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R. N. Sharan [a]; H. Ryo [a]; T. Nomura [a]
[a] Department of Radiation Biology and Medical Genetics, Graduate School of Medicine, Osaka University, Osaka, Japan

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Critical role of *RecA* and *RecF* proteins in strand break rejoining and maintenance of fidelity of rejoining following γ-radiation-induced damage to pMTa4 DNA in *E. coli*

R. N. SHARAN, H. RYO & T. NOMURA

Department of Radiation Biology and Medical Genetics, Graduate School of Medicine, Osaka University, Osaka, Japan

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Abstract

Purpose: This study was undertaken to understand the roles of *RecA* and *RecF* proteins in strand break rejoining and maintenance of fidelity of the process following exposure of *E. coli* to γ-radiation *in vivo*.

Materials and methods: A plasmid DNA construct, pMTa4, was transformed into isogenic repair proficient (wild) and deficient (*recF* and *recA*) *E. coli* strains and γ-irradiated up to 30 Gy *in vivo*. The plasmid DNA was isolated under repair non-permissive (R^-)and permissive (R^+) conditions and analyzed by gel electrophoresis for the yields of single strand breaks (SSB) and double strand breaks (DSB) and their repair. The clonogenic survival of the *E. coli* was also recorded. The effects of γ-irradiation on *recA* reconstituted with cell free extract of wild strain or ultra-violet (UV)-irradiation were also monitored.

Results: None of the strains used in this investigation showed effects of radiation-induced oxidative base damage. The dose dependent increase in SSB and DSB on pMTa4 in wild and *recF* mutants in R^- condition were abolished upon repair incubation. The *recA* mutant exhibited a disturbed yield of SSB and DSB along with formation of γ-radiation-induced 'ladder'. The 'ladder' was not observed after repair incubation, UV-irradiation or γ-irradiation in presence of cell-free extract of wild strain. The survival of *recA* mutants was seriously compromised.

Conclusions: Wild, *recF* and *recA* strains of *E. coli* could repair γ-irradiation-induced oxidative damage to base or nucleotide (NT) *in vivo*. In absence of either *RecA* or *RecF* proteins, efficiency of rejoining of strand went down; *RecA* proteins seemed more critical than *RecF* in this. High fidelity or correct rejoining of strand breaks, on the other hand, seemed to require simultaneous presence of both *RecA* and *RecF* proteins.

Keywords: E. coli, *γ-radiation, repair proficient and deficient strains, strand break, repair,* recA *and* recF *mutants,* in vivo

Introduction

Radiation interaction with living systems causes direct and indirect damage triggering complex biological responses (Budworth et al. 2002, Lindahl & Wood 1999, Sutherland et al. 2000). The DNA is, for obvious reasons, a critical target of radiation damage with serious biological consequences. Depending on the quality and dose of radiation, damage such as, nucleotide (NT) or base modifications including other oxidative alterations, single strand break (SSB), double strand break (DSB), etc. form distinct lesions in a DNA molecule (Blaisdell & Wallace 2001, Budworth et al. 2002, Goodhead 1994, Klungland et al. 1999, Sutherland et al. 2000). The strand break type of damage is generated by several events. SSB can be generated by radiation-induced direct disjoining of phosphodiester bonds between successive NT. Similarly, radiation can cause direct disjoining of phosphodiester bonds on both strands simultaneously, producing a DSB. Two SSB in close proximity on opposite strands also effectively create a DSB. Some unrepaired NT damage can also get converted to SSB (Bhattacharjee & Sharan 2005, Milligan et al. 1999). Strand break type of DNA damage should be quickly and efficiently repaired. The repair should also be carried out with high fidelity. There are chances that both efficiency and fidelity of repair are compromised due to complexity of the repair processes involved (reviewed in

Correspondence: R. N. Sharan, Radiation and Molecular Biology Unit, Department of Biochemistry, North-Eastern Hill University, Shillong 793 022, India. Tel: (0364) 272 2121. Fax: (0364) 255 0108. E-mail: rnsharan@nehu.ac.in

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Kuzminov 1999, Peterson & Côté 2004). The former may lead to loss of DNA segments or genetic information while the latter would lead to wrong strand rejoining or misrepair producing new genetic combinations. In effect it means that both unrepaired as well as misrepaired strand break(s) could potentially become sources of large mutations and lead to similar consequences (e.g., cell death).

E. coli employs *RecA* mediated recombination repair (RR) pathway to repair strand break type of damage (reviewed in Kuzminov 1999). In a simplistic overview, following strand breakage, *RecA* protein leads the process of repair by recruiting a host of other proteins including other *Rec*-family proteins (e.g., *RecBCD*, which have helicase and nuclease functions). In a highly complex sequence of events that follows, the *RecBCD* (or, alternately *RecEQ*) unwinds and degrades one strand leaving a single stranded DNA (ssDNA) overhang(s) (Kuzminov 1999, Lusetti & Cox 2002). *RecA* protein monomers, competing against or coexisting with other single strand binding proteins, coat ssDNA. The *RecA* coated DNA strand can pair with undamaged homologous DNA strand and initiate strand exchange utilizing DNA pol I, ligase & homologous strand as a template. Understandably, *RecA* mediated homologous pairing and strand exchange must be highly accurate and supervised processes to maintain genetic integrity. These critical *RecA* mediated steps of RR pathway is guided by *RecF* protein (along with *RecO* and *RecR* proteins creating *RecFOR* moiety) (Kuzminov 1999). Thus, critical involvement of *RecA* and *RecF* proteins in repair of strand break type of damage is obvious. Furthermore, *RecA* protein is also involved in cellular replication and recombination besides interstrand cross link, SOS and mutagenic lesion bypass repair pathways (Kuzminov 1999, Lusetti & Cox 2002, Peterson & Côté 2004), suggesting that a reasonable basal level of the multifunctional *RecA* protein should be maintained all the time in *E. coli*. Indeed, $1-10\times10^3$ monomers of *RecA* are normally present in an *E. coli* cell, which can jump 50 folds when needed or induced (Kuzminov 1999). In comparison, *recF* gene is part of an inducible operon with uninduced basal level of *RecF* estimated to be <190 monomers per cell. Despite apparent multiplicity and criticality of roles, null mutants of *recA* as well as *recF* are viable (Kuzminov 1999). Differential survivals are also exhibited by *recA* and *recF* mutants of *E. coli* following radiation exposure suggesting that contributions of *RecA* and *RecF* proteins in repair of radiation-induced DNA damage were not identical. While it is clear that both have roles to play in repair of DNA strand breakage, their relative contributions in strand rejoining and maintaining fidelity or correctness of rejoining remain obscure. To the best of our knowledge, this has not been investigated.

