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UV- and MMS-induced mutagenesis of λ*O*(am)8 phage under nonpermissive conditions for phage DNA replication✪

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Mutagenesis in *Escherichia coli*, a subject of many years of study is considered to be related to DNA replication. DNA lesions nonrepaired by the error-free nucleotide excision repair (NER), base excision repair (BER) and recombination repair (RR), stop replication at the fork. Reinitiation needs translesion synthesis (TLS) by DNA polymerase V (UmuC), which in the presence of accessory proteins, UmuD', RecA and ssDNA-binding protein (SSB), has an ability to bypass the lesion with high mutagenicity. This enables reinitiation and extension of DNA replication by DNA polymerase III (Pol III). We studied UV- and MMS-induced mutagenesis of λ*O*(am)8 phage in *E. coli* 594 *sup*$^+$ host, unable to replicate the phage DNA, as a possible model for mutagenesis induced in nondividing cells (e.g. somatic cells). We show that in *E. coli* 594 *sup*$^+$ cells UV- and MMS-induced mutagenesis of λ*O*(am)8 phage may occur. This mutagenic process requires both the UmuD' and C proteins, albeit a high level of UmuD' and low level of UmuC seem to be necessary and sufficient. We compared UV-induced mutagenesis of λ*O*(am)8 in nonpermissive (594 *sup*$^+$) and permissive (C600 *supE*) conditions for phage DNA replication. It appeared that while the mutagenesis of λ*O*(am)8 in 594 *sup*$^+$ requires the UmuD' and C proteins, which can not be replaced by other SOS-inducible protein(s), in C600 *supE* their functions may be replaced by other inducible protein(s), possibly DNA polymerase IV (DinB). Mutations induced under nonpermissive conditions for phage DNA replication are resistant to mismatch repair (MMR), while among those induced under permissive conditions, only about 40% are resistant.

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Abbreviations: AP-site, apurinic/apyrimidinic site; BER, base excision repair; MMR, mismatch repair; MMS, methyl-methane sulfonate; m.o.i., multiplicity of infection; NER, nucleotide excision repair; RR, recombination repair; SSB, ssDNA-binding protein; TA*, thymidylyl (3'-5')-deoxyadenosine; Tg, thymine glycol; TLS, translesion synthesis.

The mechanisms of mutagenesis induced by UV and ionizing radiation, as well as by chemical mutagens, have long been a subject of great interest. It is now well established that mutations constitute the first step in carcinogenesis, and are the cause of many genetic diseases in man. The most widely studied model for UV-induced and chemical mutagenesis is *Escherichia coli*. Many mutagens, including UV light, induce mutations in *E. coli* by the SOS pathway (SOS-mutagenesis) (for reviews see: Friedberg *et al.*, 1995; Koch & Woodgate, 1998; Sutton *et al.*, 2000; 2002; Shina & Hader, 2002; Taylor, 2002). According to the current hypothesis on the mechanism of UV-induced mutagenesis, and mutagenesis by some chemicals, mutations are generated during replication of damaged DNA with an involvement of DNA polymerase V (Pol V), which is an error-prone translesion synthesis (TLS) DNA polymerase (for reviews see: Sutton *et al.*, 2000; 2002; Goodman & Tippin, 2000; Livneh, 2001; Kobayashi *et al.*, 2002). Pol V encoded by the *umuC* gene is an extremely weak DNA polymerase without a proofreading activity and with low processivity (about 6 bp), is activated for lesion bypass by the UmuD', RecA and SS DNA-binding protein (SSB) proteins, and stimulated by the processivity subunits of DNA polymerase III, the β subunit sliding clamp and the γ complex clamp loader (Tang *et al.*, 1999; Sutton *et al.*, 2001; Becherel *et al.*, 2002). Inactivation of TLS by *umuC* mutation strongly decreases mutagenesis implying that most mutations are caused by Pol V-depended TLS. Activated Pol V replicates effectively apurinic/apyrimidinic sites (AP-sites), T^T photodimers and 6–4 photoproducts (Rajagopalan *et al.*, 1992; Tang *et al.*, 2000). The proposed model for TLS by Pol V is as follows. As the replication fork is stopped by an unrepaired lesion in DNA, single-stranded DNA (ssDNA) is bound by the SSB protein, RecA displaces it and forms a nucleoprotein filament, covering the ssDNA region including the primer termini near the lesion (Reuven *et al.*, 2001). Pol V binds to the primer–template complex guided by the RecA filament. In loading of Pol V, UmuD' interacts with the RecA filament and with UmuC, and then UmuC interacts with the primer. Pol V holoenzyme starts DNA synthesis and is able to replicate through the lesion. The RecA filament disassembles in a reaction requiring ATP hydrolysis which leads to dissociation of Pol V, so that Pol III can take over and continue replication. It is believed that there is a coordinated action of Pol III with V as well as with other translesion bypass DNA polymerases, e.g. Pol IV (DinB) (Kobayashi *et al.*, 2002). Thus, according to the hypothesis, the mutagenic process is related to the replication of damaged DNA. Such a mechanism of mutagenesis is possible only in actively metabolising cells, but not in resting or nondividing ones, e.g. somatic cells. However, it is well known that mutations may be induced by environmental mutagens, even in nondividing somatic cells, leading to cancer, implying that such mutagenesis would follow a different pathway, most probably one, independent of replication of damaged DNA. So, we asked the question whether mutations may be induced under nonpermissive conditions for DNA replication, and if so, by what mechanism. We have previously observed that UV-induced mutations in *E. coli* MV1178 *recA recF* may be formed prior to the resumption of DNA replication in UV-irradiated cells (Pietrzykowska & Felczak, 1991). This suggested that mutations may be generated in a manner independent of replication of damaged DNA. To verify this hypothesis we studied UV-induced mutagenesis of λ*O*(am)8 phage in a host nonpermissive for phage DNA synthesis, *E. coli* 594 *sup*$^+$. Phage λ*O*(am)8 with an *amber* mutation in gene *O*, is unable to replicate its DNA in a host which does not possess a suppressor of the *amber* mutation (*supE*). The product of the *O* gene – λO protein, is known to be responsible for initiation of phage DNA replication (Zylicz *et al.*, 1984; 1988) and λ*O*(am)8 phage is unable to repli-

