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Research Letter

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Influence of *uvrA*, *recJ* and *recN* gene mutations on nucleoid reorganization in UV-treated *Escherichia coli* cells

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One sentence summary: Influence of mutations in DNA repair genes on nucleoid reorganization post-irradiation in *Escherichia coli*
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ABSTRACT

Exposure to ultraviolet (UV) radiation blocks DNA replication and arrests cellular division in *Escherichia coli*. Restoration of chromosome replication involves nucleoid reorganization, which involves the participation of the recombination-catalyzing proteins RecA, RecO, RecR and RecN. In this work, we evaluated the influence of *recN*, *uvrA* and *recJ* gene mutations on post-irradiation nucleoid reorganization. We used isogenic *E. coli* strains that are defective for these genes to study post-irradiation kinetics of the nucleoid shape fractions using fluorescence microscopy. The results showed that in the wild-type strain, post-irradiation nucleoid reorganization occurs, which restores the nucleoid shape fractions in the cells to those observed prior to irradiation. First, the nucleoid condenses into the central area of the irradiated cell. Second, the nucleoid disperses along the cell. Third, the cell enters the chromosome replicative phase and cytokinesis. *Escherichia coli* cells with a *recN* mutation did not exhibit increased nucleoid condensation, but chromosome replication and cytokinesis occurred. In the *uvrA* and *recJ* strains, the condensation step was delayed compared to the wild-type strain, and chromosome replication and cytokinesis did not occur. The results are discussed with an emphasis on the functions of RecN, UvrA and RecJ in nucleoid reorganization in UV-irradiated *E. coli* cells.

Keywords: *Escherichia coli*; ultraviolet radiation; SOS response; nucleoid shape; cell division; fluorescence microscopy

INTRODUCTION

When *Escherichia coli* is exposed to ultraviolet (UV) radiation, DNA lesions are formed (i.e. cyclobutane pyrimidine dimers (CPD) and 6-4-pyrimidine pyrimidone photoproducts (6-4PP)), which are an obstacle to chromosome replication and cause cell cycle arrest (Courcelle *et al*. 2003; Rudolph, Upton and Lloyd 2007) and SOS response activation (Witkin 1976). Most of the SOS response genes are involved in the repair of, or recovery from, DNA damage (Courcelle *et al*. 2001; Quillardet, Rouffaud and Bouige 2003). These SOS response genes are transcriptionally repressed by LexA dimers, which bind to so-called SOS boxes located in the promoter regions of all SOS response genes (Little and Mount

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Table 1. *Escherichia 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)
IN250	As PQ30 but *tyrA*::*Tn10 recN*	Defective 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)

DSB: DNA double-strand break.

1982; Lewis *et al.* 1994). Induction of the SOS response is mediated by RecA (a positive regulator), which also plays a central role in homologous recombination repair (Kowalczykowski *et al.* 1994). RecA shows strong affinity for single-stranded DNA (ssDNA) regions, which serve as an initial signal for the induction of the SOS response (Higashitani, Higashitani and Horiuchi 1995). Once DNA damage occurs, RecA binds to ssDNA regions formed at stalled replication forks (Heller and Marians 2006), sites where lesions are bypassed (Marians 2008), and sites of intensive DNA repair throughout the genome (Anderson and Kowalczykowski 1998; Hishida *et al.* 2004), thereby creating RecA-ssDNA nucleoprotein filaments that promote self-digestion of the LexA repressor and allowing transcription of the SOS response genes (Courcelle *et al.* 2001).

CPD and 6-4PP are primarily removed by nucleotide excision repair and photoreactivation (Sancar and Rupp 1983; Kneuttinger *et al.* 2014). However, the reactivation of stalled forks has been shown to relate to recombinational pathways, allowing for the repair or bypass of the blocking lesion in a non-mutagenic manner (McGlynn and Lloyd 2002; Kreuzer 2005; Heller and Marians 2006). RecQ/RecJ helicase/nuclease and RecA/RecFOR recombination pathways appear to be essential for the recovery of DNA replication in *E. coli* after UV exposure (Courcelle and Hanawalt 1999; Courcelle *et al.* 2001; Chow and Courcelle 2004; Rudolph, Upton and Lloyd 2008). The RecJ exonuclease partially degrades the nascent DNA at blocked replication forks (Courcelle *et al.* 2006) and increases RecA strand-transfer activity during recombination DNA repair (Corrette-Bennett and Lovett 1995).