Using a plasmid DNA construct, pMTa4, we have studied consequences of DNA damage following γ-ray (Humtsoe et al. 1998), ^{7}Li swift ion (Humtsoe et al. 2003), and ultra-violet (UV) ray (Bhattacharjee & Sharan 2005) irradiation or exposure to free radical generating radiomimetic chemicals (Odyuo & Sharan 2005) *in vitro*. The plasmid DNA was particularly convenient as qualitative and quantitative monitoring of induction and repair of SSB and DSB were rather easy. It was possible to quantify yield of SSB and DSB by densitometric quantification of relative quantities of bands of OC and L topological forms of the plasmid on an agarose gel, respectively. Monitoring the radiation-induced change in the restriction profile of the plasmid provided evidence for induced NT modification (Humtsoe et al. 1998, 2003). In the experimental condition used in those investigations, there was no influence of repair pathways on the radiation-induced damage to pMTa4 DNA molecules. This investigation was designed to understand the contributions of *RecA* and *RecF* proteins in strand rejoining process and maintenance of fidelity of the process *in vivo*. To achieve this, pMTa4 was transformed into isogenic repair proficient (wild) and repair deficient *E. coli* hosts and then irradiated. Following irradiation of *E. coli in vivo*, the plasmid DNA was isolated under repair non-permissive or permissive condition and analyzed. Simultaneously, the overall survival of the *E. coli* was assayed by clonogenic survival assay.

Materials and methods

Bacterial culture and preparation of plasmid DNA

E. coli harboring the 6173 bp plasmid construct, pMTa4, was grown at 37°C in Luria-Bertani (LB) medium containing Ampicillin (100 μg ml^{-1}) as described previously (Bhattacharjee & Sharan 2005, Odyuo & Sharan 2005). From the overnight culture, the plasmid was isolated in sterile water using High Pure Plasmid Isolation kit (Boehringer Mannheim GmbH, Mannheim, Germany). The purity of the isolate (pMTa4 DNA) was determined by measuring the absorption ratio A_{260}:A_{280}.

Repair proficient and deficient E. coli strains

A repair proficient wild (AB1157; F^{-} *recA*$^{+}$ *recB*$^{+}$ *recC*$^{+}$ *sbcB*$^{+}$ *thr-1 leu-6 his-4 argE3 proA2 thi-1lacY1 galK2 ara-14 xyl-5 mtl-1 tsx-33 rpsL31 supE44*) and its isogenic repair deficient *recF* (JC9239; as AB1157, but *recF143*) and *recA* (KY1056; as AB1157, but *recA56 srlR::Tn10*) strains of *E. coli* were used in this

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investigation (details in Horii & Clark 1973, Yamamoto et al. 1984). AB1157, JC9239 and KY1056 were kindly provided by Prof. K. Yamamoto (Tohoku University, Japan).

Transformation of E. coli *hosts with pMTa4*

Transformation was based on Sambrook and Russell (2001) with some modifications. In brief, to 100 μl of frozen competent cells or freshly prepared competent cells in a pre-cooled tube, 1 μl ($\approx$300 ng) of pMTa4 was added, gently mixed and kept on ice for 30 min. The tube was then incubated, in sequence, at 42°C for 30 sec and on ice for 180 sec. Glucose supplemented rich LB medium (popularly named 'SOC medium') was added (900 μl, pre-warmed to 37°C) into the tube, gently mixed and incubated at 37°C for 60 min. The content (100 μl) was then plated on SOC agar plates (with 100 μg ml^{-1} Ampicillin) at 10^{-3} and 10^{-5} dilutions and incubated overnight at 37°C. Control was SOC-agar plate without Ampicillin.

Irradiation source and experimental protocols

Transformed *E. coli* cultures in LB medium (2–5 ml of overnight, top log phase culture; $A_{600} \leq 1$) in sterile tubes were pre-cooled and irradiated on ice.

γ-irradiation. Cells were exposed to γ-rays in a ^{137}Cs Gamma Cell 40 Exactor (Nordion International Inc., Vancouver, British Columbia, Canada) to accumulate doses of 10, 20 and 30 Gy (1.07 Gy min^{-1}). The irradiated samples were either subjected to isolation of pMTa4 immediately (repair non-permissive condition; R^{-}), or after incubation at 37°C for 60 min (repair permissive condition; R^{+}). The plasmid preparations were analyzed by agarose gel electrophoresis.

UV-irradiation. Wild and *recA* mutant were UV-irradiated (254 nm) to doses equivalent to survival of the *E. coli* strain at 30 Gy of γ-radiation, the plasmid isolated under R^{-} and R^{+} conditions and analyzed by gel electrophoresis.

