Acta Biochimica Polonica

Vol. 52 No. 1/2005

139–147

QUARTERLY

Effect of SOS-induced Pol II, Pol IV, and Pol V DNA polymerases on UV-induced mutagenesis and MFD repair in *Escherichia coli* cells

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Received: 29 December, 2004; accepted: 12 March, 2005

Key words: *E. coli,* UV induced mutagenesis, MFD*, mutD5, umuDC, dinB, polB*

Irradiation of organisms with UV light produces genotoxic and mutagenic lesions in DNA. Replication through these lesions (translesion DNA synthesis, TSL) in *Escherichia coli* requires polymerase V (Pol V) and polymerase III (Pol III) holoenzyme. However, some evidence indicates that in the absence of Pol V, and with Pol III inactivated in its proofreading activity by the *mutD5* mutation, efficient TSL takes place. The aim of this work was to estimate the involvement of SOS-inducible DNA polymerases, Pol II, Pol IV and Pol V, in UV mutagenesis and in mutation frequency decline (MFD), a mechanism of repair of UV-induced damage to DNA under conditions of arrested protein synthesis. Using the *argE3*→Arg$^+$ reversion to prototrophy system in *E. coli* AB1157, we found that the *umuDC*-encoded Pol V is the only SOS-inducible polymerase required for UV mutagenesis, since in its absence the level of Arg$^+$ revertants is extremely low and independent of Pol II and/or Pol IV. The low level of UV-induced Arg$^+$ revertants observed in the AB1157*mutD5*Δ*umuDC* strain indicates that under conditions of disturbed proofreading activity of Pol III and lack of Pol V, UV-induced lesions are bypassed without inducing mutations. The presented results also indicate that Pol V may provide substrates for MFD repair; moreover, we suggest that only those DNA lesions which result from *umuDC*-directed UV mutagenesis are subject to MFD repair.

Irradiation of organisms with UV light produces lesions in DNA that stop replication. The two primary UV induced lesions in DNA are *cis,syn*–cyclobutane pyrimidine dimers

✪50th Anniversary of the Institute of Biochemistry and Biophysics, Polish Academy of Sciences

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Abbreviations: CPD, cyclobutane pyrimidine dimer; MFD, mutation frequency decline; Pol, DNA polymerase; TSL, translesion DNA synthesis.

(CPD) and the pyrimidine (6–4) pyrimidone photoproducts formed at a ratio of 3:1 at moderate doses of UV (Mitchell & Nairn, 1989).

Several observations indicate that translesion DNA synthesis (TSL) past CPD is almost entirely dependent on the activity of DNA polymerase V (Pol V) encoded by the *umuDC* operon (Szekeres *et al.*, 1996; Vendewiele *et al.*, 1998). However, efficient TSL has also been observed after SOS-induction in strains with *umuDC* deletion, if these strains were deficient in the 3′→5′ exonuclease proofreading activity of DNA polymerase III (Pol III) (Vendewiele *et al.*, 1998; Borden *et al.*, 2002). The proofreading function is ensured by the ε subunit of Pol III and is inactivated by the *mutD5* mutation in *dnaQ* gene (Takano *et al.*, 1986). Deprivation of the proofreading activity leads to a large increase in spontaneous mutations, chronic induction of SOS response (Nowosielska *et al.*, 2004a; 2004b; Janion *et al.*, 2002), and expression of over 40 genes, among them *polB, dinB,* and *umuDC,* encoding repair polymerases Pol II, Pol IV, and Pol V, respectively (Friedberg *et al.*, 1995; Tang *et al.*, 1999; Janion, 2001). A common feature of the SOS-inducible DNA polymerases is their ability to replicate past a non-instructive lesion (Szekeres *et al.*, 1996; Napolitano *et al.*, 2000). Participation of a particular polymerase in TSL depends on the sequence and type of damage that blocks DNA replication (Wagner *et al.*, 1999; 2002; Napolitano *et al.*, 2000).