Table 1. *Escherichia coli* K-12 strains, bacteriophages and plasmids

Strain	Genotype	Source or reference
	***Escherichia coli* K-12 strains**	
	donors of the mutations	
GC2364	*thr leu pro his arg lac gal* ***uvrA6 mal*::Tn*9***	laboratory collection
RP821	*Δ(lac proB) nalA* $argE_{am}$ *rif thi met*B ***dnaQ49zae*::Tn*10***	Piechocki, 1986
RW82	***ΔumuDC595*::*cat*** *uvrA6*	Woodgate, 1992
NR9559	Δ(*lac proB*)*thi* ***mutL*::Tn*5***	Fijalkowska, 1995
	nonpermissive strains (*sup*$^+$)	
594	*sup*$^+$ *lac gal rpsL*	Campbell, 1961
594 *uvrA*	as 594 but *uvrA6 mal*::Tn*9*	This work
594 *dnaQ*	as 594 but *dnaQ49zae-502*::Tn*10*	This work
594 *uvrA dnaQ*	as 594 *uvrA* but *dnaQ49zae-502*::Tn*10*	This work
594 *mutL*	as 594 but *mutL*::Tn*5*	This work
594 *ΔumuDC*	as 594 but *ΔumuDC595*::*cat*	This work
594 / pSE117	as 594 but pSE117 (UmuDC)	This work
594 / pGW2122	as 594 but pGW2122 (UmuD')	This work
594 / pGW2123	as 594 but pGW2123 (UmuD'C)	This work
594 *ΔumuDC* / pGW2122	as 594 *ΔumuDC* but pGW2122 (UmuD')	This work
594 *ΔumuDC* / pGW2123	as 594 *ΔumuDC* but pGW2123 (UmuD'C)	This work
594 *ΔumuDC* / pSE117	as 594 *ΔumuDC* but pSE117 (UmuDC)	This work
	permissive strains (*supE*)	
C600	*supE44*$_{amber}$ *hsdR*? *thi-1 thr-1 leuB6 lacY1 tonA21*	Appleyard, 1953
C600 *uvrA*	as C600 but *uvrA6 mal*::Tn*9*	This work
C600 *dnaQ*	as C600 but *dnaQ49*::Tn*10*	This work
C600 *uvrA dnaQ*	as C600 *uvrA* but *dnaQ49*::Tn*10*	This work
C600 *mutL*	as C600 but *mutL*::Tn*5*	This work
C600 *ΔumuDC*	as C600 but *ΔumuDC595*::*cat*	This work
C600/ pSE117	as C600 but pSE117 (UmuDC)	This work
C600 / pGW2122	as C600 but pGW2122 (UmuD')	This work
C600 / pGW2123	as C600 but pGW2123 (UmuD'C)	This work
C600 *ΔumuDC* / pGW2122	as C600 *ΔumuDC* but pGW2122 (UmuD')	This work
C600 *ΔumuDC* / pGW2123	as C600 *ΔumuDC* but pGW2123 (UmuD'C)	This work
C600 *ΔumuDC* / pSE117	as C600 *ΔumuDC* but pSE117 (UmuDC)	This work
	Bacteriophages	
P1	(vir)	laboratory collection
λ*O*(am)8	λ with an *amber* mutation in *O* gene	Pietrzykowska, 1973
	Plasmids	
pBR322	medium-copy number plasmid (Ap^R, Tc^R)	laboratory collection
pSE117	as pBR322 but overproducing UmuDC (Ap^R, Kn^R)	Nohmi, 1988
pGW2122	high-copy number of *umuD'* (Ap^R)	GC Walker collection
pGW2123	high-copy number of *umuD'C* (Ap^R)	GC Walker collection

cate its DNA in the suppressor-less host *E. coli* 594 *sup*$^+$ (Ogawa & Tomizawa, 1968; Shuster & Weissbach, 1969).

We now present results indicating that UV- and MMS-induced mutations may arise under nonpermissive conditions for DNA replication.

MATERIALS AND METHODS

Media and growth conditions. Luria-Bertani (LB) medium (containing, per litre, 10 g tryptone, 5 g of yeast extract, and 5 g NaCl) (Miller, 1972) was used for growth of *E. coli* cultures and, when necessary, was supplemented with the appropriate antibiotic at the following final concentrations: chloramphenicol, 35 μg/ml; ampicillin, 100 μg/ml; tetracycline, 50 μg/ml; kanamycin, 50 μg/ml. Solid medium contained 1.2% Bacto-Agar, and all incubations were at 37°C. Liquid cultures were carried out overnight in LB with aeration, diluted 1:50 in fresh LB medium, and incubated to a density of 2–3 × 10^8 cells per ml. Large scale preparation of bacterio-

phage λ*O*(am)8 was performed as described by Sambrook *et al.* (1989).

Bacterial strains and plasmids. The *E. coli* strains and plasmids used in this study are listed in Table 1. *E. coli* 594 *sup*$^+$ and C600 *supE* were used as host strains for λ*O*(am)8 phage and for construction of host mutants used in this work. Bacterial strains were constructed using standard methods of P1 transduction (Miller, 1972) and plasmid transformation (Sambrook *et al.*, 1989).

SOS induction. Bacteria grown to a density of 2×10^8 cells per ml were centrifuged and resuspended in half the original volume of 10 mM $MgSO_4$, and divided into two parts. One part was UV-irradiated (254 nm) with a dose of 70 J/m^2 for *E. coli* 594 *sup*$^+$ and 40 J/m^2 for C600 *supE* and their derivatives. The second nonirradiated part was a control. The control and irradiated samples were diluted with an equal volume of 2 × concentrated LB. The control was left on ice and the irradiated cells were incubated for SOS-induction at 37°C with shaking for 20 min.

Survival of the bacteria after irradiation with UV (254 nm) was 20–30%.

Treatment of* λ*O*(am)8 *phage with UV or MMS. For both UV- and MMS-induced mutagenesis, λ*O*(am)8 phage was diluted in 100 mM Tris/HCl (pH 7.0) supplemented with 10 mM $MgSO_4$ to a final concentration of $1–2 \times 10^9$ phages per ml. Then, a sample of the diluted phage was treated with a mutagen, the rest was left as a control. For UV mutagenesis the phage was irradiated on a Petri dish with UV light (254 nm) with a dose of 150 J/m^2 or 25 J/m^2 (for details see Results). For MMS-induced mutagenesis the phage was treated with methyl-methane sulfonate (MMS) at final concentration of 0.33% for 45 or 75 min (for details see Results) at 37°C, and to stop the reaction an equal volume of cold 40% sodium thiosulfate was added. This mixture was incubated at 37°C for 10 min and then cooled on ice (Ebisuzaki *et al.*, 1975). As a control phages were treated for 75 min at 37°C with MMS previously inactivated with sodium thiosulfate. Survival of phages treated with MMS or UV was estimated in C600 *supE* cells or their derivatives and was between 2–6%. Phages prepared in this way were used for examination of mutagenesis.

UV and MMS mutagenesis of* λ*O*(am)8 *phage. The number of mutated phages was estimated as the number of ineffective centres (IC). λ*O*(am)8 phage treated with a mutagen (UV or MMS) was adsorbed to 594 *sup*$^+$ host cells preirradiated with UV light (+SOS) or not (–SOS) with multiplicity of infection (m.o.i.) about 0.2. After 15 min of adsorption at 37°C, additional drop of 594 *sup*$^+$ indicator strain and 3 ml of warm soft agar were added, plated and incubated overnight at 37°C. Appropriate dilutions of phage were plated on C600 *supE* strain to estimate the number of surviving phages. In the case of mutagenesis in the C600 *supE* strain, infected cells with mutagen treated phages after adsorption and plating were incubated at 37°C for 3 h for expression of mutations, then chloroform was added on a paper disc in the cover of the Petri dish (for 20 min) to kill C600 *supE* cells. After removal of the paper discs and aeration, a second layer of soft agar with 594 *sup*$^+$ indicator cells for λO$^+$ revertants was added and incubated overnight at 37°C. Mutation frequency was calculated as the number of revertants per 10^7 surviving phages. All manipulations with UV-irradiated phages and bacteria were done under yellow light, and overnight incubation of plates was performed in the dark to avoid photoreactivation.