γ-irradiation after reconstitution with cell free extract of wild type. Equimolar cell free extract (1.26 mg protein) of AB1157 (wild) strain of *E. coli* was first added to cultures of *recA* cells in Eppendorf tubes and incubated at 37°C for 15 min. The *recA* mutants in cultures were then γ-irradiated, plasmid isolated and analyzed.

Agarose gel electrophoresis

Plasmid preparations (~1 μg) isolated from unirradiated (control) and irradiated cultures were subjected to 1% agarose gel electrophoresis using tris-acetate-ethylenediaminetetraacetic acid (EDTA) or TAE buffer (50 V, 120 min). The *Nci I* and *Hae II* restricted plasmid preparations (restriction according to conditions recommended by manufacturers; Boehringer Mannheim GmbH, Mannheim, Germany) were also subjected to agarose gel electrophoresis (100 V, 45 min). SYBR green I (Cambrex Bio Science, Baltimore, MD, USA) stained gel was scanned immediately using a FluorImager 595 LASER scanner (Amersham Biosciences Inc., Sunnyvale, CA, USA).

Clonogenic survival assay

Irradiated *E. coli* cultures (top log phase; $A_{600} \leq 1$) were diluted (typically, between 10^{-3} and 10^{-7}) in Ampicillin supplemented LB medium and 100 μl of the diluted cultures were plated on LB plates (with 100 μg ml^{-1} Ampicillin). The plates were incubated at 37°C and colonies counted.

Data analysis and statistical treatment

Quantitative analyses of different topological forms of the plasmid were done by ImageQuant (Molecular Dynamics, version 4.0) software. Most experiments were independently repeated 2–3 times, except the UV and reconstitution experiments, which were repeated only once. Agarose gel electrophoretic runs were carried out in duplicate for each sample. The quantification data for SSB and DSB from each gel were pooled together to calculate their mean $\pm$ SD. Student's *t*-test was appropriately used; $p \leq 0.05$ value was taken as biologically significant.

Results

Transformation frequency was moderate to high for all strains used in this investigation. Different transformants of *E. coli*, however, showed slightly different rates of growth in culture. Therefore, for irradiation, the cultures were grown for different periods of time (between 13 and 20 h) to get cells in top log phase. The cultures were pre-cooled and irradiated on ice. Controls were sham-irradiated. A part of the culture was used for clonogenic survival assay. From the other part, plasmid was either isolated immediately (R^{-}) or after a repair incubation (R^{+}). The absorption ratio A_{260}:A_{280} of the pMTa4 isolates was usually about 1.95. The plots, representing yield of SSB or DSB, have been plotted as % of respective controls. Therefore, the slope of curve of yields of SSB and DSB only indicates the relative yield within the category (SSB or DSB) and not their absolute values. The corresponding OC and L bands on the agarose gels visually give

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clear indication that the absolute yield of SSB was significantly more than DSB following γ-irradiation.

Effect of γ-radiation on survival

The results of clonogenic survival assay for different strains of *E. coli* transformants are shown in Figure 1. The repair proficient (AB1157) strain showed high radioresistance to γ-rays; $\approx$80% survival at the highest dose (30 Gy) used in the investigation. The repair deficient mutants, *recF* (JC9239) and *recA* (KY1056) exhibited nearly similar survivals up to 20 Gy ($\approx$43%). Beyond the dose, *recA* (KY1056) was hypersensitive to γ-rays (survival $\approx$17% at 30 Gy) compared to *recF* (JC9239), which had $\approx$37% survival at 30 Gy.

Effect of γ-radiation on pMTa4 isolated from wild and recF strains

The agarose gel in Figure 2A shows different topological forms of the plasmid isolated from unirradiated (control) and irradiated wild (AB1157) and *recF* (JC9239) strains under R^- (lanes 1 – 4) and R^+ (lanes 5 – 8) conditions. The yields of OC and L forms of pMTa4, representing DNA with SSB and DSB, respectively, were calculated and plotted (Figure 2B). The wild and *recF* strains exhibited dose dependent increase in OC and L forms under

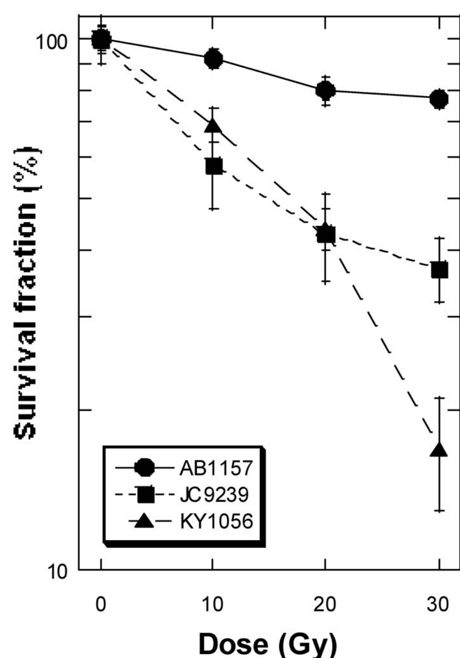

Figure 1. Graph showing per cent clonogenic survival (mean $\pm$ SD) of wild (AB1157) *E. coli* and its isogenic *recF* (JC9239) and *recA* (KY1056) mutants following exposure to 0 (Control), 10, 20 and 30 Gy of ^{137}Cs γ-radiation.

R^- condition (Figure 2B, closed symbols and Figure 2A, lanes 1 – 4). Under R^+ condition, the OC and L forms essentially plateaued in wild but dipped significantly below the control in *recF* strain (Figure 2B, open symbols and Figure 2A, lanes 5 – 8).