In addition, it has become increasingly evident that microbial exposure to DNA-damaging agents, including UV radiation, results in a massive SOS response-dependent genome condensation (Levin-Zaidman *et al.* 2000; Smith, Grossman and Walker 2002; Delmas, Duggin and Allers 2013; Shechter et al. 2013; Odsbu and Skarstad 2014). In *E. coli*, genome condensation is mediated by specific stress-induced DNA-binding proteins, such as RecA, RecO, RecR and RecN (Levin-Zaidman *et al.* 2000; Odsbu and Skarstad 2014; Vickridge *et al.* 2017). The *E. coli* RecN protein (Finch, Chambers and Emmerson 1985) is a structural maintenance chromosome protein family member (Pellegrino *et al.* 2012) that is required for DNA double-strand break (DSB) repair (Picksley, Attfield and Lloyd 1984; Meddows *et al.* 2005). In *Bacillus subtilis*, RecN participates in the recognition of double-stranded DNA breaks produced by gamma radiation, aggregating the enzymatic machinery that repairs this type of lesion (Meddows *et al.* 2005). *Escherichia coli recN* cells are hypersensitive to gamma rays, mitomycin C and ionizing radiation (Picksley, Attfield and Lloyd 1984; Breña-Valle and Serment-Guerrero 1998; Serment-Guerrero, Breña-Valle and Espinosa-Aguirre 2008) but not UV radiation (Picksley, Attfield and Lloyd 1984).

We have recently shown that isogenic *E. coli* strains deficient in UvrA or RecJ, but not RecN, are highly sensitive to UV radiation (Prada Medina *et al.* 2016). In this work, we sought to determine the function of RecN, UvrA and RecJ in post-irradiation *E. coli* nucleoid organization by studying nucleoid shapes using fluorescence microscopy. We showed that the restoration of post-irradiation chromosome replication and cytokinesis in *E. coli* is an SOS response-dependent event that restores the nucleoid shape in the cell prior to irradiation. RecN has a central role in post-irradiation *E. coli* nucleoid compaction events. The nucleoid reorganization is delayed in absence of the *uvrA* and *recJ*, demonstrating the importance of photoproduct repair to restart chromosome replication in UV-exposed *E. coli* cells.

MATERIALS AND METHODS

Chemical compounds

The compounds 4.6-diamino-2-phenyl-indole (DAPI), Eosin Y, and poly-L-lysine, as well as Luria-Bertani (LB) media and the antibiotics ampicillin and tetracycline were obtained from Sigma-Aldrich Co. Inc. (St. Louis, Missouri, USA). All other reagents were obtained from J.T. Baker (Phillipsburg, NJ, USA) or Merck (Kenilworth, NJ, USA).

Bacterial strains and culture

The *E. coli* strains used in this study are presented in Table 1. The details of the strains origin are described by Quillardet and Hofnung (1985) and Breña-Valle and Serment-Guerrero (1998). *Escherichia coli* PQ30 is a wild-type (wt) strain while the strains IN400, IN250 and PQ37 are defective in *recJ*, *recN* and *uvrA* genes, respectively. The strains were grown overnight at 37°C with constant shaking at 100 rpm in LB broth (10 g tryptone/L, 5 g yeast extract/L, 10 g sodium chloride/L, pH 7.4), supplemented with ampicillin (50 μg/mL) and tetracycline (17 μg/mL), until an optical density (OD600) of 0.4 ($\sim 2 \times 10^8$ cells/mL) was reached.

Cell irradiation

Cell cultures were distributed into 9 cm-diameter Petri dishes for UVB (280–315 nm) irradiation, which was performed in darkness using a BS-02 UVA/UVB irradiation chamber equipped with a UV-MAT radiation controller (Opsytec Dr. Groebel, Ettlingen,

Table 2. Effects of UVB on cell survival in the PQ30, PQ37, IN400 and IN250 *E. coli* strains. Lethal dose (LD) mean values and the corresponding standard errors are presented (N = 27).

