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The Effects of umuC on spontaneous and UV-induced mutagenesis in *Escherichia coli*

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mutagenesis in *Escherichia coli***

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San Jose State University, 1993

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THE EFFECTS OF *umuC* ON SPONTANEOUS AND UV-INDUCED
MUTAGENESIS IN *ESCHERICHIA COLI*

A Thesis

Presented to

The Faculty of the Department of Biological Sciences
San Jose State University

In Partial Fulfillment
of the Requirements for the Degree
Master of Arts

By

Jefferson Woodard

August, 1993

APPROVED FOR THE DEPARTMENT OF BIOLOGICAL SCIENCES

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ABSTRACT

The effects of *umuC*
on spontaneous mutagenesis
by Jefferson Woodard

Escherichia coli cells that are deficient in the *umuC* gene product are nonmutable by ultraviolet (UV) light. In this study the effect of the *umuC* gene on spontaneous mutagenesis was characterized. With *umuC* mutant strains, base-pair substitution mutation and reversion frequencies were generally decreased when compared to coisogenic *umuC*⁺ strains. Using the *trpA* reversion system, it was also shown that the mutational spectrum for base-pair substitutions was also altered in *umuC* strains. Transition and transversions at A:T base pairs were markedly reduced compared with *umuC*⁺ strains whereas substitutions at G:C sites were less affected by the presence of the *umuC* allele. It is also shown that not all UV-induced mutagenesis is dependent on the presence of the *umuC*⁺ allele.

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Introduction

Normally when the bacterium *Escherichia coli* receives damage to its DNA by certain chemical mutagens or ultraviolet light (UV), a multigene inducible SOS system is activated (Witkin, 1976; Walker, 1984). Consequently the gene products from the SOS system are expressed and appear in the cell. The *umuC* gene is one member gene in the SOS regulatory network. Mutants defective in this gene when challenged with UV light exhibit a severe reduction in UV mutagenesis (Kato and Shinoura, 1977; Steinborn, 1978). SOS induction is required for UV mutagenesis which is thought to be the result of an error-prone form of DNA repair dependent upon the *umuC*⁺ allele.

Central to this inducible system is the RecA protein, which plays a critical regulatory role, once activated, by mediating two proteolytic events. Proteolytic cleavage of the LexA repressor results in derepression of a network of chromosomally encoded SOS genes including the *umuDC* genes which are necessary for mutagenesis (Sweasy and Witkin, 1990). Posttranslational proteolytic processing converts the UmuD protein to UmuD', the active carboxyl terminal fragment that is required for SOS mutagenesis (Burckhardt et al., 1988; Shinagawa et al., 1988). The precise role of the UmuC and UmuD proteins in UV mutagenesis remains unclear. However it is known that the UmuC protein forms a complex with chaperone proteins GroES and GroEL to avoid

proteolysis (Donnelly and Walker, 1989). Once enough UmuD has been processed to umuD', it forms a complex with UmuC and together (UmuD'- UmuC) interact with the cellular replication machinery to bypass noncoding DNA lesions, resulting in an elevated mutation rate (Woodgate *et al.*,1989; Smith *et al.*,1990).

The *umuDC* operon encodes both the UmuD and UmuC proteins. UmuC, the less promoter proximal of the two genes, produces a protein synthesized from an overlapping (1bp) reading frame (Perry *et al.*,1985). UmuC is a basic protein with a molecular weight of 45,000 daltons that is thought to be a DNA lesion recognition protein given its low cellular levels of approximately 200 molecules per induced cell and does not seem to be required for efficient processing of UmuD to UmuD' (Elledge and Walker, 1983). However, biochemical evidence supports the suggestion that UmuC forms a complex with a homodimer of UmuD' and may be required to bring UmuD' to the DNA for *recA*-mediated cleavage or to stabilize the UmuD' at the lesion site (Perry *et al.*,1985).