Effect of γ-radiation on pMTa4 isolated from recA strains

Figure 3A shows an agarose gel with different topological forms of pMTa4 isolated from *recA* (KY1056) mutant under R^- and R^+ conditions. The OC and L forms were quantified and plotted (Figure 3B). As compared to the wild or *recF* strains, the yields of γ-radiation induced SSB and DSB in pMTa4 were significantly different in *recA* strain under R^- and R^+ conditions exhibiting pronounced abnormalities in induction of strand breaks as well as its repair. The *recA* strain also exhibited a conspicuous, dose dependent 'ladder' between OC and L bands on the gels under R^- condition (Figure 3A, lanes 1 – 4) that was not observed in the wild and *recF* strains. Upon repair incubation (R^+), the 'ladder' disappeared for all doses of radiation (Figure 3A, lanes 5 – 8).

Effect of UV-radiation (254 nm) on pMTa4 isolated from recA (KY1056) and wild strains

Figure 4 shows the agarose gel of pMTa4 isolated from *recA* mutant and wild strain under R^- and R^+ conditions after UV-exposure to doses survival-equivalent to 30 Gy of γ-radiation. No 'ladder' was detected in UV exposed *recA* under R^- condition (lane 1) in contrast to γ-irradiation (Figure 3A).

Effect of γ-radiation on Nci I and Hae II restriction of pMTa4

Figure 5 shows the *Nci I* restricted pMTa4 isolated from unirradiated control, and γ-ray exposed *recF* (JC9239) and *recA* (KY1056) strains under R^- and R^+ conditions. The restriction profiles were identical. The results were similar for *Hae II* (not shown).

Effect of γ-radiation on pMTa4 isolated from reconstituted recA strain

The agarose gel electrophoresis of pMTa4 isolated from *recA* strain that was supplemented or reconstituted with cell free extract of the wild strain prior to irradiation was conspicuous by absence of the expected 'ladder' under R^- condition (not shown).

Discussion

By transforming pMTa4 into isogene wild, *recF* and *recA* mutants of *E. coli* we have created *in vivo* conditions wherein repair of γ-radiation-induced

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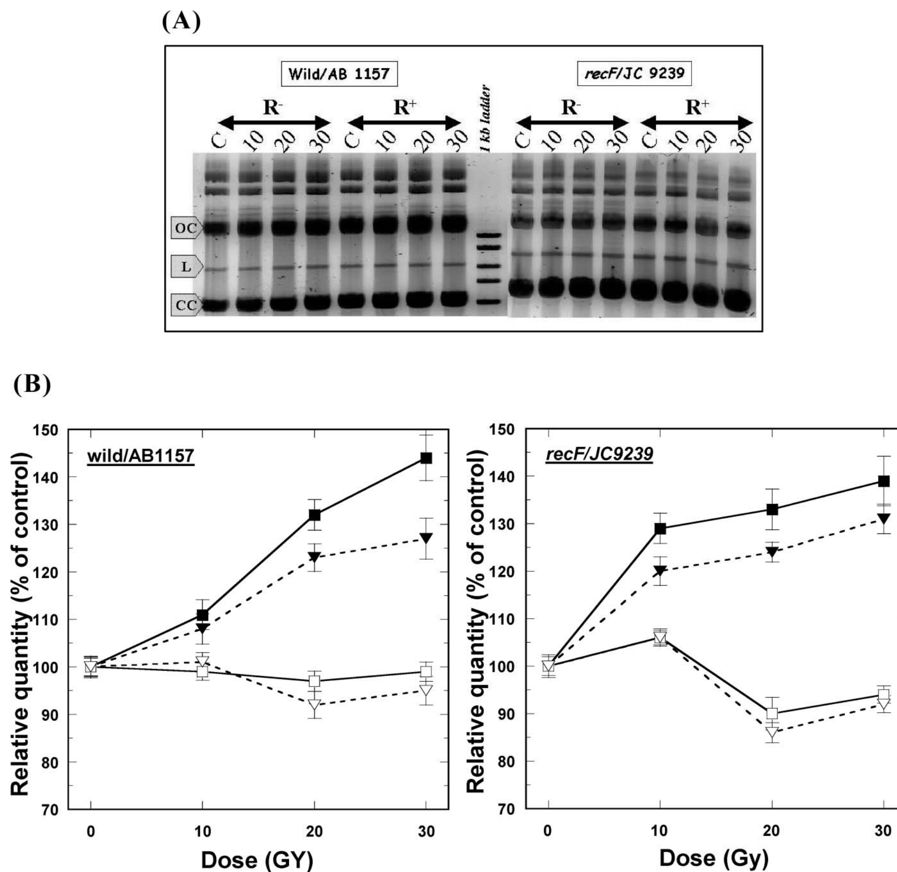

Figure 2. (A) Agarose gel showing topological forms of pMTa4 isolated from non-irradiated control (C) or γ-irradiated (10, 20 and 30 Gy) wild, AB1157, (left panel) and *recF*, JC9239, (right panel) *E. coli* under repair non-permissive (R^-) and repair permissive (R^+) conditions. Size marker, 1 kb ladder, was used in the marked lane. (B) Graphs showing the quantitative yield (mean $\pm$ SD) of γ-radiation-induced SSB (triangle) and DSB (square) on pMTa4 under repair non-permissive, R^- (closed symbol), and permissive, R^+ (open symbol), conditions in wild, AB1157, (left panel) and *recF*, JC9239, (right panel) strains of *E. coli*.

