibited *I* values significantly lower compared with the *wt* along the entire dose range. These results suggest that RecO and RecN proteins are essential for UVB-mediated SOS induction in *E. coli* cells, while the RecD protein is less important in this process. Third, the strains OG100, IN520, IN602, and IN900 showed *I* values very similar to *wt*, indicating that the corresponding defective genes are not particularly important for SOS induction by UVB-generated DNA lesions.

As indicated above, a decrease in the expression of *sulA* over time can be considered an indication of cell division recovery. The *wt* strain (PQ30) showed no relevant post-irradiation induction of the *sulA* gene when irradiated at a dose of 10 J/m^2, therefore, a dose of 120 J/m^2 was used, which significantly induced the *sulA* gene in this strain (Figure 2). This strain showed significant increases in *I* values at 30 min, reaching a maximum after an incubation time of 120 min. Afterward, a considerable reduction was observed (Table 3), indicating a shutdown of *sulA* and therefore, cell division recovery. Conversely, the PQ37 (*uvrA*) strain showed a substantial *sul*A induction after 30 min and reached its maximum *I* value at 150 min, with this level persistent for up to 240 min. Under similar conditions, the IN400 (*recJ*) strain significantly increased *sul*A expression after 120 min and reached its maximum *I* value at 240 min. The results for both the PQ37 and IN400 strains clearly indicated that SOS functions are not turned off in *uvrA* and *recJ* mutants. Additionally, the IN237

Table 3. Induction of SOS responses (*I* values) in the PQ30, PQ37, IN400, IN237 and IN250 strains for different post-irradiation incubation times.

Cellular recovery Time (minutes)	*I* values†				
	PQ30 (*wt*)	PQ37 (*uvrA*)	IN400 (*recJ*)	IN237 (*recO*)	IN250 (*recN*)
NC	1.0 ± 0.1	1.0 ± 0.0	1.0 ± 0.1	0.3 ± 0.0	1.0 ± 0.1
0	1.0 ± 0.1	1.0 ± 0.0	1.0 ± 0.1	1.0 ± 0.1	1.0 ± 0.1
30	2.9 ± 0.3$^{n.s.}$	2.7 ± 0.2*	0.8 ± 0.3$^{n.s.}$	0.6 ± 0.0$^{n.s.}$	0.7 ± 0.1$^{n.s.}$
60	3.8 ± 0.5*	5.2 ± 0.2*	2.2 ± 0.6$^{n.s.}$	0.5 ± 0.0$^{n.s.}$	0.9 ± 0.1$^{n.s.}$
90	5.1 ± 1.0*	6.7 ± 0.2*	1.5 ± 0.4$^{n.s.}$	0.5 ± 0.0$^{n.s.}$	1.0 ± 0.1$^{n.s.}$
120	5.2 ± 1.4*	6.4 ± 0.2*	5.3 ± 0.7*	0.4 ± 0.0$^{n.s.}$	1.1 ± 0.2$^{n.s.}$
150	2.8 ± 0.5$^{n.s.}$	6.8 ± 0.8*	4.9 ± 0.6*	0.6 ± 0.0$^{n.s.}$	1.0 ± 0.1$^{n.s.}$
180	2.8 ± 0.5$^{n.s.}$	5.6 ± 0.4*	5.8 ± 1.0*	0.4 ± 0.0$^{n.s.}$	1.0 ± 0.1$^{n.s.}$
210	2.5 ± 0.3$^{n.s.}$	5.8 ± 0.5*	6.3 ± 0.7*	0.5 ± 0.0$^{n.s.}$	0.9 ± 0.1$^{n.s.}$
240	2.3 ± 0.4$^{n.s.}$	5.0 ± 0.5*	8.7 ± 1.3*	0.5 ± 0.0$^{n.s.}$	0.9 ± 0.1$^{n.s.}$

Cells were treated with radiation doses that significantly induced *sul*A expression in each strain. The PQ37, IN400, IN237 and IN250 strains used a dose of 10 J/m^2, while the PQ30 strain was irradiated at 120 J/m^2 (see *Materials and methods*). NC, negative control (non-irradiated cells) was cultured for 2 h at 37 °C before enzymatic assays. †Errors indicate the standard error of the mean for $n = 12$. *Significant increases for *I* values ($p < 0.05$) when compared with the zero cellular recovery time using Tukey test. n.s., no significant difference was found.