In this work we studied the contribution of Pol III, the main replication polymerase of *E. coli,* and of SOS-inducible repair polymerases, Pol II, Pol IV, and Pol V, to UV mutagenesis and to mutation frequency decline (MFD) repair. MFD is a loss of certain UV-induced tRNA suppressor mutations under conditions of transient inhibition of protein synthesis immediately following UV irradiation (Witkin, 1956; 1994; Doudney & Haas, 1958; Selby & Sancar, 1994). This phenomenon is connected with an error-free repair system preferentially acting on the transcribed strand of DNA. Encoded by the *mfd* gene, the Mfd protein is a transcription repair coupling factor (TRCF) (Selby & Sancar, 1991) that frees DNA from RNA polymerase stalled at the damage site and directs repair proteins to the transcribed DNA strand.

We found that (i) the *umuDC*-encoded Pol V is indispensable to UV mutagenesis; (ii) the SOS-inducible Pol II and Pol IV are not involved in UV mutagenesis; (iii) in a strain deficient in Pol V and mutated in proofreading Pol III the level of UV-induced Arg^+ revertants is low which may indicate a more precise bypass of UV-induced lesions by mutated Pol III than by Pol V; (IV) UV-irradiated and transiently starved *E. coli* cells are subject to MFD but only in strains proficient in Pol V.

MATERIALS AND METHODS

Bacterial strains and plasmids. The *E. coli* strains and plasmids used in this study are listed in Table 1. Strains RW82, NR9458, YG7207, and NR11501 were donors of the Δ*umuDC*, *mutD5*, Δ*dinB*, and Δ*polB* markers, respectively. The remaining strains were constructed according to Miller (1972) by P1-mediated transduction of the desired gene into the recipient AB1157 strain and its *mutD5* derivative. Bacteria were selected on LB plates containing the appropriate antibiotic: 50 μg/ml kanamycin or spectinomycin, 30 μg/ml chloramphenicol, or 12.5 μg/ml tetracycline.

Media and plates. LB (Luria-Bertani) medium contained 1% Bacto-tryptone, 0.5% yeast extract and 0.5% NaCl. The E medium consisted of C-salts (Vogel & Bonner, 1956) supplemented with 0.5% glucose, 0.2% casamino acids, 10 μg/ml thiamine, and 25 μg/ml each of arginine, histidine, threonine, proline, and leucine. E-Arg medium was E medium deprived of arginine. For starvation bacteria were incubated in C-salts supplemented with 0.5% glucose. Plates were solidified with 1.5% Difco agar.

UV treatment and MFD assay. Overnight bacterial cultures in LB were diluted 1:50 in LB-medium, grown to a density of $2–4 \times 10^8$ cells/ml at 37°C with shaking, centrifuged, and resuspended in C-salts. Samples of 5 ml were irradiated for 35 s in Petri plates (ϕ 80 mm) with a Philips 15 W UV lamp, placed 8 cm above the plate, emitting mainly 254 nm light. The UV dose was 45 J/m^2 except for the AB1157 *mfd-1uvrA6* strain which was irradiated with 2.7 J/m^2 and the UV lamp was placed 16 cm above the plate. Before and after irradiation, samples were withdrawn to estimate the number of viable cells. The irradiated bacteria were diluted 10-fold in LB medium either immediately or after further incubation (usually for 20 min) in C salts supplemented with 0.5% glucose (starvation conditions), then incubated overnight and plated on LB for viable cells and E-Arg for Arg$^+$ counts. Viable cells were counted after one day of incubation, whereas Arg$^+$ colonies required 2 days to be well visible. The frequency of UV-induced *argE3*→Arg$^+$ revertants per viable cells was then calculated.