While the *umuC* gene is essential for most UV mutagenesis, what role, if any, it has in spontaneous mutagenesis remains to be established. The purpose of this study is to determine whether or not the *umuC* mutation affects the frequency and/or specificity of spontaneous mutagenesis.

Literature Review

Both transcription and replication of DNA are inhibited as a direct result of the disruptive effect on hydrogen bonding and the formation of pyrimidine dimers by ultraviolet (UV) light (Lewin, 1974). Bacterial cells that lack repair mechanisms due to mutations in the corresponding genes prove nonadaptive and even the presence of a single dimer can be lethal (Hanawalt *et al.*, 1979). In *Escherichia coli* dimer repair is carried out either before replication of its DNA (prereplication repair) or after DNA replication (postreplication repair). While both types of repair systems utilize enzymes, the mechanisms and components of each are uniquely different (Witkin, 1976).

Photoreactivation and excision repair are both components of prereplication repair. Photoreactivation makes use of a single enzyme (photoreactivating enzyme) in *E. coli* that binds to the dimer. However a single photon of light is required to supply the energy needed to split the dimer and restore the DNA to its normal state (Beukers and Berends, 1960; Witkin, 1966).

Excision repair is a multi-enzyme repair process and makes use of DNA's complementary double-stranded nature. In *E. coli* a correndonuclease makes a strand incision (on the 5' side) close to the dimer. Excision of the fragment of DNA that contains the dimer is completed by an exonuclease. The gap left behind is subsequently filled in by using DNA polymerase 1 and the opposite complimentary

strand of the DNA as a template (Witkin, 1976). Finally polynucleotide ligase seals the gap in the phosphodiester backbone completing the repair process. Excision repair is abolished in *uvr⁻* mutants. These mutants contain no endonuclease to excise dimers.

Prereplication repair is thought to be mostly error free and does not contribute to the UV mutation frequency to a significant extent (Sutherland, 1981; Lindahl, 1982). Dimers that are not removed by prereplication repair may impair DNA strand elongation during replication. Faulty base pairing (hydrogen bonding) at dimer sites may activate DNA polymerase's 3'→5' exonuclease editing function (Chi *et al.*, 1986) and eventually stall the polymerase. Replication is initiated again at sites about 1000 base pairs from the dimer and this results in daughter strand gaps and secondary lesions (Witkin, 1976). DNA continuity and cell survival would be at risk if not for additional repair pathways. Postreplication repair includes both SOS repair and one or more forms of recombination repair.

Recombination and SOS repair are both dependent on the *recA* gene product, which is regulated by the *lexA* gene (Little *et al.*, 1981). Recombination repair uses DNA from the intact daughter DNA helix to fill in replication gaps left behind in the daughter strand synthesized from the dimer-containing parental DNA in the other daughter helix.

While recombination repair is thought to be error-proof in *E. coli*, SOS repair is error-prone and is the most significant

contributor to UV-induced mutagenesis. DNA damage not quickly corrected by error-free forms of repair will induce the error prone repair pathway (Witkin, 1976). A number of physiological responses are induced in *E. coli* after DNA damage and/or inhibition of DNA replication and are collectively called the SOS response. One such inducible physiological response leads to UV mutagenesis of the bacterial chromosome (Witkin, 1976). The SOS response is controlled by the *recA* and *lexA* genes and *E. coli* *recA* and *lexA* mutants display a host of pleiotrophic phenotypic effects and interfere with UV mutagenesis (Walker, 1984). The LexA protein acts as a repressor of SOS genes, keeping them at low but variable levels of basal expression (Little and Mount; 1982). Activation of the RecA protein by DNA damage, possibly the presence of single-stranded DNA (Lu and Echols, 1987), leads to autocleavage of the LexA protein (Horii *et al.*, 1981). Derepressed SOS genes now express elevated levels of protein products.