RESULTS

UV-induced reversion of λ*O*(am)8 to λO$^+$ in *E. coli* 594 *sup*$^+$ host

To test the hypothesis that mutations may occur before resumption of DNA replication, we studied reversion of λ*O*(am)8 to λO$^+$ in

E. coli 594 *sup*$^+$ host cells unable to support growth of the λ*O*(am)8 mutant due to inability to replicate its DNA. In this host, UV-induced reversion of λ*O*(am)8 to λO$^+$ could be observed only if the mutation occurred before initiation of DNA replication and enable the phage to form plaques on 594 *sup*$^+$ host. The results presented in Fig.1 show that λ*O*(am)8 phage efficiently reverts to λO$^+$ in *E. coli* 594 *sup*$^+$ cells nonpermissive for phage DNA replication. The frequency of reversion is dependent on the UV dose to the phage. Preirradiation of the host cells before infection with UV-irradiated phage increases the mutation frequency about 10-fold, indicating that some SOS function(s), induced in the host, stimulate the mutagenic process. It is worth to note that a significant increase in the number of λ mutants is seen also when only the phage was irradiated.

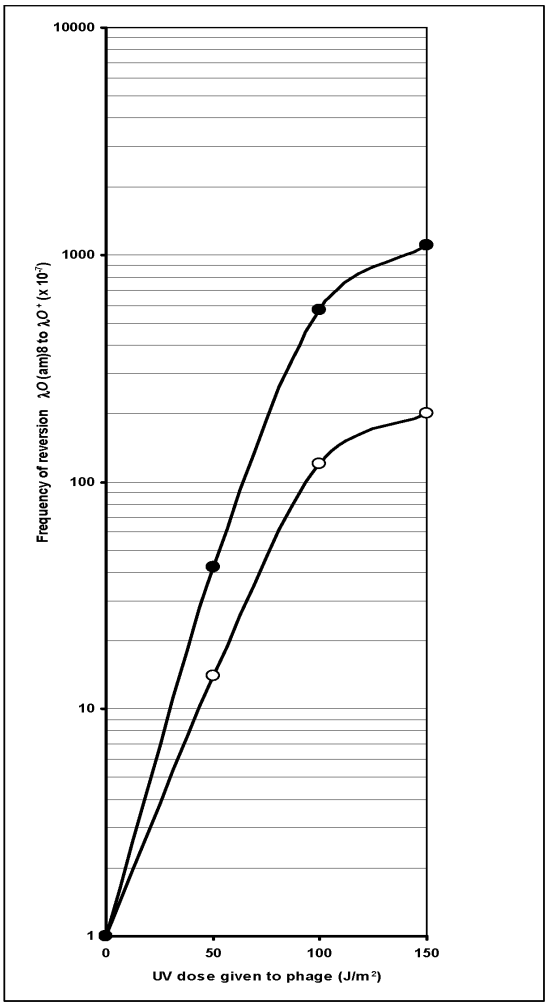

Figure 1. UV-induced reversion of λ*O*(am)8 phage to λO$^+$ in *E. coli* 594 *sup*$^+$ nonpermissive for DNA replication.

(○) irradiated phage in non irradiated bacteria (–SOS); (●) irradiated phage in irradiated bacteria (+SOS).

UV-induced mutagenesis of λ*O*(am)8 phage in *E. coli* 594 *sup*$^+$ cells is not dependent on nucleotide excision repair (NER)

There were two reasons to study the effect of the *uvrA* mutation on UV-induced mutagenesis of λ*O*(am)8 phage in the host nonpermissive for phage DNA replication: (1) to check whether DNA replication of the irradiated phage in the nonpermissive host may be initiated by UvrABC nuclease incision; (2) whether nucleotide excision repair (NER) is involved in the generation of mutations under nonpermissive conditions for phage DNA replication. Cohen-Fix & Livneh (1992) described UV mutagenesis in ColE1 plasmid independent of DNA replication, but dependent on the UvrABC-excinuclease. We therefore examined the effect of the *uvrA* mutation on the UV-induced mutagenesis of λ*O*(am)8 phage in *E. coli* 594 *sup*$^+$ *uvrA* cells. The results (Table 2) show that at a UV-dose giving similar phage survival (about 2%) in *uvr*$^+$ and *uvrA* hosts (after 150 and 25 J/m^2, respectively), similar levels of mutants were observed in 594 *sup*$^+$ and 594 *sup*$^+$ *uvrA* cells. This suggests that: (1) UvrABC-excinuclease is not involved in the mutagenic process occurring under nonpermissive conditions for λ*O*(am)8 phage replication, and (2) that incision by UvrABC can not initiate DNA replication of UV-irradiated λ*O*(am)8 phage in 594 *sup*$^+$ host. If the incision could initiate DNA replication in 594 *sup*$^+$ cells, the level of λO$^+$ revertants in 594 *sup*$^+$ should be similar to that observed in C600 *supE* host permissive for phage DNA replication. As may be seen in Table 5, the efficiency of UV-induced mutagenesis of λ*O*(am)8 is 3-fold lower in the

594 *sup*$^+$ then in C600 *supE*. Secondly, the deficiency in UvrABC activity in 594 *sup*$^+$ *uvrA* host does not affect the level of UV-induced mutagenesis of λ*O*(am)8 when UV-doses were used to give the same survival. At similar survival the same levels of mutants were observed in 594 *sup*$^+$ *uvrA* and 594 *sup*$^+$ hosts (Table 2). On the other hand, at a low UV dose to a high mutator effect, related to DNA replication (Horiuchi *et al.*, 1978; Piechocki *et al.*, 1986). We introduced the *dnaQ49* mutation into 594 *sup*$^+$ and C600 *supE* cells and studied its effect on spontaneous and UV-induced mutagenesis of λ*O*(am)8 phage at 37°C, a temperature nonpermissive for the *dnaQ49* mutant.

Table 2. Comparison of UV-induced mutagenesis of λ*O*(am)8 phage in *E. coli* 594 *sup*$^+$ and its *uvrA* derivative

E. coli strain	UV-dose (J/m^2)		Mutation frequency ($\times 10^{-7}$)
	phage	bacteria	
594 *sup*$^+$	0	0	5.2 ±1.23
	0	70	5.2 ±2.09
	25	0	17.8 ±5.12
	25	70	78.6 ±15.9
	150	0	123.0 ±32.1
	150	70	276.0 ±45.5
594 *sup*$^+$ *uvrA*	0	0	8.6 ±2.22
	25	0	142.0 ±41.1
	25	6	270.0 ±52.3

Mutation frequencies and (±S.D.) are average values from at least four independent experiments, each in duplicate.

(25 J/m^2) given to the phage, a 3–4 times higher level of λO$^+$ revertants is observed in 594 *sup*$^+$ *uvrA* than in 594 *sup*$^+$ *uvr*$^+$, indicating that UV lesions in the DNA of λ*O*(am)8 phage, which are processed by a mutagenic mechanism operating in the host nonpermissive for DNA replication, may be repaired by the error-free NER.