Table1. Bacterial strains

Strain	Genotype	Origin
AB1157	*thr-1 ara-14 leuB6 Δ(gpt-proA)62 lacY1 tsx-33 supE44*$_{\text{amber}}$ *galK2 hisG4 rfbD1 mgl-51 rpsL31 kdgK51 xyl-5 mtl-1 argE3 thi-1*	Bachmann (1987)
NR9458	*mutD5 zaf-13*::Tn*10 ara thi* Δ(*prolac*)	Schaaper & Cornacchio (1992)
YG7207	Δ(*dinB-yafN*)::*kan* derivative of AB1157	Kim *et al.* (1997); McKenzie *et al.* (2003)
RW82	Δ*umuDC595::cat* derivative of AB1157 but *thyA325*	Woodgate (1992)
NR11501	Δ*polB::spc::str ara thi* Δ(*prolac*) derivative of KA796	Escarceller *et al.* (1994)
EC2413	AB1157 but Δ*umuDC595::cat*	Grzesiuk & Janion (1994)
EC2426	AB1157*mfd-1*	Grzesiuk E. (Fabisiewicz & Janion, 1998)
EC2424	AB1157 (*mfd-1, uvrA6*::Tn*10)*	Wójcik A. (Fabisiewicz & Janion, 1998)
AN20	AB1157 but *mutD5 zaf13*::Tn*10*	Nowosielska *et al.* (2004a)
AN21	AB1157 but *mutD5 zaf13*::Tn*10* Δ*umuDC595::cat*	Nowosielska *et al.* (2004a)
AN22	AB1157 but *mutD5 zaf13*::Tn*10* Δ*dinB::kan*	Nowosielska *et al.* (2004a)
AN23	AB1157 but *mutD5 zaf13*::Tn*10* Δ*umuDC595::cat* Δ*dinB::kan*	Nowosielska *et al.* (2004a)
AN24	AB1157 but *mutD5 zaf13*::Tn*10*. Δ*polB::spc::str*)	Nowosielska *et al.* (2004a)
AN26	AB1157 but *mutD5 zaf13*::Tn*10* Δ*umuDC595::cat* Δ*dinB::kan* Δ*polB::spc::str*	Nowosielska *et al.* (2004a)
WN1	AB1157 but Δ*dinB::kan*	this work
WN2	AB1157 but Δ*polB::spc::str*	this work
WN3	AB1157 but Δ*umuDC595::cat* Δ*dinB::kan*	this work
WN4	AB1157 but Δ*umuDC595::cat* Δ*dinB::kan* Δ*polB::spc::str*	this work
WN5	AB1157 but Δ*dinB::kan* Δ*polB::spc::str*	this work
WN6	AB1157 but *mutD5 zaf13*::Tn*10* Δ*dinB::kan* Δ*polB::spc::str*	this work
WN7	AB1157 but *mutD5 zaf13*::Tn*10* Δ*umuDC595::cat* Δ*polB::spc::str*	this work
WN8	AB1157 but Δ*umuDC595::cat* Δ*dinB::kan* Δ*polB::spc::str* pYG768	this work
WN9	AB1157 but Δ*umuDC595::cat* Δ*dinB::kan* Δ*polB::spc::str* pGW2123	this work

Experiments on separate cultures were repeated 4–6 times, each in duplicate, and the standard deviations (S.D.) were estimated. All plates were incubated at 37°C.

RESULTS

Survival of *E. coli* AB1157 derivatives after UV irradiation

The sensitivity of strains used in this study, described as the percentage of survival, to UV_{254} irradiation is shown in Table 2. AB1157 strains bearing a single mutation in genes encoding DNA polymerases Pol III, Pol II or Pol IV showed a sensitivity to UV irradiation similar to that of AB1157 wt. However, deletion of the *umuDC* operon encoding Pol V led to a noticeable increase in the sensitivity to UV irradiation in all the strains tested, including those lacking Pol II (*polB* deletion), Pol IV (*dinB* deletion), and mutated in the ε subunit of Pol III (*mutD5*). For four strains, AB1157 wt and three of its derivatives, Δ*umuDC*, *mutD5*, and *mutD5*Δ*umuDC*, sensitivity to various doses of UV_{254} was estimated (Fig. 1A). The introduction of the *mutD5* mutation led to a decreased sensitivity to UV irradiation, whereas, as already men-

Table 2. Survival and frequency of Arg^+ revertants in *E. coli* AB1157 derivatives after UV irradiation (2.7 J/m^2 for the *mfd-1 uvrA6* mutant and 45 J/m^2 for other strains)