Induction of two SOS genes, *umuC* and *umuD*, is required for UV mutagenesis in *E. coli* (Kato and Shinoura, 1977). Mutations in these genes reduce UV mutability by more than 100-fold and slightly reduce UV sensitivity without apparently affecting other SOS functions (Kato and Shinoura, 1977). The *umuC* and *umuD* genes compose the *umuDC* operon located between *hemA* and *purB* on the *E. coli* chromosome (Kato and Shinoura, 1977). *UmuC* the less promoter proximal of the two genes, produces a protein synthesised from an overlapping (1bp) reading frame (Perry and Walker, 1982). The UmuC

protein is a basic protein that has a molecular weight of 45,000 daltons while the UmuD protein also basic, has a molecular weight of 16,000 daltons (Perry and Walker, 1982). Both proteins are essential for UV mutagenesis of chromosomal DNA as well as lambda phage reactivation (Bridges and Woodgate, 1984).

The exact role of the *umuDC* gene products in mutagenesis is not yet clear. It has been suggested that these proteins may not be involved in the misincorporation step during DNA synthesis that leads to the mutagenic event. Rather they may function in increasing the ability of a stalled DNA polymerase to replicate past the blocking DNA lesion, presumably a pyrimidine dimer (Bridges and Woodgate, 1984,1985).

It has been shown that UmuD is proteolytically cleaved to yield a carboxyl-terminal fragment (UmuD') that is active in UV mutagenesis (Burckhardt *et al.*,1988; Shinagawa *et al.*,1988). This *in vivo* processing requires a high level of activated RecA protein (Burckhardt *et al.*,1988) and occurs in a manner similar to the processing of the LexA protein.

It is known that the UmuC protein forms a complex with chaperone heat shock proteins GroES and GroEL to avoid proteolysis (Donnelly and Walker, 1989). UmuC protein half-life in *groE*⁺ cells is 17 minutes compared to that of 6 minutes for *groE* mutants . It was shown that these mutants display both a reduction in the UmuC protein and a defect in UV mutagenesis (Donnelly and Walker, 1989).

No altered stability in the UmuD protein was observed in *groE* mutants.

Similar genes with homology to *umuC* and *umuD* have been found in plasmid pKM101 and are called *mucA* and *mucB* (Ciesla, 1982). Both are required for the enhancement of UV resistance and UV mutagenesis by pKM101 in host cells. The molecular weight of both MucA and UmuD is about 16,000 daltons while the molecular weight of MucB and UmuC is about 45,000 daltons each (Shanabruch and Walker, 1980). Regulation of the *muc* genes is mediated by the RecA protein which suggests homology with the chromosomal *umuDC* genes.

While the *umuC* and *umuD* genes are essential for UV mutagenesis in *E. coli*, there is little information concerning a possible role in spontaneous mutagenesis. A number of repair genes have been found to influence spontaneous mutation frequencies (Smith, 1992). The *umuC36* allele was shown to decrease spontaneous frequencies at one site in an *uvrB* background (Sargentini and Smith, 1981). However, the presence of the *uvrB* allele in the strain used in this study complicates the contribution of the *umuC* gene toward spontaneous mutagenesis.

Materials and Methods

Bacterial Strains

The tryptophan synthetase A gene mutants used in this study were obtained from Dr. Charles Yanofsky (Yanofsky *et al.*, 1966; Drapeau *et al.*, 1968). The *umuC* 122: Tn5 (*umuC*⁻) mutant allele was originally isolated and characterized in the laboratory of Dr. Graham C. Walker (Elledge and Walker, 1983). This mutant carries a 5.7-kb insertion sequence (Tn5) near the carboxyl terminus of its *umuC* gene (Elledge and Walker, 1983), and is assumed not to be leaky. The *umuC* 122:Tn5 allele was previously transduced into each of the *trpA* strains by Dr. Neil J. Sargentini using phage PI and selection for kanamycin resistance that is conferred by Tn5.