Escherichia coli strains[a]	Lethal doses (J/m^2) ± standard error[b]		
	LD_{50}	LD_{30}	LD_{10}
PQ30 (wild-type)	620 ± 150	409 ± 115	241 ± 79
PQ37(*uvrA*)	50 ± 10[c]	31 ± 4[c]	14 ± 1[c]
IN400 (*recJ*)	160 ± 40[c]	56 ± 31[c]	19 ± 15[c]
IN250 (*recN*)	560 ± 90	370 ± 45	214 ± 88

[a]*Escherichia coli* cells growing in exponential phase were used.
[b]Lethal doses were calculated from the previously published raw data of Prada Medina *et al.* (2016).
[c]Significant differences for DL values ($P \leq 0.05$) when compared with the wild-type strain using a Tukey test.

Baden-Würtemberg, Germany). The radiation controller continuously measured the irradiance, calculated the irradiation dose and switched the lamps off after the target dose was reached. The *E. coli* strains defective in *uvrA*, *recJ*, and *recN* genes were treated with a radiation dose of 10 J/m^2, which considerably induced the *sulA* gene, which did not affect their survival (Table 2). 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 induced the SOS response to a level that was similar to that observed in the PQ37 (*uvrA*) strain (Prada Medina *et al.* 2016). For each experiment, a non-irradiated control (negative control) was included.

SOS response induction kinetics

The post-irradiation induction kinetics of the *sulA*::*lacZ* reporter was studied in *E. coli* strains defective in *uvrA*, *recJ*, and *recN* genes and in the wt strain as described by Prada Medina *et al.* (2016), with minor modifications. Briefly, UV-treated cells were diluted 1:10 and incubated at 37°C in the dark. Aliquots were taken at different incubation times between zero and 240 min and β-galactosidase and alkaline phosphatase assays were performed in 96-microwell plates (Brand GmbH, Wertheim, Baden-Württemberg, Germany). For the former assay, bacteria were disrupted by mixing 15 μL of each cell suspension with 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) 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. To assess alkaline phosphatase activity, cell membranes were disrupted by mixing 15 μL of each cell suspension with 135 μL of T buffer (1 M Tris HCl and 0.1% SDS, pH 8.8). The enzyme reaction was started by adding 30 μL of PNPP solution (4 mg/mL in T buffer) and was stopped after 40 min by adding 50 μL of 2 M HCl. After waiting 5 min for the color to be restored, 50 μL of 2 M Tris was added. The final absorbance of the β-galactosidase and alkaline phosphatase assay samples 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 as follows: enzyme units = (A420/t × 1000), where A420 is the optical density at 420 nm and t is the incubation time in minutes. Finally, the ratios of both enzymatic units were determined (R = β-galactosidase units/alkaline phosphatase units). This R value at the post-irradiation incubation time x was normalized to the R value at zero. Therefore, the induction factor ($I_x = R_x/R_0$) represents the normalized post-irradiation induction of *sulA* (Prada Medina *et al.* 2016).

Post-irradiation dynamic of the *E. coli* nucleoid

The post-irradiation nucleoid dynamics in *E. coli* cells was studied in defective and wt strains. Briefly, the UV-treated cells were grown in darkness for different times (0, 15, 30, 45, 60 and 120 min) post-irradiation, collected by centrifugation at 0°C, washed twice with sterile saline solution (0.84% NaCl) and suspended in an appropriate volume of saline solution (~5 × 10^7 cells/mL) as previously described (Hiraga *et al.* 1989). Cell suspensions (12 μL) were spread on clean slides, dried at 37°C and fixed with methanol (10 μL) and poly-L-lysine (10 μL; 0.5 mg/mL of distilled water). Nucleoids were stained in darkness with 10 μL of DAPI solution (0.125 mg/mL of saline solution). After 2 min, cell cytoplasm components were stained with Eosin Y (8 μL; 0.7 mg/mL of distilled water) to contrast nucleoids. Fluorescent nucleoid images were observed using a Carl Zeiss Axio Scope A1 epi-fluorescence microscope (Jena, Germany) equipped with a narrow bandpass filter (Zeiss Fs 02 HE) with an excitation wavelength of ~350 nm and an emission range ≥420 nm. Images were captured using a Zeiss Axio Cam ICm 1 digital camera connected to computerized image analysis software (Zeiss ZEN 2012 blue edition) and images were processed using ImageJ (Schneider, Rasband and Eliceiri 2012).