(*recO*) and IN250 (*recN*) strains did not show increased *sulA* expression at any incubation time.

Discussion

Survival

According to the cell survival data (Figure 1), three groups (I, II, and III) showing different levels of sensitivity to UVB were identified. Group I includes strains with *uvrA*, *recB* and *recO* mutations, which produced high sensitivity; group II contained strains with *recJ* and *xonA* mutations revealing an intermediate sensitivity; finally, group III contained strains with *oxyR*, *recN*, *exoX*, and *recD* mutations, which displayed a sensitivity similar to the *wt* PQ30 strain.

Group I results indicated that the UvrABC, RecBCD and RecFOR pathways are essential for survival after UVB-induced damage. As expected, the *E. coli* strains carrying a *uvrA* mutation were highly sensitive to this agent. The UvrA protein forms part of the UvrABC excinuclease complex that recognizes CPD and 6-4PP and removes single-stranded DNA fragments containing the photoproducts (Kisker et al. 2013). This explains our results with the PQ37 strain. Recombination pathways were essential for survival in UVB-treated *E. coli* cells. *E. coli recO* and *recB* defective strains (i.e., IN237 and IN602) were highly sensitive to UVB, in accordance with previous reports (Wang and Smith 1983; Kolodner et al. 1985). The RecO protein is part of the RecFOR recombination pathway that simultaneously catalyzes the withdrawal of the single-strand binding protein (SSB) and recruits the RecA protein onto the single-stranded DNA (Morrison et al. 1989; Umezu et al. 1993; Courcelle et al. 2003; Chow and Courcelle 2004). Conversely, the RecB protein is a key part of the RecBCD helicase/nuclease complex involved in DNA double-strand breaks (DSB) recombination repair (Thoms and Wackernagel 1998; Dillingham and Kowalczykowski 2008; Khan and Kuzminov 2012). The RecFOR and RecBCD recombination pathways contribute differently to the repair of replication forks blocked or broken due to photoproducts not previously removed by nucleotide excision repair. Therefore, the components of both pathways are necessary for survival.

As indicated above, group II (containing the *recJ* and *xonA* defective strains) showed intermediate *E. coli* cell sensitivity to UVB. The RecJ exonuclease (Lovett and Kolodner 1989) is part of the RecQ-RecJ helicase-nuclease complex that processes the blocked replication forks from UV-photoproducts (Courcelle and Hanawalt 1999; Courcelle et al. 2003). Previous studies reported similar results regarding the *recJ* defective strain (Viswanathan and Lovett 1998). Because RecJ participates with the RecQ-RecJ and RecBCD helicase-nuclease complexes in DNA fragmentation repair in UVR-treated bacteria (Thoms and Wackernagel 1998; Dillingham and Kowalczykowski 2008; Khan and Kuzminov 2012), we expected the IN400 strain to show sensitivity to this agent.

The ExoI protein is a 3′→5′ single-strand exonuclease implicated in DNA recombination and repair (Breyer and Matthews 2000). ExoI exonuclease participates in processing DNA double-strand ends with a protruding single strand and permits the RecA/RecBCD recombination pathway to repair broken replication forks by recombination (Thoms and Wackernagel 1998; Viswanathan and Lovett 1998; Thoms et al. 2008). The UV sensitivity of the IN900 strain contrasted with previous observations in a △*xonA300* mutant (Viswanathan and Lovett 1998). These contrasting results could be due to a difference in the dose rate used for cell irradiation. Indeed, we observed that IN900 sensitivity was similar to the wild type when irradiated at 1 J/m^2/s (data not shown), which is a dose rate 10 times lower than the one used in this study (10 J/m^2/s). Interestingly, the survival pattern observed in this work for the IN900 strain was similar to the one observed for gamma rays (Serment-Guerrero et al. 2008). This suggests that cell irradiation at high UV dose rates could produce photoproduct clusters that eventually become DNA double-strand breaks (Khan and Kuzminov 2012).