Strain	Survival (%) after UV irradiation	Spontaneous Arg^+ revertants/10^8 cells	UV-induced Arg^+ revertants/10^8 cells	UV-induced Arg^+ revertants/10^8 cells in cultures starved for 20 min
wt	26.3 ± 9.5	10.9 ± 6.6	2242.3 ± 325.6	259.3 ± 73.7
mfd-1	13.9 ± 6.1	5.1 ± 0.5	1283.3 ± 72.7	643.0 ± 162.2
mfd-1 uvrA6	21.2 ± 3.2	13.6 ± 2.5	340.0 ± 56.4	315.7 ± 75.7
Δ*umuDC*	11.4 ± 5	4.1 ± 1.6	1.1 ± 0.5	1.9 ± 1.0
Δ*polB*	23.1 ± 16.3	5.4 ± 1	2440.0 ± 62.6	347.7 ± 19.1
Δ*dinB*	36.7 ± 3.7	52.9 ± 30.5	1815.0 ± 144.0	431.7 ± 203.8
Δ*dinB* Δ*polB*	46.3 ± 5.9	6.0 ± 2.3	3750.0 ± 284.0	331.5 ± 293.4
Δ*umuDC* Δ*dinB*	3.2 ± 0.7	1.8 ± 0.2	1.3 ± 0.2	0.9 ± 0.2
Δ*umuDC* Δ*dinB* Δ*polB*	2.8 ± 1.6	4.4 ± 1.5	10.7 ± 3.2	1.6 ± 0.8
Δ*umuDC* Δ*dinB* Δ*polB* pYG768	7.8 ± 5.1	2.2 ± 0.8	11.9 ± 1.9	2.0 ± 0.1
Δ*umuDC* pGW2123	54.7±7.3	28.7±2.3	2810.0 ± 358.4	618.3 ± 105.9
Δ*umuDC* Δ*dinB* Δ*polB* pGW2123	24.7 ± 10.3	20.5 ± 4.6	3069.3 ± 711.1	285.3 ± 45.1
mutD5	58.2 ± 9.1	357.6 ± 42.1	2732.2 ± 123.4	944.7 ± 131.5
mutD5 Δ*umuDC*	7.1 ± 5.1	115.2 ± 36.9	351.3 ± 114.4	210.7 ± 34.1
mutD5 Δ*dinB*	14.0 ± 5.8	70.3 ± 19.7	1538.0 ± 182.1	405.0 ± 140.8
mutD5 Δ*polB*	20.2 ± 2.3	146.0 ± 13.9	2720.0 ± 161.7	497.0 ± 63.2
mutD5 Δ*umuDC* Δ*dinB*	6.9 ± 4.1	219.8 ± 47	223.0 ± 39.1	184.3 ± 26.2
mutD5 Δ*umuDC* Δ*polB*	4.3 ± 0.3	72.9 ± 26.1	401.3 ± 97.1	121.8 ± 34.2
mutD5 Δ*dinB* Δ*polB*	41.7 ± 1.7	73.6 ± 14.0	1985.5 ± 129.0	64.7±7.2
mutD5 Δ*umuDC* Δ*dinB* Δ*polB*	31.3 ± 2.1	92.8 ± 20.2	194.0 ± 43.3	147.5 ± 27.3

tioned, additional deletion of the *umuDC* operon sensitised the cells to UV light.

Single mutation in the *mfd* gene did not influence the sensitivity of the AB1157 strain to UV irradiation, however, the double mutant *mfd-1uvrA6* was extremely sensitive to UV light and therefore in further experiments had to be irradiated with an about 17-fold lower dose (2.7 J/m^2) than the other strains.