Cells were classified into three categories depending on nucleoid shapes as follows. (i) Cells with a spherical and condensed nucleoid located in the mid region of the cell, which does not occupy more than 1/3 of cell volume (Odsbu and Skarstad 2014). Cells in this state are preparing to repair DNA damage caused by UV radiation. (ii) Cells with nucleoids dispersed throughout the cell, suggesting active DNA metabolic processes (e.g. DNA repair and gene expression, etc.). In this category, both cells (≈3 nm in length) and filaments (≥4.4 μm in length) can be observed (Volkmer and Heinemann 2011). (iii) Cells with bilobed nucleoids in some state of the chromosomal segregation and cytokinesis. Cells in this state have overcome arrest produced by UV exposure. At least two independent experiments per treatment with two replicates were performed. Nucleoid shape fractions (%) were determined by counting at least 500 cells per slide and were expressed as percentages.

Statistical analysis

The average *I* values and nucleoid shape fractions (%) of the kinetics experiments with their corresponding standard errors were calculated. The normality of the data was tested using the Kolmogorov-Smirnov test. Variance homogeneity and analysis of variance (ANOVA) tests were also conducted. For each *E. coli* strain, the average values per treatment were compared with controls using a Tukey test. For all statistical analyses, $P < 0.05$ was considered significant. The R program (R core Team 2013) was used for all analyses.

RESULTS

The post-irradiation induction kinetics of the *sulA* gene in wt *E. coli* (PQ30) showed a significant rise in the first 30 min of incubation (Fig. 1). Over the next 60 min, the *I* value continued to increase, although to a lesser extent, and peaked at 90 min

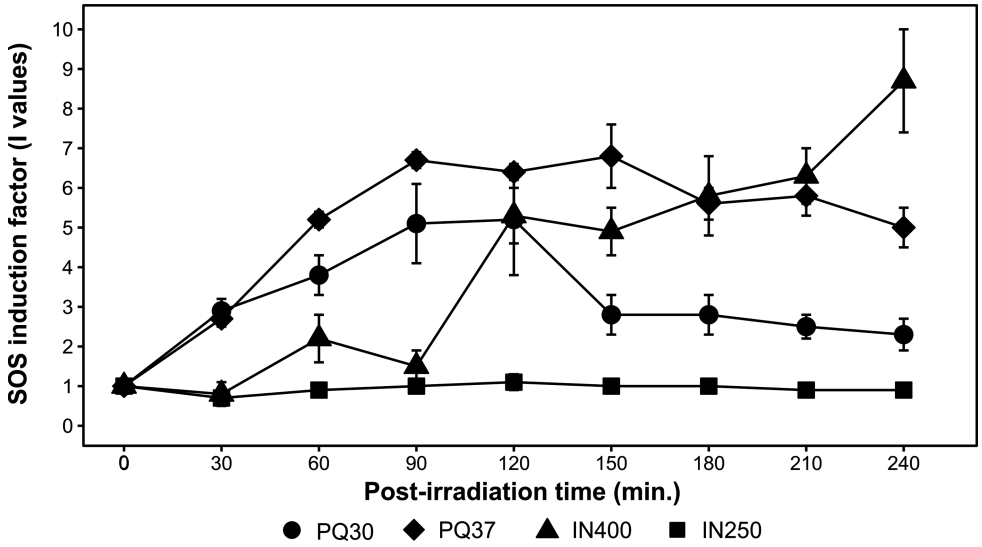

Figure 1. Induction of sulA expression in the *E. coli* strains PQ30, PQ37, IN400 and IN250 for different post-irradiation incubation times. The average I value and their standard errors are presented (n = 12).

post-irradiation. Afterwards, the expression of *sulA* remained stable for 30 min, followed by a considerable reduction of the *I* value during the remainder of the incubation, which indicated an arrest in *sulA* expression. In contrast, *sulA* induction kinetics were altered in each defective strain evaluated. In the *E. coli* IN250 (*recN*) strain, an increase in the *sulA* expression was not observed during the complete post-irradiation incubation time. Conversely, the PQ37 (*uvrA*) strain showed a notable *sulA* induction during the first 90 min of incubation, which stabilized and remained generally steady, with significant *I* value observed during the rest of the post-irradiation time. Finally, the IN400 (*recJ*) strain did not show any significant *sulA* expression until 90 min after the start of incubation, with the *I* value increasing dramatically after 120 min and kept increasing during the rest of the incubation time.