In contrast with groups I and II, group III (*oxyR*, *recN*, *recD* and *exoX* defective strains) showed similar sensitivities comparable to the *wt* strain (Figure 1), indicating that products of these genes are not essential for survival after UVB-induced damage. Strains defective in *recN* and *oxyR* showed higher sensitivity to γ-rays, while lethality of the *recD* and *exoX* defective strains was less severe (Breña-Valle and Serment-Guerrero 1998; Almeida et al. 2004; Serment-Guerrero et al. 2008). Contrasting results with *recN* and *oxyR* mutations suggest that these gene products are essential to remove γ-ray induced DNA damage but not UV photoproducts.

As stated above, UVB radiation produces a variety of DNA damage, including CPD, 6-4PP and oxidized DNA bases (Cadet et al. 2005, Courdavault et al. 2005; Schuch et al. 2013); however, CPD and 6-4PP play the main role in the induction of DNA inactivation and mutagenesis (Chandrasekhar and Van Houten 2000; Schuch et al. 2009; Schuch and Menck 2010). Indeed, these lesions disturb DNA structure and block DNA replication and cell division; if these photoproducts are not repaired, cell death occurs (Courcelle et al. 2006). The results presented here support the idea that UvrABC excinuclease and RecBCD and RecFOR recombination repair pathways are essential for cell survival and act as if they synchronize to work together in genome reconstitution

and the final removal of lethal photoproducts. In addition, these data indicate that cell survival depends on the restoration of blocked replication forks through RecQ-RecJ activity. Finally, results for the IN250 (*recN*) and OG100 (*oxyR*) strains strongly suggest that oxidative stress caused by UVB is not enough to affect *E. coli* cell survival.

SOS response activity

UVB-treated *E. coli* strains carrying the *uvrA* and *recJ* mutations showed elevated levels of SOS induction. In PQ37 (*uvrA*) and IN400 (*recJ*) strains, *sulA* gene expression was not shut down (Table 3) suggesting that UvrA and RecJ proteins are essential to restart DNA replication, and therefore, cell division in UVB- treated *E. coli*. The poor ability of the PQ37 (*uvrA*) strain to remove photoproducts, and therefore the persistence of these photoproducts in its genome, can explain these results. Conversely, the RecJ exonuclease is part of the RecQ-RecJ helicase-nuclease complex that is required to process the blocked replication forks and the rapid recovery of DNA synthesis (Courcelle and Hanawalt 1999; Courcelle et al. 2003; 2006). Corrette-Bennett and Lovett (1995) also showed that the RecJ exonuclease increased RecA strand-transfer activity, demonstrating the role of these proteins in genetic recombination. In short, the IN400 strain defective in RecJ exonuclease activity cannot perform the replication forks reversal process; therefore, DNA replication cannot be recovered, thus maintaining a high level of SOS activity.

In contrast, no SOS induction was observed in the IN237 (*recO*) and IN250 (*recN*) strains even after 240 min post-irradiation incubation (Figure 2b, Table 3). These findings suggest that the RecO and RecN proteins contribute to the generation of single-stranded DNA, the primary signal of SOS induction. Using standard protocols (Quillardet and Hofnung 1985), *recO* deficiency in the IN237 strain prevents SOS induction through different genotoxins, such as cisplatin, MMC, 4-NQO, UV and γ-rays (Keller et al. 2001, Serment-Guerrero et al. 2008; and present work). Previous studies (Hegde et al. 1995; Whitby and Lloyd 1995) have shown that a *recO* mutation delays, but does not prevent, SOS induction in *E. coli*. The differences in the UV dose used in all these studies could explain the contrasting results. Molecular interactions between RecO, RecR and probably RecF with RecA and single-stranded DNA have been shown (Umezu et al. 1993), indicating that the RecFOR complex catalyzes interactions between the RecA protein and single-stranded DNA to finally trigger SOS induction (Thoms and Wackernagel 1987, 1988).