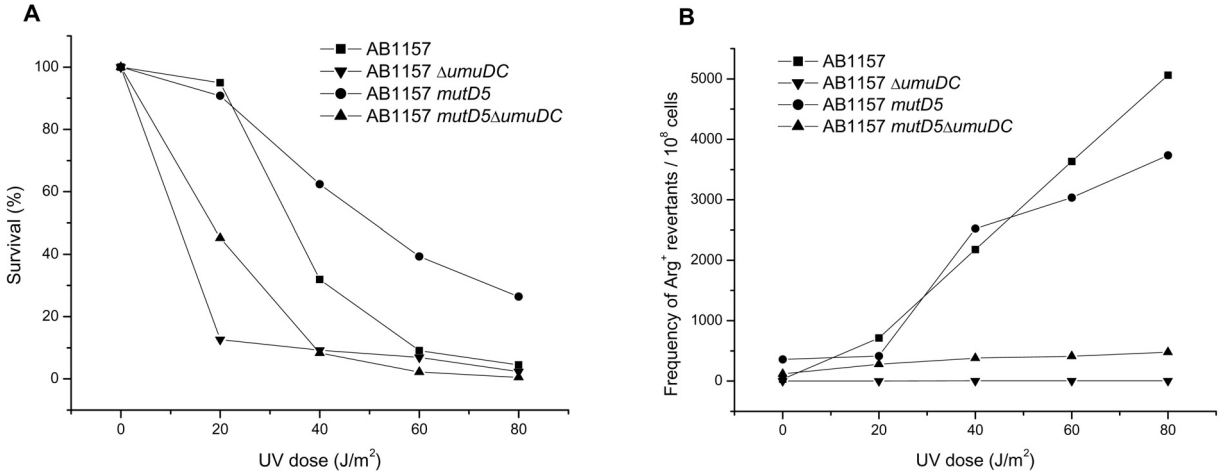

Figure 1. (A) survival and (B) *argE3*→Arg$^+$ reversion frequency in UV-irradiated AB1157 strains.

The data points shown are the averages of 4–6 independent experiments, each performed in duplicate. S.D. value was 1–10%.

UV-induced *argE3*→Arg$^+$ reversion frequency in AB1157 *mutD5* strains deficient in Pol II, Pol IV or Pol V polymerases

The studies were performed on AB1157 strains bearing an ochre mutation in the *argE3* locus. Only *argE3*→Arg$^+$ reversion allows growth of these bacteria on a minimal medium without arginine. Table 2 shows frequencies of UV-induced Arg$^+$ reversion in the AB1157 strain and its *mutD5* derivative mutated in the ε subunit of Pol III. It also includes the frequencies of Arg$^+$ reversion in strains deleted in the *polB*, *dinB*, and *umuDC* genes encoding Pol II, Pol IV and Pol V, respectively. The presence of the *mutD5* allele led to an increased level of spontaneous Arg$^+$ revertants but a level of UV-induced Arg$^+$ revertants in *mutD5* strain was comparable to that in the *mutD*$^+$ background. However, among all strains tested, even in the *mutD5*, only those with the *umuDC* deletion showed a low level of UV-induced mutations. Thus, the results in Table 2 indicate that the only polymerase essential for UV mutagenesis is Pol V. This conclusion is confirmed by application of different doses of UV to four strains, AB1157, Δ*umuDC*, *mutD5*, and *mutD5*Δ*umuDC*, and estimation of the frequency of Arg$^+$ revertants (Fig. 1B). In both *umuDC*$^+$ strains a dose dependent increase in UV induced Arg$^+$ revertants was observed. In strains lacking the *umuDC* genes, the level of Arg$^+$ revertants remained low (in *mutD5*Δ*umuDC*) or extremely low (in Δ*umuDC*), independently of the UV dose.

Additional proof for the dependency of UV mutagenesis on Pol V was provided by results obtained in the AB1157Δ*umuDC* strain harbouring the pGW2123 plasmid overproducing UmuD′C proteins. In this strain the presence of Pol V of plasmid origin led to a significant increase in the frequency of UV-induced Arg$^+$ revertants equalling or even outnumbering the level of such revertants in the AB1157*umuDC*$^+$ strain (Table 2, column 4, rows 2 and 12) which was not observed in a strain bearing the pYG768 plasmid overproducing Pol IV polymerase (Table 2, column 4, row 11).