In the wt *E. coli* PQ30 strain, the condensed-nucleoid shape fraction increased during the first 15 min post-irradiation and remained constant for up to 30 min (Fig. 2). Afterwards, the percentage of this nucleoid shape decreased until 60 min and remained constant for up to 120 min post-irradiation. The bilobed-nucleoid shape decreased during the first 60 min post-irradiation while the dispersed-nucleoid shape increased, after which the bilobed-nucleoid shape increased while dispersed-nucleoid shape fraction decreased for up to 120 min post-irradiation. The values of the condensed-, dispersed- and bilobed-nucleoid shape fractions at time zero post-irradiation were identical to those values observed in the cells prior to irradiation (data not shown). These results indicated that in wt-type cells, there is a post-irradiation nucleoid reorganizational dynamic that tended to restore the nucleoid shape fractions prior to irradiation. First, the nucleoid is condensed into the central area of the irradiated cell. Second, the nucleoid is dispersed along the cell, so it can be repaired, expressed, etc. Third, the cell goes into the chromosome replicative phase and cytokinesis, as demonstrated by the observed bilobed nucleoid shape.

The post-irradiation nucleoid reorganization described above was affected in the *E. coli* cells defective in the *recN*, *uvrA* and *recJ* genes (Fig. 2). In the *E. coli* IN250 cells (*recN*), an increase in the condensed-nucleoid shape fraction post-irradiation was not observed, in accordance with a previous study (Odsbu and Skarstad 2014), indicating that RecN has a role in nucleoid condensation. After 45 min post-irradiation, an increase in the bilobed-nucleoid shape fraction and a decrease in the dispersed-nucleoid shape fraction was observed for this strain for up to 60 min post-irradiation, after which both shapes remained constant up to 120 min. This result indicates that the RecN protein did not affect the chromosome replicative phase.

In PQ37 (*uvrA*) and IN400 (*recJ*) cells, the nucleoid condensation event was delayed to 30 and 60 min post-irradiation, respectively. After condensation, the dispersed-nucleoid shape fraction abruptly increased in these strains, while the bilobed-nucleoid shape fraction decreased. Finally, at 120 min post-irradiation, cell filaments ($\geq$4.4 μm in length), always with dispersed-nucleoid shape, were commonly observed in both strains (data not shown).

DISCUSSION

This study showed the influence of UvrA, RecJ and RecN on nucleoid reorganization and cell division restoration in *E. coli* cells exposed to UV. We showed that in the wt strain, a post-irradiation nucleoid reorganization dynamic occurred that tended to restore cells to a pre-irradiated state. First, the nucleoids condensed into the central area of the irradiated cells; second, the nucleoids dispersed along the cells such that they could be repaired, expressed, etc.; and third, the cell entered the chromosome replicative phase and cytokinesis. Although some differences in the magnitude of nucleoid shape fractions were observed due to different UV doses used, our result confirms the nucleoid shape kinetics observed in an earlier study (Odsbu and Skarstad 2014). Since the shapes and number of nucleoids in *E. coli* cells is dependent on growth medium, differences may also be due to the different media used in these studies.

In the IN250 strain (*recN*), the condensed-nucleoid shaped fraction did not increase post-irradiation, suggesting that RecN is essential for nucleoid condensation, in accordance with previous study (Odsbu and Skarstad 2014). As mentioned above, genome condensation is an SOS response-dependent event