The effect of *dnaQ49* mutation on spontaneous and UV-induced reversion of λ*O*(am)8 to λO$^+$ in hosts nonpermissive and permissive for phage DNA replication

Looking for additional evidence that λ*O*(am)8 phage DNA is not replicated in the nonpermissive *E. coli* 594 *sup*$^+$ host, we examined the mutator effect of the *dnaQ49* mutation on λ*O*(am)8 phage in *E. coli* 594 *sup*$^+$ *dnaQ49* cells.

The *dnaQ* gene, coding for the ε-subunit of DNA polymerase III holoenzyme, is responsible for the proofreading activity of the DNA replicating complex. Mutation *dnaQ49* leads

Introduction of the *dnaQ*49 mutation into 594 *sup*$^+$ and C600 *supE* cells increased 100–200-fold spontaneous and UV-induced mutations *Rif*^{S} to *Rif*^{R} in the bacterial cells, but had no mutator effect on UV-induced or spontaneous mutagenesis of λ*O*(am)8 phage in the nonpermissive host, 594 *sup*$^+$ *dnaQ49* (Table 3). By contrast, the *dnaQ49* mutation in the permissive, C600 *supE dnaQ49* cells, leads to a high mutator effect in λ*O*(am)8 phage, increasing the mutation frequency by about two orders of magnitude. The results suggest that in the nonpermissive host, *E. coli* 594 *sup*$^+$, replication of λ*O*(am)8 DNA does not occur. This is in accordance with the data reported by Ogawa & Tomizawa (1968), Shuster & Weissbach (1969), Wyatt & Inokuchi (1974) as well as with *in vitro* studies on the role of λO protein in the initiation of phage DNA replication (Zylicz *et al.*, 1984; 1988; Dodson *et al.*, 1989). This supports the hypothesis that UV-induced mutagenesis of λ*O*(am)8 in the *E. coli* 594 *sup*$^+$ host is not related to replication of damaged DNA, and sug-

gests the existence in *E. coli* cells of more than one mechanism by which UV-induced mutations may be generated; one related to stimulated UV-induced reversion of λ*O*(am)8 to λO$^+$ in 594 *sup*$^+$ cells (Fig. 1), pointing to the role of inducible functions in mutagenesis

Table 3. Influence of *dnaQ49* mutation on UV-induced mutagenesis of λ*O*(am)8 in the 594 *sup*$^+$ host and its derivatives

UV-dose (J/m^2)		Mutation frequency (× 10^{-7})	
phage	bacteria	*dnaQ*$^+$	*dnaQ49*
		594 *sup*$^+$	
0	0	16.8 ±8.4	22.3 ±16
0	70	17.2 ±8.1	21.9 ±13
150	0	99.8 ±61.5	144.7 ±90
150	70	399.3 ±148	437.0 ±53
		594 *sup*$^+$ *uvrA*	
0	0	12.77 ±1.25	18.3 ±1.26
0	6	13.63 ±1.43	17.6 ±2.03
22	0	162.01 ±119	182.0 ±33.2
22	6	304.02 ±128	364.4 ±99.5

Mutation frequencies and (±S.D.) are average values from at least five independent experiments, each in duplicate.

error-prone replication of damaged DNA, and another independent of DNA replication.

Effect of UmuD' overproduction on UV-induced reversion of λ*O*(am)8 to λO$^+$ in 594 *sup*$^+$ cells

According to the current hypothesis on the mechanism of UV-induced mutagenesis, and mutagenesis by some chemicals, mutations are generated during replication of damaged DNA with an involvement of TLS by Pol V. DNA Pol V, the product of *umuC* gene, is activated by the UmuD', RecA and SSB proteins for TLS (see introduction). The requirement for the UmuD/D' and C proteins in mutagenesis induced by various mutagens has been known for years, but only lately have their functions in TLS elucidated (Tang *et al.*, 1998; 1999; 2000; Ruven *et al.* 1999; Maor-Shoshani *et al.*, 2000; Sutton *et al.*, 2000). To learn more about the possible mechanism of mutagenesis induced under nonpermissive conditions for phage DNA replication, we studied the role of the UmuD/D' and C proteins in this mutagenic process. As mentioned above, induction of SOS functions occurring under nonpermissive conditions for DNA replication. We examined the role of UmuD/D' and C proteins in this mutagenic pathway by studying the effect of overproduction of these proteins on the efficiency of UV-induced reversion of λ*O*(am)8 phage to λO$^+$ in the nonpermissive host cells, *E. coli* 594 *sup*$^+$ and the permissive C600 *supE*. The results (Table 4) show that overproduction of UmuD/D' and C proteins significantly increases the frequency of UV-induced reversion of phage λ*O*(am)8 to λO$^+$ in non-induced for SOS 594 *sup*$^+$ host (–SOS). Overproduction of UmuDC from pSE117, UmuD'C from pGW2123 and UmuD' from pGW2122 increased the frequency of mutations 7-, 24- and 12-fold, respectively. On the other hand, the enhancing effect of the overproduction of UmuD/D' and C proteins in the C600 *supE* cells permissive for phage DNA replication is much lower, being 1.5-fold for pSE117 (UmuDC) and pGW2122 (UmuD') and 3-fold for pGW2123 (UmuD'C) (Table 4). Pre-irradiation of the 594 *sup*$^+$ cells to induce the SOS functions (+SOS) reduces the effect of overproduction of UmuD/D' and C proteins (Table 4). This is most probably due to deliv-

ery of chromosomal UmuD/D' and C. The enhancing effect in 594 *sup*$^+$ host is seen with both the low copy plasmid pSE117 (UmuDC) and the high copy pGW2123 (UmuD'C) (about 20 and 100 copies per cell, respectively). The difference in the enhancing effect by these two plasmids, 7- and 24-fold, respectively, may be related either to the amount of UmuD and C proteins overproduced by the low copy pSE117 and the high copy pGW2123 plasmids, or to the fact that the protein encoded by pGW2123 is in the UmuD' form, known to be active in mutagenesis (Nohmi *et al.*, 1988). It is most interesting that overproduction of UmuD' alone in 594 *sup*$^+$ (pGW2122) strain so efficiently (12-fold) stimulates UV-induced reversion of λ*O*(am)8 to λO$^+$ in non-SOS-induced host cells. This suggests that a high level of UmuD', and low, non induced, of UmuC are necessary and sufficient for UV-induced mutagenesis of λ*O*(am)8 in the nonpermissive host and points to an important role of UmuD' protein in this mutagenic pathway.

Table 4. Effect of overproduction of UmuD and C proteins on UV-induced reversion of λ*O*(am)8 to λO$^+$ in non-SOS- and SOS-induced 594 *sup*$^+$ and C600 *supE* hosts

	−SOS		+SOS	
	E.coli 594 *sup*$^+$	*E.coli* C600 *supE*	*E.coli* 594 *sup*$^+$	*E.coli* C600 *supE*
control	1.0	1.0	1.0	1.0
pSE117 (UmuDC)	7.0	1.5	2.1	1.9
pGW2123 (UmuD'C)	24.0	3.3	2.3	1.6
pGW2122 (UmuD')	12.0	1.5	1.7	1.4

UV doses used: 150 J/m^2 to phage and 70 J/m^2 or 40 J/m^2 to induce SOS in 594 *sup*$^+$ and C600 *supE* hosts, respectively (+SOS); nonirradiated hosts (−SOS); numbers in the table are ratios of mutation frequency in the host with a plasmid overproducing Umu proteins to the host with a control plasmid (pBR322 for pSE117) or without plasmid for pGW2123 and pGW2122.