Media

VB medium is the minimal salts medium of Vogel and Bonner (1956). Minimal plates contained minimal medium solidified with 1.5% agar and supplemented with 0.2% glucose and 1 µg/ml thiamine. Required amino acids were added at 50 µg/ml to liquid and solid media; and indole was added to solid media at 10 µg/ml. Tryptone plates consisted of 1% tryptone and 0.5% NaCl solidified with 1.5% agar. Mutation frequencies to nalidixic acid resistance (Nal^R) and rifampicin resistance (Rif^R) were measured on tryptone plates containing 50 µg/ml nalidixic acid and 100 µg/ml rifampicin.

respectively. UV-induced Trp⁺ revertants were selected on minimal plates supplemented with casamino acids (Difco Laboratories) at 2.5 mg/ml and limiting tryptophan at 0.4 ug/ml. While allowing initial cell division, this amount of tryptophan was not enough to sustain further growth and, therefore, colony formation of non-revertants. L-broth was 1% tryptone, 0.5% yeast extract and 0.5% NaCl. The saline for dilutions and washing was 0.85% NaCl.

Spontaneous Mutation and Reversion Frequencies

Mutation frequencies to Nal^R and Rif^R were determined for each strain in both wild-type (*umuC*⁺) and *umuC* backgrounds. Four cultures of each strain were started from a small inoculum (approximately 1000-2000 cells) and grown to saturation in L-broth. Each culture was concentrated 10x by centrifugation (approximately 10¹⁰ cells per ml) in saline, diluted when appropriate and plated with and without nalidixic acid (50ug/ml) and rifampicin (100ug/ml). Mutation frequencies were calculated by dividing the average number of nalidixic acid and rifampicin-resistant mutants by the average number of total cells.

A Trp⁺ reversion frequency was calculated for each base-pair substitution and frameshift *trpA* strain in the *umuC*⁺ and *umuC* backgrounds using the same cultures as described above. Cultures were plated on minimal medium to select for Trp⁺ revertants and on

minimal medium supplemented with tryptophan to titer total cells. The average number of Trp⁺ revertants was divided by the average number of total cells to obtain the reversion frequency for each strain.

UV Mutagenesis

To measure frequencies of UV-induced reversion to Trp⁺ for each *trpA* strain in *umuC*⁺ and *umuC* backgrounds, 0.1ml washed samples (10⁸ cells) were taken from overnight L-broth cultures and spread on minimal plates supplemented with casamino acids and limiting tryptophan (0.4ug/ml). Four to six plates were exposed to 39.0 J/m² UV light from a Sylvania germicidal lamp, and the other plates were left unirradiated (to detect spontaneous revertants). The UV dose was measured with a UV meter. Irradiated and unirradiated plates were wrapped in aluminum foil to prevent possible photorepair and Trp⁺ colonies were counted after five days of incubation at 37° C. The number of UV-induced revertants per 10⁸ survivors was calculated by subtracting the average number of colonies per unirradiated plate from the average per irradiated plate and then correcting for the fraction of irradiated bacteria surviving as determined by viable counts described below.

Classification of Trp⁺ revertants

After 5 days at 37° C revertants were selected for classification. The classification of Trp⁺ revertants of *umuC*⁺ and *umuC* strains was based upon two physiological tests. First, purified revertants were tested for indole glycerol phosphate (IGP) accumulation. Full revertants do not accumulate IGP; partial revertants do (Allen and Yanofsky, 1963). All Trp⁺ revertants were tested for IGP accumulation. Second, various partial revertants can often be distinguished quantitatively by their sensitivity to 5-methyl tryptophan (5-MT) (Lester and Yanofsky, 1961). Partial or suppressed revertants growing on agar plates show much larger zones of inhibition than full revertants around disks impregnated with 5-MT (Cox *et al.*, 1972; Fowler *et al.*, 1974). The 5-MT inhibition test was used to classify partial revertants of *trpA* alleles 23, 46 and 58. Since the IGP accumulation test is accurate for full revertants, 5-MT inhibition tests were not performed on revertants of *trpA* alleles 3, 11 and 78 where further separation of partial revertants provides no additional useful data.