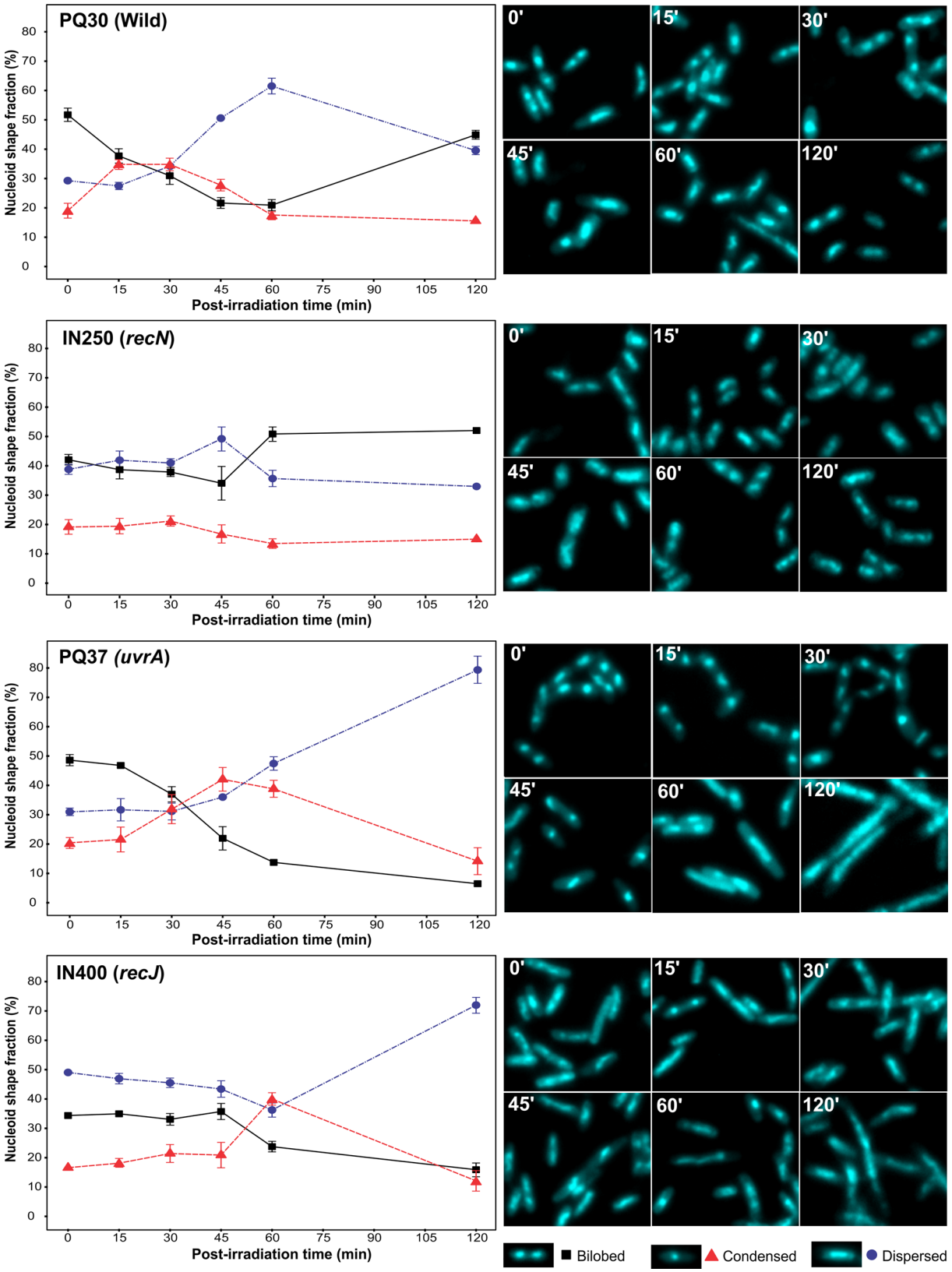

Figure 2. Post-irradiation nucleoid kinetic in the *E. coli* PQ30 (wt), PQ37 (uvrA), IN400 (recJ) and IN250 (recN) cells. The average values of the nucleoid shapes (%) and their standard errors are presented (n = 4).

(Levin-Zaidman *et al.* 2000; Smith, Grossman and Walker 2002; Odsbu and Skarstad 2014). The IN250 (*recN*) does not induce SOS response at non-lethal UV-dose (Fig. 1), explaining why nucleoid condensation was not observed post-irradiation in this strain, as was previously observed in *E. coli* strains carrying *lexA*, *recA* and *recO* mutations (Levin-Zaidman *et al.* 2000; Smith, Grossman and Walker 2002; Odsbu and Skarstad 2014).

According to our results and previous work (Odsbu and Skarstad 2014), RecN is clearly involved in nucleoid condensation. Based on studies done in *B. subtilis* and *Deinococcus radiodurans*, two different functions have been proposed for RecN during DSB repair, cohesion (Reyes *et al.* 2010) and end-joining (Ayora *et al.* 2011; Pellegrino *et al.* 2012) However, the function of RecN during nucleoid condensation remains unknown. Odsbu and Skarstad (2014) hypothesized that RecN stabilizes damaged DNA for recombination and repair before chromosome replication occurs. Delmas, Duggin and Allers (2013) indicated that nucleoid condensation in the archaeon *Haloferax volcanii* is part of a DNA damage response that is independent from homologous recombination and accelerates cell recovery by helping DNA repair proteins to both to locate their targets and facilitate the search for intact DNA sequences during homologous recombination. Vickridge *et al.* (2017) have recently demonstrated that in *E. coli*, RecN is primarily involved in the SOS response-dependent sister chromatid cohesion process. These authors proposed that RecN acts as a cohesion factor that promotes strict realignment of an extensive part of the nucleoid, which initiates at the location of a damaged replication fork.