pMTa4 DNA damage was achieved to varying extents. Logically the repair pathways are optimally operational in the repair proficient wild host, AB1157, but sub-optimally active in *recF* and *recA* mutants due to different component(s) of repair pathways being defective in them. The isogene *E. coli* hosts harboring pMTa4 were irradiated on ice in complete medium to inflict DNA damage. The dose of radiation was relatively low for *E. coli*, maximum being 30 Gy. After irradiation the plasmid DNA was isolated to study the effect of repair pathways under two conditions. In the first, the conventional repair non-permissive (R^-) situation, plasmids were isolated soon (approximately 10 – 15 min) after irradiation on ice. In the second, the irradiated culture tubes were subjected to a post-irradiation incubation at 37°C for 60 min creating a repair permissive (R^+) condition. In the experimental set-up, the quantities of *RecA* and *RecF* proteins are likely to be different in different strains and experimental conditions (Table I). Under R^- condition, *RecA* protein ($1-10\times10^3$ molecules/cell; indicated as ++ in Table I) in wild and *recF* strains is reasonably high as compared to *RecF* (<190 molecules/cell; indicated as + in Table I) in wild and *recA* strains (Kuzminov 1999). On the other hand, under R^+ condition both proteins should be induced and optimally present (indicated as +++ in Table I) only in wild strain. The other two mutants would contain only one protein (Table I).

The repair proficient wild (AB1157) strain of *E. coli* exhibited about 80% survival at 30 Gy of γ-radiation (Figure 1). Its pMTa4 isolate showed dose dependent increase in SSB and DSB under R^- condition (Figure 2B). The increase was abolished for all doses of radiation under R^+ condition (Figure 2B). The plasmid DNA in γ-irradiated *recF* strain, which displayed 37% survival at 30 Gy (Figure 1), also sustained identical spectrum of damage (Figure 2). This is evident from the yield of strand breaks under R^- condition (Figure 2B). The repair of strand break type of damage under R^+ condition was, however, different than the wild type (Figure 2B). The level of SSB and DSB in *recF* strain

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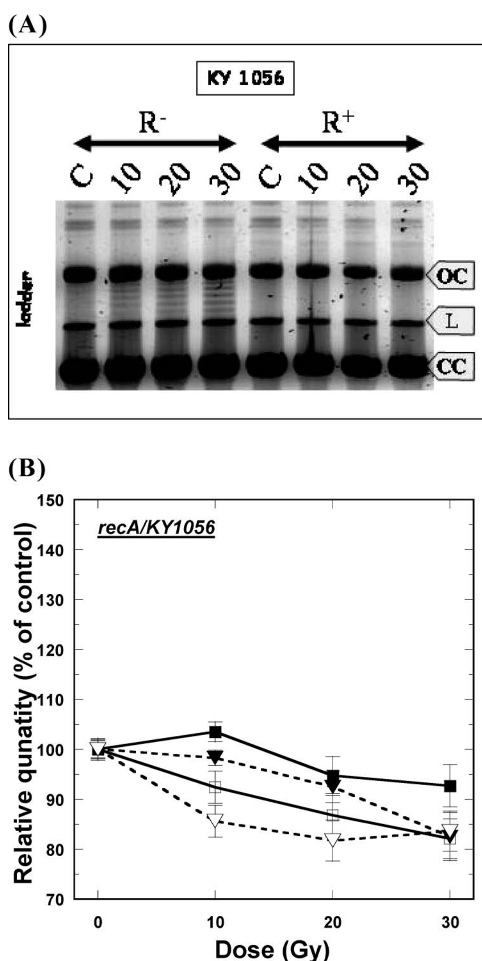

Figure 3. (A) Agarose gel showing topological forms of pMTa4 isolated from *recA* mutants of *E. coli* (KY1056) following γ-irradiation (0 (C), 10, 20 and 30 Gy) under R^- and R^+ conditions. Note a dose dependent 'ladder' between OC and L bands in lanes 2 to 4. (B) Graph showing the quantitative yield (mean $\pm$ SD) of γ-irradiation-induced SSB (triangle) and DSB (square) on pMTa4 under repair non-permissive, R^- (closed symbols), and permissive, R^+ (open symbols), conditions in the mutant.

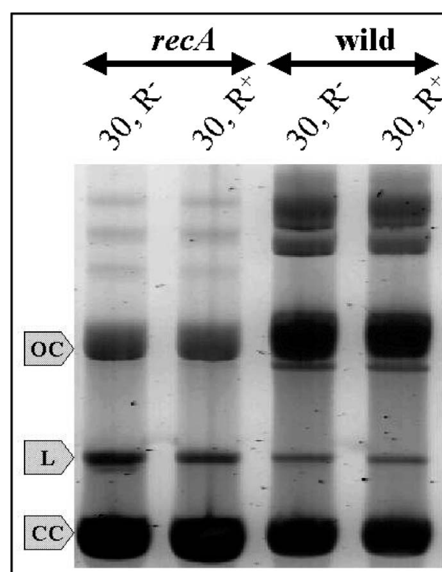

Figure 4. Agarose gel showing topological forms of pMTa4 isolated from UV-irradiated (equivalent to 30 Gy γ-radiation in survival term) *recA*, KY1056, (lanes 1 and 2) and wild, AB1157, (lanes 3 and 4) *E. coli* under R^- and R^+ conditions, respectively.