UV Survival

Since all of the *trpA* strains used are coisogenic except for the *trpA* allele, UV survival curves were determined for the *trpA* 23 *umuC*⁺ and *umuC* strains and the results assumed to be similar for the other strains. To determine UV survival, cultures were

inoculated from single colonies of the *trpA23 umuC⁺* and *umuC* strains and grown to saturation in L- broth. The cells were washed in saline, serially diluted to approximately 10^3 cells/ml and exposed to varying UV doses under a Sylvania germicidal lamp. Control plates were left unirradiated to determine the total cell count. Irradiated and unirradiated plates were wrapped in aluminum foil to prevent possible photorepair, incubated for 48 hours at 37° C and colonies counted. The total number of viable cells was then deduced from the number of colonies present on each plate. UV survival was expressed as a percentage of the control values.

Results

Cell survival in UV-exposed *umuC*⁺ and *umuC trpA23* strains

UV survival curves were determined for coisogenic *umuC*⁺ and *umuC trpA23* strains and are shown in Figure 1. The percentage of surviving bacteria was measured at UV fluences of 19.5, 39.0, 58.5 and 78.0 J/m². The *umuC* strain shows a small increase in UV sensitivity compared to the *umuC*⁺ strain

Spontaneous mutation and reversion frequencies

Mutation frequencies to Rif^R and Nal^R for *umuC* and *umuC*⁺ strains are shown in Table 1. Both Rif^R and Nal^R mutation frequencies are lower in the *umuC* background compared to *umuC*⁺ values. Since all of the *trpA* strains are coisogenic, the average *umuC* Rif^R and Nal^R mutation frequencies were compared to average *umuC*⁺ frequencies using the student t-test. For both the Rif^R and Nal^R mutation frequencies, *umuC*⁺ values were significantly higher than *umuC* values at the 0.01 level. Only the Nal^R mutation frequency for the *trpA46* strain showed an obviously larger value in the *umuC* strain compared to the *umuC*⁺ frequency and this can be attributed to the large variation in mutation frequencies found in individual strains because of the difficulty in measuring such low values accurately.

UV Survival Curve for TrpA23

Figure 1.

Table 1. Mutation frequencies for *trpA* *Escherichia coli* strains in *umuC*⁻, and *umuC* backgrounds.

<i>trpA</i> allele	Genotype	Rif ^R mutants per 10 ⁸ cells	Rif ^R <i>umuC</i> / <i>umuC</i> ⁻ ratio ^a	Nal ^R mutants per 10 ⁸ cells	Nal ^R <i>umuC</i> / <i>umuC</i> ⁻ ratio ^a
A3	<i>umuC</i> ⁻	8.08	.14	.42	.21
	<i>umuC</i>	1.12		.09	
A11	<i>umuC</i> ⁻	3.91	.15	.36	.75
	<i>umuC</i>	.58		.27	
A23	<i>umuC</i> ⁻	.602	.80	6.5	.03
	<i>umuC</i>	.48		.20	
A46	<i>umuC</i> ⁻	2.13	.25	.22	2.64
	<i>umuC</i>	.54		.58	
A58	<i>umuC</i> ⁻	1.85	.89	.89	.17
	<i>umuC</i>	1.65		.15	
A78	<i>umuC</i> ⁻	4.40	.12	.91	.21
	<i>umuC</i>	.51		.19	
A540 ^b	<i>umuC</i> ⁻	6.71	.09	.25	.48
	<i>umuC</i>	.59		.12	
A9813 ^b	<i>umuC</i> ⁻	1.53	.39	.19	1.11
	<i>umuC</i>	.60		.21	

a. Ratio denotes the increase above the wild-type level.

b. Frameshift mutation

Trp⁺ reversion frequencies were measured for six *trpA* alleles that revert by base-pair substitutions (Yanofsky *et al.*,1966; Drapeau *et al.*,1968) and two *trpA* alleles that revert via frameshift mutations (Brammar *et al.*,1967). Five of the six base-pair substitution *trpA* alleles showed lower Trp⁺ reversion frequencies in the *umuC* background compared to *umuC*⁺ values (Table 2). Only the *trpA11* allele had a higher reversion frequency in the *umuC* strain. Of the two *trpA* frameshift alleles, one (*A540*) had a higher frequency in the *umuC* strain and the other (*A9813*) had a lower frequency.