Based on functional interactions between RecA with RecN (Keyamura *et al.* 2013) and the RecFOR complex (Umezu, Chi and Kolodner 1993), we believe that RecA plays a key role in the nucleoid condensation event. RecA should interact with newly synthesized RecN at the beginning of the SOS response (Courcelle *et al.* 2001; Khanin, Vinciotti and Wit 2006), which should contribute to maintain nucleoid condensation by its cohesion activity (Reyes *et al.* 2010). We believe that newly synthesized RecN protein during SOS response (Courcelle *et al.* 2001; Khanin, Vinciotti and Wit 2006) might also stabilize the RecA-ssDNA complex needed for SOS response induction, a role which appears to be determined by the type of DNA damage (Prada Medina *et al.* 2016). Interestingly, *recN* gene is the unique SOS gene with three SOS boxes that show very different heterology index (3.53–10.08), indicating different strength levels for LexA repression (Courcelle *et al.* 2001). In the UV-treated *E. coli* cells, *recN* gene is rapidly expressed reaching in few minutes their maximum gene expression levels (Khanin, Vinciotti and Wit 2006). According to this model, RecN should be essential to trigger and/or modulate the *E. coli* SOS response, and therefore, for nucleoid compaction which, is an SOS response-dependent event. Future studies are required to elucidate the mechanism by which the RecN interferes with SOS response induction.

In UV-treated PQ37 (*uvrA*) cells, the condensation step was delayed compared to the wt strain, reaching maximum nucleoid condensation at 45 min post-irradiation. UvrA is part of the UvrABC complex, which repairs UV-induced photoproducts in bacteria (Kisker, Kuper and Van Houten 2013). However, a role for UvrA in nucleoid condensation has not been described, although the *B. subtilis* UvrA protein showed an ability to agglutinate with chromosomal DNA after UV irradiation (Smith, Grossman and Walker 2002; Stracy *et al.* 2016). The role of UvrA in *E. coli* nucleoid condensation should be studied in the future.

Additionally, nucleoid condensation was delayed in the IN400 (*recJ*) strain compared to wt strain, reaching maximum condensation at 60 min post-irradiation. The RecJ exonuclease, together with the RecQ helicase and RecFOR, processes the blocked replication fork prior to the resumption of replication in UV-treated *E. coli* cells (Courcelle and Hanawalt 1999; Chow and Courcelle 2004; Rudolph, Upton and Lloyd 2008). Since nucleoid condensation is an SOS response-dependent phenomenon, and *recJ* mutation delays the SOS response induction (Fig. 1), this mutation must also delay nucleoid condensation, as was observed in this study. The delay in nucleoid condensation observed in the PQ37 (*uvrA*) and IN400 (*recJ*) strains can be explained by the requirement of the UvrABC excinuclease and RecQ-RecJ helicase-nuclease to remove photoproducts and to restart DNA replication, respectively (Courcelle, Crowley and Hanawalt 1999). The robust reduction in the bilobed-nucleoid shape fraction post-irradiation indicated that chromosome replication and cytokinesis did not occur in these strains.

CONCLUDING REMARKS

The restoration of post-irradiation chromosome replication and cytokinesis in *E. coli* was characterized by nucleoid reorganization through three primary sequential steps, namely, condensation, dispersion and chromosome segregation coupled with cytokinesis, which tended to restore cells to a pre-irradiated state. RecN has a role in post-irradiation nucleoid condensation events, although a basal level of nucleoid condensation post-irradiation (~between 15% and 20%) was consistently evident in IN250 (*recN*) cells, indicating that additional factors are involved in nucleoid condensation. In the absence of UvrA and RecJ, the post-irradiation nucleoid reorganization was modified, demonstrating the importance of both UvrABC pathway-mediated photoproduct repair and the reassembly of the replication fork by the RecQ/RecJ complex, respectively, for cell division restoration in UV-treated cells. These findings support the classic idea that photoproduct repair is required before chromosome replication occurs.

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Conflict of interest. None declared.

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