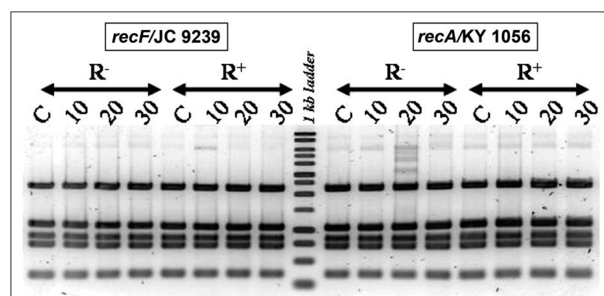

Figure 5. Agarose gel showing *Nci I* restricted pMTa4 isolated from unirradiated (C) and γ-irradiated (10, 20 and 30 Gy) *recF* (JC9239) and *recA* (KY1056) mutants under R^- and R^+ conditions. Size marker, 1 kb ladder, was used in the marked lane.

unexpectedly dipped below control (100%) at 20–30 Gy (Figure 2B; compare with the wild type). The isogene *recA* mutant used in this study showed even more significantly compromised ($\approx$17% at 30 Gy) survival (Figure 1). The yield of SSB and DSB under R^- and R^+ conditions (Figure 3B) was also noticeably different from the wild or *recF* strain (Figure 2). Lastly, a prominent dose dependent 'ladder' was visible on the agarose gel only under R^- condition (Figure 3A, lanes 1–4). The ladder disappeared completely upon repair incubation (Figure 3A, lanes 5–8). In spite of these differences among the three isogenic strains studied, none showed any difference in the *Nci I* (Figure 5) and *Hae II* (not shown) restriction profiles of pMTa4 before and after γ-irradiation – something that was prominently noticed under *in vitro* condition (Humtsoe et al. 1998).

Table I. Status of *RecA* and *RecF* proteins under R^- and R^+ conditions in three strains of *E. coli* used in the investigation and their clonogenic survivals (uninduced level in R^- condition: *RecA* $= 1-10 \times 10^3$ monomers/cell (++); *RecF* < 190 monomers/cell (+); Optimum induction in R^+ for both: +++; see text for details).

	Repair non-permissive (R^-)		Repair permissive (R^+)		
Strain↓	*RecA*	*RecF*	*RecA*	*RecF*	Survival (30 Gy)
Wild strain	++	+	+++	+++	≅80%
recF mutant	++	−	+++	−	≅37%
recA mutant	−	+	-	+++	≅17%

Normal metabolic and physiological activities (including DNA replication etc.) generate substantial physiological load of predominantly oxidative

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NT but also strand break types of damage (Beckman & Ames 1997, Lindahl & Wood 1999, Kuzminov 1999). Different 'null' mutants (e.g., *rec* family genes) exhibit reduced viabilities even in the absence of an exogenous source of DNA damage further supporting this contention. Based on a host of compelling evidences (reviewed in Kuzminov 1999), it appears that much of the multitude of repair machineries in *E. coli* must have evolved to contain the damage caused essentially by endogenous sources (Lindahl 2000). Nonetheless, the fact that different null mutants remain viable strongly suggests that different repair machineries are integrated with each other in a way that it overcame at least a part of other's deficiency (reviewed in Kuzminov 1999, Peterson & Côté 2004). In the experimental conditions employed in this investigation, even additional radiation-induced burden of NT and other oxidative damages were efficiently repaired in both *recA* and *recF* strains under R^- as well as R^+ conditions (Figure 5). As the maximum dose of radiation in this investigation was only 30 Gy, a relatively small dose for *E. coli*, the additional burden of damage due to radiation is likely to be small. This suggests that the observed compromised survival of either of repair deficient mutants (Figure 1) could not have been due to lack of repair of oxidative NT damage. The fact that under conventional repair non-permissive (R^-) as well as permissive (R^+) conditions the repair of NT damage was carried out efficiently seems overtly odd. However, on deeper analysis it may not appear so. For experiments conducted under R^- condition, top log-phase *E. coli* culture was pre-cooled (about 10 – 15 min) and irradiated on ice. In its normal growth condition, the NT repair enzymes were fully induced and optimally working to repair damages arising out of endogenous metabolic activities. Incubation on ice lowered the temperature of the culture to about 6°C. This would make the *E. coli* metabolism sluggish due to which the burden of endogenous damage would also reduce proportionately. At this temperature enzymatic processes (e. g., repair pathways) are only diminished but not eliminated (Bonura, Smith & Kaplan 1975). Therefore, the NT repair systems in *E. coli* could still handle successful repair of the reduced burden of the endogenous damage (at ice temperature) as well as the relatively small quantum of additional damage due to γ-ray doses up to 30 Gy, a relatively low dose for a bacterial system. For experiment conducted under R^+ condition, it appears from our results that the fully induced NT repair systems successfully repaired damages induced by endogenous sources as well as γ-ray doses of up to 30 Gy. Because of this, the pMTa4 isolated from it showed total recovery from NT or base modification type of damage as *Nci I* (Figure 5) and *Hae II* (not shown) restriction patterns remained invariant for all doses of radiation under R^- and R^+ conditions. These observations also bring to prominence the importance of strand break types of damage in manifestation of radiation effects.

The yield of SSB and DSB in wild and *recF* strains were similar under R^- condition but not under R^+ condition (Figure 2). In contrast, it was very different in case of *recA* under both R^- and R^+ conditions (Figure 3). The dose dependent ladder under R^- condition (Figure 3A; lanes 1 – 4) disappeared upon repair incubation (Figure 3A; lanes 5 – 8) and also upon reconstitution with cell free extract of wild cells (not shown). UV-irradiation also did not produce the ladder in *recA* (Figure 4). The complexity of these observations is in line with the complexity of expected biological response to induction of strand break type of damage and its repair, particularly in *E. coli* (Kuzminov 1999, Peterson & Côté 2004). At first, the genomic and plasmid DNA of *E. coli* are covalently closed circular molecules. Secondly, DNA replication and RR processes are intricately linked in *E. coli* (reviewed in Kuzminov 1999). Therefore, the induction of damage and its repair of pMTa4 DNA in *E. coli* are very different and complex as compared to a linear DNA chromosome. The initial topology of DNA is shown to exert significant influence on the induction of damage as well as repair (Goodhead 1994, Isaacs & Spielmann 2004). The situation is more complex in a circular DNA, e.g., pMTa4, which has complex topology because of simultaneous operation of replication and RR pathways. Because of such reasons, in many cases the final biological response tends to correlate poorly with initial damages (Goodhead 1994). While we attempt to put in perspective the complex observations, we are alive to the fact that not everything can perhaps be explained and some grey areas may be left out as also concluded by Kuzminov (1999). We do intend to undertake appropriate investigations in the future to understand the process fully. We also hope that some other research groups might also get interested in this line of investigation.