Effect of deletion of *umuDC* operon and overproduction of UmuD' or UmuD'C proteins on UV-induced mutagenesis of λ*O*(am)8 in a 594 *sup*$^+$ Δ*umuDC* host

To examine in more detail the role of the UmuD' and C proteins in the mutagenic processes occurring under nonpermissive conditions for phage DNA replication, we constructed the *E. coli* 594 *sup*$^+$ Δ*umuDC* strain, with a deleted *umuDC* operon. Plasmids overproducing either UmuD' (pGW2122) or UmuD'C (pGW2123) were introduced into this strain, and UV-induced reversion of λ*O*(am)8 to λO$^+$ was monitored. As shown in Table 5, deletion of the *umuDC* operon in the 594 *sup*$^+$ Δ*umuDC* host reduces about 10 times (from 180×10^{-7} to 20×10^{-7} of mutants) UV-induced mutagenesis of λ*O*(am)8 phage to the level observed in non-SOS-induced cells. Introduction of plasmid pGW2122 (UmuD') into this strain does not restore UV-mutagenesis of the phage. However, overproduction of both proteins, UmuD' and C, in 594 *sup*$^+$ Δ*umuDC* (pGW2123) cells does restore UV-mutagenesis of the λ phage even in non SOS-induced cells, enhancing the mutation frequency about 200-fold. It follows that UmuD' protein, although important, as shown above, alone is not sufficient for the mutagenic process occurring under nonpermissive conditions for phage DNA replication. The UmuC protein is also required, albeit at a high level of UmuD' the constitutive level of UmuC seems to be sufficient (see Table 4). Preirradiation of the host to induce the SOS functions does not increase the number of mutants in the 594 *sup*$^+$ Δ*umuDC* or in 594 *sup*$^+$ Δ*umuDC* (pGW2122) strains, show-

ing that no other induced SOS function(s) can replace the UmuD and C proteins in the nonpermissive host. On the other hand, preirradiation of 594 *sup*$^+$ Δ*umuDC* (pGW2123) cells overproducing both UmuD' and C proteins enhances two-fold the level of mutants in comparison to that in non-preirradiated 594 *sup*$^+$ Δ*umuDC* (pGW2123), indicating that some inducible function(s), other than the UmuD' and C proteins, slightly enhances the level of mutants. However, since there was no effect of preirradiation of 594 *sup*$^+$ Δ*umuDC* (pGW2122) cells on the level of mutants (Table 5), the enhancing effect observed with 594 *sup*$^+$ Δ*umuDC* (pGW2123) seems to be related to improvement of survival of the mutants, due to induction of DNA repair systems, e.g. NER or RR, rather than to induction of some other mutagenic functions increasing the level of mutagenesis.

Table 5. UV-induced reversion of λ*O*(am)8 phage to λO$^+$ in 594 *sup*$^+$ Δ*umuDC* and C600 *supE* Δ*umuDC* hosts and the effect of overproduction of UmuD' and C proteins

SOS induction	−SOS		+SOS	
UV dose to phage	0 J/m^2	150 J/m^2	0 J/m^2	150 J/m^2
Strains:	Mutation frequency (× 10^{-7})			
594 *sup*$^+$	1.05 ±0.29	17.47 ±14.1	1.75 ±0.70	179.10 ±74.47
594 *sup*$^+$ Δ*umuDC*	1.77 ±0.85	16.55 ±10.61	1.95 ±0.70	20.52 ±13.5
594 *sup*$^+$ Δ*umuDC* (pGW2122)	1.20 ±0.52	13.05 ±7.76	1.09 ±0.51	17.32 ±5.4
594 *sup*$^+$ Δ*umuDC* (pGW2123)	1.60 ±0.65	307.22 ±137.5	1.52 ±0.49	636.72 ±279
C600 *supE*	3.65 ±1.24	51.76 ±29.47	3.55 ±0.93	583.75 ±267
C600 *supE* Δ*umuDC*	4.80 ±1.22	57.22 ±27.97	5.80 ±3.76	1257.95 ±793
C600 *supE* Δ*umuDC* (pGW2122)	3.64 ±1.06	46.65 ±22.25	3.22 ±1.10	330.70 ±135
C600 *supE* Δ*umuDC* (pGW2123)	5.00 ±1.07	59.17 ±22.00	5.33 ±0.90	713.00 ±363

Mutation frequencies and (±S.D.) are average values from at least five independent experiments, each in duplicate. UV-doses to phage and bacteria as in Table 4.

Different requirement for SOS functions and UmuD' and C proteins in UV-induced mutagenesis of λ*O*(am)8 phage in the hosts nonpermissive and permissive for phage DNA replication

As shown above, in UV-induced mutagenesis of λ*O*(am)8 phage in 594 *sup*$^+$ cells, nonpermissive for phage DNA replication, UmuD' and C proteins are required, and induction of other SOS-function(s) seems to be non essential.

To compare the mechanisms of UV mutagenesis under permissive and nonpermissive conditions for phage DNA replication, we also examined the effect of deletion of the *umuDC* operon on UV-mutagenesis of λ*O*(am)8 phage in the *E. coli* C600 *supE* strain permissive for phage DNA replication. In contrast to the 594 *sup*$^+$ Δ*umuDC,* deletion of the *umuDC* operon in the C600 *supE* Δ*umuDC* host does not abolish SOS-dependent mutagenesis of λ phage, and even the level of mutants is two times higher than in C600 *supE* (Table 5). Moreover, overproduction of UmuD' or of UmuD' and C proteins leads to some reduction of the number of mutants in SOS-induced C600 *supE* Δ*umuDC* cells. This might be related to the fact that a high level of UmuC protein inhibits mutagenesis (Woodgate *et al.*, 1994) and DNA replication (Opperman *et al.*, 1996; Frank *et al.*, 2000) (see also Discussion).

The foregoing points to different requirements for UV-induced mutagenesis under permissive and nonpermissive conditions for phage DNA replication and suggests the involvement of different mechanisms. While

UmuD' and C proteins are indispensable for UV-mutagenesis in the nonpermissive host, they do not seem to be essential in the mutagenic process occurring under permissive conditions for phage DNA replication, so that some other inducible SOS-function(s) may replace the UmuDC proteins. This may be Pol IV (DinB), another DNA polymerase involved in TLS (Wagner *et al.*, 1999; Kim *et al.*, 2001). Moreover, in contrast to 594 *sup*$^+$ Δ*umuDC*, overproduction of UmuD'C in C600 *supE* Δ*umuDC* does not restore mutagenesis of λ*O*(am)8 phage in non-SOS-induced cells (Table 5).