The recN defective mutant (IN250) also showed SOS induction lower than the wt strain when exposed to UVB, which was in contrast with previous reports (Thoms and Wackernagel 1987). This finding also differed from the results observed when gamma rays (Breña-Valle and Serment-Guerrero 1998) or MMC (Table 2) were used, indicating that RecN proteins were not directly involved in the generation of primary signals for SOS induction. In light of the survival data, there is a crucial difference between the IN250 (*recN*) and IN237 (*recO*) strains because the *recO* defective strain was significantly more sensitive to UVB compared with the former. Presumably, this is because the RecO protein is required for survival following UVB-induced DNA damage, whereas the RecN protein is not. *E. coli* RecN (Finch et al. 1985) is part of a structural maintenance for the chromosome (SMC) protein family (Pellegrino et al. 2012), which is required for DSB repair (Picksley et al. 1984; Meddows et al. 2005). When bacteria are treated with UV, the recN gene is rapidly expressed, reaching its maximum level after a few minutes and remaining stable for up to 60 min (Khanin et al. 2006). Odsbu and Skarstad (2014) demonstrated that the RecN protein is required for reorganization of the nucleoid after UV irradiation, which suggests that RecN stabilizes the damaged DNA prior to recombination and repair. Based on the ability of RecN to stabilize DNA topology and previous reports showing functional interactions between RecN and RecA (Keyamura et al. 2013), we hypothesized that RecN might stabilize the RecA-ssDNA complex needed for SOS induction; but, this role could be determined by the type of DNA damage as was shown with 4-NQO and MMC (Table II). However, this does not explain the contrasting result with the previous study by Thoms and Wackernagel (1987).

The results showed that *recD* deficiency in the IN650 strain slightly reduced SOS induction by UV as previously observed in bacteria exposed to γ-rays (Serment-Guerrero et al. 2008). Inactivation of the RecD domain or removal of the RecD subunit altered the rate of DNA unwinding in the RecBCD complex (Taylor and Smith 2003; Portakal and Doğan 2008). Because RecBCD helicase/nuclease generates ssDNA regions (Anderson and Kowalczykowski 1998; Vlašić et al. 2011) that are recognized by RecA, it is expected that *recD* mutations affect, at least partially, the SOS induction occurring in the IN650 strain.

In contrast with the strains carrying *uvrA*, *recJ*, *recO*, *recN* and *recD*, strains carrying the *exoX*, *recB*, *xonA* and *oxyR* mutations showed *I* values similar to *wt* PQ30 cells. γ-irradiated *E. coli* cells carrying these mutations showed lower levels of SOS induction compared with the PQ30 cells (Breña-Valle and Serment-Guerrero 1998; Serment-Guerrero et al. 2008). These contrasting results suggest that these gene products are essential to processed DSB induced by γ-rays, but they do not remove UV-induced photoproducts.

Concluding remarks

This study showed the differential contribution of DNA repair gene products for survival and SOS induction after UVB radiation in *E. coli*. The survival data showed that strains carrying *uvrA*, *recB*, *recO*, *recJ* and *xonA* mutations were significantly more sensitive to UV compared with the *wt*. These results indicated that the UvrABC excinuclease with RecA/RecBCD and RecA/RecFOR recombination pathways are equally important for survival after UVB-induced lesions. To a lesser extent, RecJ and ExoI exonucleases also contributed to overcoming the effects of UVB, probably because they help the RecA/RecBCD recombination pathway or because they support DNA replication recovery. Conversely, *E. coli* cells carrying *uvrA* and *recJ* mutations exhibited elevated and sustained levels of SOS induction, which support the importance of the

UvrABC excinuclease and RecQ-RecJ helicase-nuclease to remove photoproducts and restart DNA replication, respectively, and therefore, to recover cell division. Finally, we showed that *recO* and *recN* defective strains prevent UVB-mediated SOS induction; however, future studies are required to elucidate the mechanism by which the RecN protein interferes with SOS induction in the IN250 strain.

Author contributions

Dr Jorge Luis Fuentes (JLF) designed and proposed the project and applied for research funds (Contract No. RC-0572–2012). The published data are part of the results from the above mentioned project, and these constitute the BSc (Biology) theses of César Augusto Prada Medina (CAPM) and Elke Tatjana Aristizabal Tessmer (ETAT). Nathalia Quintero Ruiz (NQR) developed the kinetic study of *sulA* induction after UVB irradiation. CAPM, ETAT and NQR developed all the experimental work, collected and analyzed the data, and prepared draft figures and tables. Dr Jorge Serment Guerrero (JSG), CAPM and JLF contributed to work discussions; JLF and JSG prepared the manuscript. All authors approved the final manuscript and had complete access to the study data.

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Disclosure statement

The authors report no conflict of interest. The authors alone are responsible for the content and writing of the paper.

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