Mutation frequency decline (MFD) in strains mutated in the ε subunit of Pol III and deficient or proficient in polymerases Pol II, Pol IV or Pol V

In order to evaluate the role of Pol II, Pol IV, Pol V, and the proofreading ε subunit of Pol III in the MFD type of DNA repair, the level of UV-induced Arg^+ revertants was estimated in transiently starved *polB*, *dinB*, *umuDC*, and *mutD5* mutants. After UV-irradiation at a dose of 45 J/m^2, AB1157 cells were incubated for 20 min in C-salts supplemented with glucose but not with the amino acids required for growth (starvation conditions). To express the mutations, further incubation was in the LB medium. The frequencies of spontaneous and UV-induced Arg^+ revertants in the listed AB1157 derivatives are shown in Table 2, columns 3 and 4. Column 5 contains frequencies of UV-induced Arg^+ revertants in samples starved prior to further incubation in the complete medium. High rates of MFD (7- to 30-fold decline in the frequency of UV-induced Arg^+ revertants after transient starvation) were observed for the wt strain and four of its derivatives, *mutD5*Δ*dinB*Δ*polB*, Δ*dinB*Δ*polB*, Δ*polB*, and Δ*umuDC*Δ*dinB*Δ*polB* bearing the pGW2123 plasmid overproducing UmuD′C proteins (Pol V). In contrast, all the strains lacking Pol V (Δ*umuDC*) did not show any decline in the frequency of UV-induced Arg^+ revertants after transient starvation. These two sets of data may suggest an at least indirect involvement of Pol V in MFD repair. The most spectacular result, a 30-fold decline in the frequency of UV-induced Arg^+ revertants after 20 min of starvation in the *mutD5*Δ*dinB*Δ*polB* strain may indicate better access of Pol V to DNA lesions in the absence of Pol IV and Pol II.

For the wt strain and its five derivatives, Δ*umuDC*, *mutD5*, *mutD5*Δ*polB*, *mutD5*Δ*dinB*, and *mutD5*Δ*umuDC*, Arg^+ reversion frequencies were determined at different times of starvation to estimate the kinetics of MFD repair (Fig. 2). The data indicate that a decline in mutation frequency takes place in the wt and

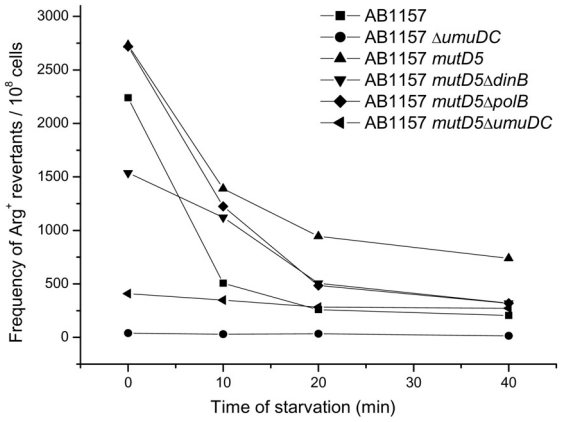

Figure 2. Effect of defect in the ε subunit of Pol III (the *mutD5* mutation) and the *umuDC* deletion on kinetics of MFD in UV-irradiated and starved for indicated time AB1157 strains.

The data points shown are the averages of 4–6 independent experiments, each performed in duplicate. S.D. value was 1–10%.

*mutD5*Δ*polB* strains, is less visible in the *mutD5* and *mutD5*Δ*dinB* ones, and absent in the strain with deleted *umuDC* genes. We suggest that in the *mutD5* background, MFD repair is less visible, being masked by mutations resulting from damage in the proofreading function of Pol III. These mutations are probably not subject to MFD.

DISCUSSION

In this work we investigated UV-induced mutagenesis in *E. coli* strains deficient in the 3′→5′ exonucleolytic proofreading activity ensured by the ε subunit of the replicative polymerase Pol III, and defective in the SOS-induced DNA polymerases Pol II, Pol IV, and Pol V. We also examined the contribution of these polymerases to MFD repair, connected with transcription. The test system used was chromosomal *argE3*→Arg^+ reversion to prototrophy in *E. coli* AB1157 strains. Only a back mutation at the *argE3* locus or tRNA suppressor formation leading to Arg^+ phenotype allows for growth of these cells on minimal medium deprived of arginine (Śledziewska-Gójska *et al.*, 1992).