Different effects of deficiency in mismatch repair on mutagenesis of λ*O*(am)8 phage induced under nonpermissive and permissive conditions for phage DNA replication

The results presented above suggest involvement of different mechanisms for UV-induced mutagenesis of λ*O*(am)8 phage under permissive and nonpermissive conditions for phage DNA replication. To learn more about the mechanisms, we compared the effect of a deficiency in MutHLS-dependent mismatch repair (MMR) on UV-induced reversion of λ*O*(am)8 phage to λO$^+$, in permissive and nonpermissive hosts. MutHLS-dependent MMR is known to repair mismatches formed during DNA replication to avoid mutations (Friedberg *et al.*, 1995; Modrich *et al.*, 1996). We constructed *mutL* deficient strains 594 *sup*$^+$ *mutL* and C600 *supE mutL* and compared the effect on UV-induced reversion of λ*O*(am)8 phage to λO$^+$. The results presented in Table 6 show that MMR deficiency does not affect either spontaneous or UV-induced mutagenesis of λ*O*(am)8 phage in the 594 *sup*$^+$ *mutL* strain nonpermissive for phage DNA replication. In contrast, in the C600 *supE mutL* host, a high level of spontaneous mutations and a two-fold enhancement of UV-induced mutagenesis are observed. This clearly indicates that all UV-induced mutations in the *O* gene of λ*O*(am)8 phage in the 594 *sup*$^+$ host are resistant to MMR, and support the notion that they are not a result of DNA replication. The mismatch repair deficiency did not affect the reversion of λ*O*(am)8 phage to λO$^+$ induced by MMS in 594 *sup*$^+$ *mutL*, either (not shown). On the other hand, in the permissive host C600 *supE*, at least half of the UV-induced mutations in the λ*O*(am)8 phage are subject to the MMR, implying that they are related to replication of the UV-damaged phage DNA. In addition, the absence of a mutator effect on λ*O*(am)8 phage in 594 *sup*$^+$ *mutL* cells supports the finding that, in the 594 *sup*$^+$ host, DNA replication of λ*O*(am)8 phage does not occur.

Table 6. Effect of *mutL* on UV-induced mutagenesis of λ*O*(am)8 phage in the host nonpermissive (594 *sup*$^+$ *mutL*) and permissive (C600 *supE mutL*) for phage DNA replication

UV-dose to phage	594 *sup*$^+$	594 *sup*$^+$ *mutL*	C600 *supE*	C600 *supE mutL*
	Mutation frequency × 10^{-7}			
0 J/m^2	0.31 ±0.17	0.45 ±0.46	32.85 ±5.13	1325 ±591
150 J/m^2 (−SOS)	22.51 ±9.28	25.56 ±7.67	243.00 ±14.71	2211 ±112
150 J/m^2 (+SOS)	262.66 ±74.54	384.10 ±119.7	2317.00 ±114	4472 ±2087

Mutation frequencies and (±S.D.) are average values from at least six independent experiments, each in duplicate. UV doses to the hosts used for induction SOS as in Table 4.

MMS-induced reversion of λ*O*(am)8 to λO$^+$ under nonpermissive conditions for phage DNA replication. The effect of UmuD' protein

We were wondering which of the photoproducts in UV-irradiated phage DNA may be the premutagenic lesion leading to reversion of λ*O*(am)8 phage to λO$^+$. UV irradiation of DNA leads to the formation of several photoproducts, the most frequent being pyrimidine photodimers and 6–4 photoproducts (Friedberg *et al.*, 1995). Among the minor photoproducts in UV-irradiated DNA are pyrimidine photohydrates and thymine glycols (Tg) (Yamane *et al.*, 1967; Fisher *et al.*, 1976; Demple & Linn, 1982; Boorstein *et al.*, 1990; Doetsch *et al.*, 1995). Cytosine photohydrates dehydrate readily and may also be converted by deamination to the more stable uracil photohydrates in irradiated DNA. Cytosine photohydrates, as well as uracils and Tg, are recognised by specific DNA-glycosylases (Demple & Linn, 1980), leading to generation of AP-sites, which are known to be efficient premutagenic lesions (Loeb & Peterson, 1986; Woodgate & Levine, 1996; Rothwell & Hickson, 1997; Kow, 2002), as well as thymidylyl-(3'-5')-deoxyadenosine (TA*), reported to be a potential premutagenic lesion (Zhao & Taylor, 1996; Otoshi *et al.*, 2000). Sequence analysis of the *O* gene of λ*O*(am)8 phage (data unpublished) shows that in the coding strand the TAG stop codon, the *amber* mutation in λ*O*(am)8 phage, is in the sequence 5' GTG TAG ATC 3' in which Tg or the TA* photoproduct could be formed as the main photoproduct, and could be responsible for mutagenic events. So the possibility that AP-sites, a result of DNA glycosylase(s) activity, are the premutagenic lesions, can not be excluded. MMS is known to induce mutations mainly by formation of AP-sites as a result of the activity of the two 3-mA-DNA glycosylases recognising methylated purines in DNA (Friedberg *et al.*, 1995). It has been reported that MMS-induced mutagenesis in *E. coli* is highly dependent on the level of UmuDC proteins (Doyle & Strike, 1995; Grzesiuk & Janion, 1996). Assuming that, in UV-induced mutagenesis of λ*O*(am)8 phage, AP-sites might be the main premutagenic lesions we compared the effect of overproduction of UmuD' on MMS- and UV-induced mutagenesis of the phage.

The effect of overproduction of UmuD' on MMS- and UV-induced reversion of λ*O*(am)8 to λO$^+$ in non-SOS-induced *E. coli* 594 *sup*$^+$ (pGW2122) host was studied. As shown in Table 7 treatment of phage with UV light (150 J/m^2) or 0.33% MMS for 75 min leads to some induction of λO$^+$ revertants in 594 *sup*$^+$ cells. However, overproduction of UmuD' in the strain 594 *sup*$^+$ (pGW2122) increases the

Table 7. Effect of overproduction of UmuD' protein on UV- and MMS-induced reversion of λ*O*(am)8 to λO$^+$ in non-SOS-induced 594 *sup*$^+$ strain

Host	Mutation frequency ($\times 10^{-7}$)		Effect of UmuD'
	−UV	**+UV**	**fold increase**
594 *sup*$^+$	1.26 ±0.53	27.7 ±13.53	1.0
594 *sup*$^+$ (pGW2122)	2.11 ±0.27	399.8 ±136.58	15.0
	−MMS	**+MMS**	
594 *sup*$^+$	0.59 ±0.17	38.6 ±21.65	1.0
594 *sup*$^+$ (pGW2122)	2.91 ±0.54	692.33 ±245.98	18.0

Mutation frequencies and (±S.D.) are average values from at least five independent experiments, each in duplicate. Phage was irradiated with UV light (150 J/m^2) or treated with 0.33% MMS for 75 min.

mutation frequency 15-fold for UV and 18-fold for MMS mutagenesis relative to 594 *sup*$^+$ cells without the plasmid. It follows that in both UV- and MMS-induced mutagenesis, overproduction of UmuD' and the basal noninduced level of UmuC are sufficient for λ*O*(am)8 phage reversion under nonpermissive conditions for phage DNA replication. Since MMS induces mutations mainly by formation of AP-sites, the fact that overproduction of UmuD' in 594 *sup*$^+$ (pGW2122) affects in a similar way the mutagenesis of λ*O*(am)8 induced by UV and MMS, suggests that AP-sites might be the premutagenic lesions in UV- and MMS-induced mutagenesis of the phage. The primary mutagenic lesion could be Tg or 3-methyloadenine (3-mAmet)known to be recognized by DNA-glycosylases. The sequence surrounding the *amber* codon in which mutation occurs also suggests the possibility that Tg or 3-mAmet may be formed.