Genotypically, *recA* and *recF* strains are identical to the wild strain except a defective *recA* 56 and *recF 143* gene, respectively (Horii & Clark 1973). Therefore, under R^- condition wild and *recF* strains would have uninduced levels of *RecA* protein while *recA* would be completely devoid of it (Table I). The uniduced level of *RecA* is significantly high ($1 - 10 \times 10^3$ monomer/cell). Since the integrity of genome is maintained in normal physiological conditions, it can be visualized that *RecA* must keep interacting with DNA continuously to repair any endogenously inflicted strand break type of damage. Therefore, the topology of pMTa4 DNA in wild and *recF* is likely to be different than in *recA*, which is devoid of

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RecA (Table I) and, consequently, to this avenue of repair machinery. Thus, there seems to be two special situations operating here. At first, there was initial difference in the topology or 3-D conformation of pMTa4 DNA in wild and *recF*, on one hand, and *recA*, on the other, due to presence or absence of *RecA*. High affinity sequence independent, helical and filamentous binding of *RecA* (starting from ssDNA and later extending to duplex DNA as well) under physiological conditions *in vitro* has been shown to relax DNA conformation by 1.5x (Stasiak & DiCapua 1982). Secondly, *recA* strain would be devoid of *RecA* mediated RR leading to accumulation of strand break type of damage in *recA*. These might be the cause of the observed difference of the yield of SSB and DSB in *recA* strain (Figure 3) as compared to wild and *recF* (Figure 2).

Since *RecF* protein guides binding of *RecA* protein onto ssDNA in preparation to initiation of RR (Kuzminov 1999, Lusetti & Cox 2002), the absence of *RecF* protein is likely to suppress the *RecA* mediated strand break rejoining process. This would result in increased quantum of unrepaired strand breaks. Reduced rejoining of DNA strand breaks could lead to loss of DNA fragments. This may explain the unexpected dip of OC and L forms of pMTa4 (representing SSB and DSB, respectively) below the control in *recF* strain under R^+ condition (Figure 2B). This could also be the cause of compromised survival of the mutant following irradiation (Figure 1).

The situation in *recA* was more complex. Both under R^- and R^+ conditions, the strain was devoid of *RecA* protein (Table I). Consequently, its genomic and plasmid DNA are likely to have different topological organization as well as accumulated strand break type of damage under R^- condition when even *RecF* (and other RR proteins) were present at uninduced or low levels. At this stage we observed a γ-ray dose dependent 'ladder' formation in *recA* mutant (Figure 3A, lanes 1–4). The ladder disappeared upon repair incubation (R^+) (Figure 3A, lanes 5–8). The level of γ-ray induced strand break type of damage would rise in a dose dependent manner due to the absence of *RecA* mediated RR pathway. Accumulation of such damage on top of initial difference in the DNA topology is likely to create different degrees of supercoiling on plasmid DNA giving it differential mobility on agarose gel. This might explain the observation of a ladder (Figure 3A). There is another likely possibility which shall be subject of future investigations. Many bacteriophages (e.g., Lambda, Rac prophage, etc.) employ a simpler strategy of single strand annealing (SSA) to repair strand break type of damage. In comparison to highly complex RR pathway, SSA is significantly simpler but very prone to mistake and, therefore, considered non-conservative (reviewed in Kuzminov 1999). SSA simply involves λ exonuclease-β protein-DNA ligase mediated joining two DNA ends carrying direct repeats in the overlapping configuration, as in the repair of a double-strand break between direct repeats (Kmiec & Holloman 1981, Takahashi & Kobayashi 1990). The pathway is shown to be operational in absence of *RecA* protein (Zieg & Kushner 1977). Therefore, in *recA* strain this could have been another way by which uncontrolled rejoining, possibly wrong rejoining or misrepair of strand break type of damages occurred. These misrepaired pMTa4 molecules are likely to have different degrees of supercoiling because of which the ladder was visible under R^- condition. This attractive hypothesis derives support from the fact that SSA mediated plasmid recombination leads to (a) invasive type repair in absence of *RecA* (Kuzminov 1999) and (b) formation of linear multimers of plasmids (Cohen & Clark 1986). Further, UV-irradiation, which does not directly produce strand breaks, also did not exhibit ladder formation in *recA* in R^- condition (Figure 4). Lastly, reconstitution of *recA* strain with cell free extract of wild strain prevented ladder formation (not shown). However, upon repair incubation (R^+) and consequent induction of *RecF* protein (Table I), the ladder disappeared (Figure 3A). This suggests that in the optimized presence of *RecF* the operation of SSA pathway was stalled in some unknown way. Stalled SSA pathway would also leave many strands without rejoining leading to loss of genetic information. The dip below control in the yield of SSB and DSB in *recA* under R^+ condition (Figure 3B), likely to be due to loss of DNA, supports this contention. It would be worthwhile to mention that dose dependent ladder formation under R^- condition has also been observed in three other unrelated *recA* mutants of *E. coli* (unpublished results).