There is strong evidence that TLS past UV-induced CPD requires *E. coli* UmuD′$_2$C proteins (Friedberg *et al.*, 1995; Gonzalez & Woodgate, 2001; Goodman & Woodgate, 2000; Sutton & Walker, 2001; Sutton *et al.*, 2001), known as SOS-inducible mutagenic DNA polymerase Pol V (Reuven *et al.*, 1999; Tang *et al.*, 1999). Our observations confirmed that almost all the UV-induced Arg$^+$ revertants were Pol V dependent. The tested strains, regardless of whether Pol II and Pol IV were present or absent, did not show UV mutagenesis in the absence of Pol V. In the strains bearing the high copy number plasmid pGW2123, producing numerous copies of the UmuD′$_2$C proteins, the level of UV-induced Arg$^+$ revertants was slightly higher compared to the plasmid-free strain containing Pol V of chromosomal origin independently of the presence of Pol II or Pol IV. Introduction of the pYG768 plasmid producing Pol IV into the AB1157Δ*umuDC*Δ*dinB*Δ*polB* strain preserved UV mutagenesis at the same extremely low level as in the strain without the plasmid.

Pol III, the main replicative polymerase, demonstrates the high accuracy of replication and usually involves replication of undamaged templates. However, it has been found that in the absence of the 3′→5′ exonucleolytic proofreading activity encoded by the ε subunit, Pol III can bypass CPD similarly to Pol V (Borden *et al.*, 2002). The observed almost 8-fold lower frequency of Arg$^+$ revertants in the AB1157*mutD5*Δ*umuDC* strain than in AB1157*mutD5* seems to confirm this finding, suggesting more precise TSL by Pol III mutated in its proofreading function compared to a properly functioning Pol V. Another explanation is that in the absence of Pol V, less TLS occurs and so lesions are repaired prior to replication.

Transcription and DNA repair are coupled in *E. coli* by the Mfd protein which dissociates transcription elongation complexes blocked at non-pairing lesions such as CPD. Decline in mutation frequency, i.e., the MFD phenomenon, can be observed in UV-irradiated and transiently starved *E. coli* cells (Witkin, 1994). In our experiments, an 8.6-fold decline in the frequency of UV-induced Arg$^+$ revertants was observed in AB1157 wt strain, 2-fold in AB1157*mfd1* strain, and none in AB1157*mfd1uvrA6* strain mutated in both MFD and nucleotide excision repair. The occurrence of MFD in the AB1157Δ*umuDC* strain was impossible to estimate, since the level of UV-induced Arg$^+$ revertants was extremely low. However, in the AB1157Δ*umuDC* strain harbouring pGW2123, a plasmid encoding UmuD′C proteins, a high level of UV-induced Arg$^+$ revertants but only a 4.5-fold decline in their frequency after starvation were seen. Surprisingly, in AB1157Δ*umuDC*Δ*dinB*Δ*polB* bearing pGW2123 not only a high level of UV-induced Arg$^+$ revertants but also an intense MFD (11-fold decline in mutation frequency) were observed. The intensity of MFD in this strain can be explained by creation of more mismatches during TLS that serve as substrates for MFD-related repair. A better access of an elevated number of Pol V molecules to UV-induced DNA lesions in the absence of Pol II and Pol IV may also be taken into consideration.

Among all the strains tested the highest rate (over 30-fold) of decline of Arg$^+$ revertants in UV-irradiated and starved cultures was observed in the case of the *mutD5*Δ*dinB*Δ*polB* strain. Interestingly, the strain deleted for *dinB* and *polB* but with a properly functioning proofreading activity of Pol III has shown an only 11-fold decline of such revertants. Thus, in *E. coli* cells lacking Pol IV and Pol II, and mutated in the ε subunit of Pol III, Pol V seems to be a very efficient support of MFD-related repair.

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