This supports and underlines the role of UmuD' protein in the mutagenic process occurring under nonpermissive conditions for DNA replication, and suggests that the premutagenic lesions processed by UmuD' might be AP-sites.

DISCUSSION

The current hypothesis on the mechanism of mutagenesis induced in *E. coli* by UV light and many chemicals assumes that mutations are a result of translesion synthesis and replication of damaged DNA. It is now well documented that UmuD'$_2$C complex possesses an intrinsic DNA polymerase activity (DNA Pol V) that facilitates error-prone translesion DNA synthesis at replication forks stalled by a lesion, which is followed by resumption and continuation of replication by DNA polymerase III complex (Tang *et al.*, 1998; 1999; 2000; Reuven *et al.*, 1999; Goodman & Tippin, 2000; Livneh, 2001). Thus, the mutagenic process is considered to be related to replication of damaged DNA. Our results indicate that SOS-dependent mutagenesis induced by UV or MMS may occur under nonpermissive conditions for DNA replication, and support our previous finding that UV-induced mutagenesis in *E. coli* MV1178 *uvrA recF* may occur before resumption of DNA replication in UV-irradiated cells (Pietrzykowska & Felczak, 1991). Our present findings show that UV-induced mutagenesis of λ*O*(am)8 phage may occur in a host nonpermissive for phage DNA replication, *E. coli* 594 *sup*$^+$. λ*O*(am)8 phage has the *amber* mutation in gene *O* encoding the λO protein which is responsible for initiation of phage DNA replication. Lack of λ*O*(am)8 replication in the 594 *sup*$^+$ host was first shown by Ogawa & Tomizawa (1968) and Shuster & Weisbach (1969) and the role of the gene *O* product in initiation of phage DNA replication is now well established (Wyatt & Inokuchi, 1974; Zylicz *et al.*, 1984; 1988; Dodson *et al.*, 1989; Stephens & McMacken, 1997). The absence of λ*O*(am)8 phage DNA replication in the 594 *sup*$^+$ host is also supported by our experiments with mutants 594 *sup*$^+$ *dnaQ49* and 594 *sup*$^+$ *mutL*. Both mutations are known to cause a high mutator effect related to DNA replication (Horiuchi *et al.*, 1978; Piechocki *et al.*, 1986; Fridberg *et al.*, 1995; Rasmussen *et al.*, 1998). Inactivation of the proof-reading activity of the 3'→5' exonuclease in a *dnaQ49* mutant at 37°C markedly increases the number of errors introduced during DNA replication. On the other hand, the mutation *mutL* leads to a high mutator effect due to deficiency in post-replicative MutHSL-dependent MMR (Friedberg *et al.*, 1995; Rasmussen *et al.*, 1998). The absence of mutator effects of these two mutations on the λ*O*(am)8 phage in 594 *sup*$^+$, and high mutator effect in C600 *supE* support the notion that phage DNA replication does not occur in 594 *sup*$^+$ host cells. The possibility that DNA replication of UV-irradiated λ*O*(am)8 phage might be initiated by UvrABC nuclease seems to be excluded by the experiments with the *E. coli* 594 *sup*$^+$ *uvrA* host. If

incision by UvrABC nuclease could initiate replication of UV-irradiated DNA, one would expect the absence of UV-induced mutagenesis of λ*O*(am)8 phage in the excision-repair-defective 594 *sup*$^+$ *uvrA* host. Our experiments show that this is not the case. In addition, if UvrABC nuclease could initiate replication of irradiated phage DNA, the level of UV-induced mutagenesis of λ*O*(am)8 phage in 594 *sup*$^+$ *uvr*$^+$ should be comparable to that in C600 *supE,* a permissive host. As can be seen in Table 5 the efficiency of mutagenesis in 594 *sup*$^+$ *uvr*$^+$ constitutes about 30% of that in C600 *supE,* permissive for phage DNA replication. The absence of λ*O*(am)8 phage DNA replication in the *E. coli* 594 *sup*$^+$ host is also supported by the fact that untargeted mutagenesis (UTM) of the phage, known to be related to the replication of undamaged λ phage DNA in preirradiated cells (Brotcorne-Lannoye & Maenhaut-Michel, 1986; Kim *et al.,* 1997; 2001) does not occur in this host. It may be seen in Table 2 that when λ*O*(am)8 phage is not irradiated with UV light, the level of λO$^+$ revertants is the same in nonirradiated (–SOS) and irradiated (+SOS) 594 *sup*$^+$ cells. This is in agreement with *in vitro* studies on the crucial role of the λO protein in initiation of λ phage DNA replication (Zylicz *et al.,* 1984; Stephens & McMacken, 1997). This is good evidence that DNA replication of λ*O*(am)8 phage in the 594 *sup*$^+$ host does not occur, and strongly suggests that the observed UV- and MMS-induced mutations of λ*O*(am)8 phage in 594 *sup*$^+$ cells are generated before initiation of DNA replication. Thus, we are dealing with a new mutagenic pathway in *E. coli,* independent of DNA replication. Another mutagenic pathway independent of DNA replication was described by Cohen-Fix & Livneh (1992) under *in vitro* conditions. It differs from that presented here in several respects, in that it requires the UvrABC excinuclease and DNA polymerase III, but not the functions of *umuC*$^+$ and *recA*$^+$ gene products. In contrast, the mutagenic pathway we describe does require the UmuD' and C proteins and is independent of the UvrABC excinuclease.

What may be the mechanism of UV-induced mutagenesis independent of DNA replication? We consider that UV-induced mutation of λ*O*(am)8 in the 594 *sup*$^+$ host is not a result of recombination. It has been reported that recombination is not involved in UV mutagenesis of λ phage (Miura & Tomizawa, 1970), and the m.o.i. of 0.2 used in our experiments was too low to promote recombination. In general, recombination repair is considered to be error-free. This mutagenic pathway clearly differs from that occurring under permissive conditions for phage DNA replication. It is absolutely dependent on the UmuD' and C proteins, since we showed that deletion of the *umuDC* operon significantly reduces UV-mutagenesis of λ*O*(am)8 in the 594 *sup*$^+$ Δ*umuDC* strain. The requirement for both proteins is illustrated by the fact that UV-mutagenesis of λ*O*(am)8 in the 594 *sup*$^+$ Δ*umuDC* host may be recovered by delivery, from plasmid pGW2123, of both UmuD' and C proteins, but not by UmuD' alone. On the other hand, overproduction of UmuD' from pGW2122 in the wild type 594 *sup*$^+$ strain increases 12–15 times UV- and 18 times MMS-induced frequency of λO$^+$ revertants in the non-SOS-induced cells (Table 4 and 7). This suggests that for the mutagenic pathway a high level of UmuD' and a low, noninduced level of UmuC protein are necessary and sufficient, and implies an important role of UmuD' protein in the mutagenic process. A possible role of UmuC (Pol V) on the survival of mutants, in 594 *sup*$^+$ rather than in the mutagenic process itself, can not be excluded. We observed that overproduction of UmuD' and C proteins in the C600 (pGW2123) strain enhances 2–6 times (depending on the UV-dose) survival of UV-irradiated λ*O*(am)8 phage (Table 8). How can one explain that under nonpermissive conditions the mutagenic process needs a high level of UmuD' and low (non-induced) level of UmuC? If UmuD' plays an important role in