In conclusion, using pMTa4 plasmid in isogenic wild, *recF* and *recA* strains of *E. coli*, we show that *recF* and *recA* mutants possessed ability to repair oxidative NT damage of DNA induced by γ-rays up to 30 Gy, a relatively low dose for a bacterium. Repair of the accompanying strand break types of damage required optimal quantum of both *RecA* and *RecF* proteins. Absence of *RecF* protein lowered strand rejoining efficiency leading to partial loss of genetic information. On the other hand, in the absence of *RecA* protein the fidelity of strand rejoining was also compromised leading to misrepairs. The results suggest that for overall repair of strand break type of repair, *RecA* protein is more critical than *RecF* in *E. coli*. More studies are planned for the future.

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References

Beckman KB, Ames BN. 1997. Oxidative decay of DNA. Journal of Biological Chemistry 272:19633 – 19636.

Bhattacharjee C, Sharan RN. 2005. UV-C radiation induced conformational relaxation of pMTa4 DNA in *Escherichia coli* may be the cause of single strand breaks. International Journal of Radiation Biology 81:919 – 927.

Blaisdell J, Wallace SS. 2001. Abortive base-excision repair of radiation-induced clustered DNA lesions in *Escherichia coli*. Proceedings of National Academy of Science of the USA 98:7426 – 7430.

Bonura T, Smith KC, Kaplan HS. 1975. Enzyme induction of DNA strand breaks in γ-irradiated *Escherichia coli* K-12. Proceedings of National Academy of Science of the USA 72: 4265 – 4269.

Budworth H, Dianova II, Podust VN, Dianov GL. 2002. Repair of clustered DNA lesions: Sequence specific inhibition of long-patch base excision repair by 8-oxoguanine. Journal of Biological Chemistry 277:21300 – 21305.

Cohen A, Clark AJ. 1986. Synthesis of linear plasmid multimers in *Escherichia coli* K-12. Journal of Bacteriology 167:327 – 335.

Goodhead DT. 1994. Initial events in the cellular effects of ionizing radiations: Clustered damage in DNA. International Journal of Radiation Biology 65:7 – 17.

Horii Z.-I, Clark AJ. 1973. Genetic analysis of *RecF* pathway to genetic recombination in *Escherichia coli* K12: Isolation and characterization of mutants. Journal of Molecular Biology 80:327 – 344.

Humtsoe JO, Schneeweiss FHA, Srivastava A, Sarma A, Sharan RN. 2003. Biological effects induced by swift heavy ions of Lithium on aqueous solution of plasmid pMTa4. Radiation Effects and Defects in Solids 158:603 – 607.

Humtsoe JO, Schroeder CH, Sharan RN. 1998. Is there a relationship between nucleotide sequence and radiation induced DNA damage? In: Sharan RN, editor. Trends in radiation and cancer biology, Jülich: Forschungszentrum Jülich GmbH), International Co-operation Bilateral Seminars series volume 29, pp 29 – 32.

Isaacs RJ, Spielmann HP. 2004. A model for initial DNA lesion recognition by NER and MMR based on local conformational flexibility. DNA Repair 3:455 – 464.

Klungland A, Rosewell I, Hollenbach S, Larsen E, Daly G, Seeberg E, Lindahl T, Barnes E. 1999. Accumulation of premutagenic DNA lesions in mice defective in removal of oxidative base damage. Proceedings of National Academy of Science of the USA 96:13300 – 13305.

Kmiec E, Holloman WK. 1981. β protein of bacteriophage λ promotes renaturation of DNA. Journal of Biological Chemistry 256:12636 – 12639.

Kuzminov A. 1999. Recombinational repair of DNA damage in *Escherichia coli* and bacteriophage λ. Microbiology and Molecular Biology Reviews 63:751 – 813.

Lindahl T, Wood RD. 1999. Quality control by DNA repair. Science 286:1897 – 1905.

Lindahl T. 2000. Suppression of spontaneous mutagenesis in human cells by DNA base excision-repair. Mutation Research 462:129 – 135.

Lusetti SL, Cox MM. 2002. The bacterial *recA* protein and the recombinational DNA repair of stalled replication forks. Annual Review of Biochemistry 71:71 – 100.

Milligan JR, Aguilera JA, Nguyen T.-TD, Ward JF, Kow YW, He B, Cunningham RP. 1999. Yield of DNA strand breaks after base oxidation of plasmid DNA. Radiation Research 151: 334 – 342.

Odyuo MM, Sharan RN. 2005. Differential DNA strand breaking abilities of $^{\circ}$OH and ROS generating radiomimetic chemicals and γ-rays: Study of plasmid DNA, pMTa4, *in vitro*. Free Radical Research 39:499 – 506.

Peterson CL, Côté J. 2004. Cellular machineries for chromosomal DNA repair. Genes and Development 18:602 – 616.

Sambrook J, Russell DW. 2001. Molecular cloning: A laboratory manual. New York: Cold Spring Harbor Laboratory Press.

Stasiak A, Dicapua ED. 1982. The helicity of DNA in complexes with *RecA* protein. Nature 299:185 – 186.

Sutherland BM, Bennet PV, Sidorkina O, Laval J. 2000. Clustered DNA damages induced in isolated DNA in human cells by low doses of ionizing radiation. Proceedings of National Academy of Science of the USA 97:103 – 108.

Takahashi N, Kobayashi I. 1990. Evidence for the double-strand break repair model of bacteriophage λ recombination. Proceedings of National Academy of Science of the USA 87: 2790 – 2794.

Yamamoto K, Satake M, Shinagawa H. 1984. A muticopy *phr*-plasmid increases the ultraviolet resistance of a *recA* strain of *Escherichia coli*. Mutation Research 131:11 – 18.

Zieg J, Kushner SR. 1977. Analysis of genetic recombination between two partially deleted lactose operons of *Escherichia coli* K12. Journal of Bacteriology 131:123 – 132.

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