the generation of the mutations in λ*O*(am)8 phage in the 594 *sup*$^+$ host, reversion of the *amber* mutation in gene *O* of the phage would enable initiation of DNA replication. However, phage irradiated with a dose of 150 J/m^2 has a number of unrepaired lesions which will stop the replication fork. This needs TLS synthesis with involvement of Pol V (UmuD'$_2$C) for survival of the phage. The increasing effect of overproduction of UmuD'C on survival of λ*O*(am)8 phage in C600 *supE* host seen in Table 8 seems to be in agreement with the hypothesis.

The absence of the UmuD' and C protein functions in the deletion mutant 594 *sup*$^+$ Δ*umuDC* can not be replaced by any other SOS-inducible function(s) in mutagenesis of λ*O*(am)8 phage since no effect of preirradiation of this host cells on the level of mutants was seen (Table 5). In contrast to the 594 *sup*$^+$ Δ*umuDC* host, deletion of the *umuDC* operon in C600 *supE* Δ*umuDC* did not abolish UV-induced mutagenesis of λ*O*(am)8 phage, indicating that a different mutagenic processes independent of UmuD'C may occur in the host permissive for phage DNA replication. It requires preirradiation of the host cells to induce some SOS function(s) that are not UmuD and C proteins. Overproduction of UmuD', and to a lesser extent of UmuD'C, reduces the level of mutants (Table 5), which is in agreement with the reported inhibition of mutagenesis by a high level of UmuC protein (Woodgate *et al.*, 1994). What could be the SOS function(s) responsible for the UmuD'C-independent mutagenesis in C600 *supE* Δ*umuDC* host? One possible candidate is the product of the *dinB* gene, a homologue of UmuC protein, reported to be Pol IV (Wagner *et al.*, 1999; Tang *et al.*, 2000). Overproduction of the *dinB* gene product was reported to highly enhance UTM of λ phage (Kim *et al.*, 1997; 2001). In the last few years the roles of several DNA polymerases controlled by SOS in *E. coli* has been elucidated: Pol II (PolB), Pol IV (DinB) and Pol V (UmuC) (for a review see: Goodman & Tippin, 2000; Livneh, 2001; Wagner *et al.*, 2002). Which of them may be responsible for the UV- and MMS-induced mutagenesis of λ*O*(am)8 in the permissive C600 *supE supE* Δ*umuDC* cells? Pol II, being known to be involved in frameshift mutagenesis in the case of base substitution mutagenesis studied in λ*O*(am)8 phage, seems to be excluded. On the other hand Pol IV, able to produce frameshifts and base substitutions in λ phage (Kim *et al.*, 1997; 2001) may be the translesion DNA poly-

Table 8. Effect of overproduction of UmuD' and UmuD'C proteins on survival of UV-irradiated λ*O*(am)8 phage (%)

Host	Phage survival (%)		
	100 J/m^2	**150 J/m^2**	**200 J/m^2**
C600 *supE*	19.6 ±8.5	12.6 ±3.1	2.4 ±1.1
C600 *supE* (pGW2122)	36.3 ±23.1	21.9 ±12.9	6.7 ±2.8
C600 *supE* (pGW2123)	46.9 ±22.3	29.0 ±19.2	14.2 ±8.3
C600 *supE ΔumuDC*	–	6.0 ±0.21	–
C600 *supE ΔumuDC* (pGW2122)	–	5.0 ±0.83	–
C600 *supE ΔumuDC* (pGW2123)	–	18.0 ±3.01	–
	10 J/m^2	**20 J/m^2**	**40 J/m^2**
C600 *supE uvrA*	46.6 ±14.9	11.5 ±5.6	0.25 ±0.14
C600 *supE uvrA* (pGW2122)	30.8 ±14.3	11.5 ±6.5	0.53 ±0.20
C600 *supE uvrA* (pGW2123)	53.7 ±23.9	23.1 ±2.0	1.43 ±0.84

Survival (%) of λ*O*(am)8 phage irradiated with UV-dose (J/m^2) and (±S.D.) are average values from at least five independent experiments, each in duplicate.

merase which can replace Pol V in the C600 Δ*umuDC* host. It was shown *in vitro* that both Pol IV and Pol V may bypass photochemical lesions and AP-sites, but Pol V is much more efficient than Pol IV (Tang *et al.*, 2000; Napolitano *et al.*, 2000). In the absence of Pol V, Pol IV may become active in mutagenic TLS. Studies on the role of SOS-controlled DNA polymerases in induced mutagenesis in λ*O*(am)8 phage under nonpermissive and permissive conditions are under way.

Our results have shown that in *E. coli* two different mutagenic pathways may operate, one dependent and the other independent of DNA replication. This is also corroborated by the fact that deficiency in MutHLS-dependent mismatch repair has no effect on UV- and MMS-induced mutagenesis of λ*O*(am)8 phage in the 594 *sup*$^+$ *mutL* host, but increases it about 2.5-fold in C600 *supE mutL* (Table 6). This implies that about 60% of the mutations induced in the host permissive for DNA replication undergo postreplicative mismatch repair, and about 40% are resistant to MMR. By contrast, all mutations generated in 594 *sup*$^+$ cells are not subject to mismatch repair. This is in agreement with the observation that the efficiency of UV-induced mutagenesis of λ*O*(am)8 in the 594 *sup*$^+$ host is 30–40% of that observed in C600 *supE* (see Table 5). Moreover, sequence analysis of λO$^+$ revertants showed that most mutations induced in the 594 *sup*$^+$ host are transversions, and in C600 *supE* transitions prevail (data unpublished). These two pathways could not be separated in C600 *supE* cells able to replicate phage DNA, but it proved possible with the use of the 594 *sup*$^+$ host unable to replicate λ*O*(am)8 phage DNA.

We believe that our studies on mutagenesis independent of DNA replication may be useful for understanding the mechanism of generation of mutations in nondividing cells e.g. somatic cells. Studies on DNA repair and mutagenesis in eukaryotic cells, including man, indicate that general rules may be common for both Prokaryota and Eukaryota (Sutton & Walker, 2001; Goodman, 2002; Stary *et al.*, 2003). Most significant is the identification in eukaryotic cells, including humans, of DNA polymerases belonging to the Y family (earlier called the UmuC/Rad30/DinB family) which promote translesion replication (for reviews see: Woodgate, 1999; Goodman & Tippin, 2000; Livneh, 2001; Wang, 2001; Woodgate, 2001; Boudsocq *et al.*, 2002).

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