Table 2. Spontaneous reversion frequencies for base-pair substitution and frameshift *trpA* *Escherichia coli* strains in *umuC*⁺, and *umuC* backgrounds.

<i>trp A</i> allele	Genotype	Trp ⁺ revertants per 10 ⁸ cells	<i>umuC</i> / <i>umuC</i> ⁺ ratio ^a
A3	<i>umuC</i> ⁺	.59	.54
	<i>umuC</i>	.32	
A11	<i>umuC</i> ⁺	.23	2.13
	<i>umuC</i>	.49	
A23	<i>umuC</i> ⁺	3.18	.42
	<i>umuC</i>	1.32	
A46	<i>umuC</i> ⁺	1.15	.11
	<i>umuC</i>	.13	
A58	<i>umuC</i> ⁺	1.65	.26
	<i>umuC</i>	.43	
A78	<i>umuC</i> ⁺	4.23	.71
	<i>umuC</i>	3.00	
A540 ^b	<i>umuC</i> ⁺	1.23	5.85
	<i>umuC</i>	7.19	
A9813 ^b	<i>umuC</i> ⁺	11.0	.24
	<i>umuC</i>	2.62	

a. Ratio denotes the increase above the wild-type level

b. Frameshift mutation

Characterization and distribution of spontaneous Trp⁺ revertants

Trp⁺ revertants of the six *trpA* base-pair substitution alleles were characterized in order to determine whether or not the *umuC* allele affected the mutational spectrum. The reversion pattern and thus specific base-pair substitutions of some *trpA* alleles can be deduced from IGP accumulation and 5-MT inhibition tests independently of amino acid sequence data (Yanofsky *et al.*,1966). The characterization and distribution of Trp⁺ revertants for the six *trpA* alleles in *umuC* and *umuC*⁺ backgrounds are shown in Table 3.

The proportions of Trp⁺ revertants that fall into the various revertant classes for *trpA* alleles, A23, A58 and A78 obviously differ between the *umuC* and *umuC*⁺ backgrounds. With *trpA*23, full revertants (FR), which result from A:T→G:C transitions and A:T→C:G transversions (Yanofsky *et al.*,1966), predominate (68%) in the *umuC*⁺ spectrum while this class is reduced (29%) in the *umuC* strain. In contrast the partial revertant class (III PR) resulting from G:C→T:A transversions (Yanofsky *et al.*,1966) which is a minority (32%) class in the *umuC*⁺ background becomes the largest (71%) class with *umuC*. For the *trpA*58 allele the full revertant (I FR) and a partial (II PR) class decrease in proportion in the *umuC* background as compared to *umuC*⁺ while the second partial revertant class (III PR) increases (Table 3). The I FR and II PR classes of *trpA*58 result

Table 3. Characterization and distribution of spontaneous *trpA* base-pair substitutions.

<i>trpA</i> allele	Revertant class	Substitution pathway	Fraction of revertants in pathway <i>umuC</i> ^a	<i>umuC</i>	<i>umuC</i> ^a	<i>umuC</i>	<i>umuC</i> ^a
A3	I FR	A:T → T:A	1.0(45) ^b	.965(29)	.59	.31	.53
A11	I FR	G:C → C:G	.885(87)	.975(40)	.20	.48	2.40
A23	I FR	A:T → G:C A:T → C:G A:T → T:A	.681(78)	.294(85)	2.16	.39	.18
	II PR	G:C → C:G	0(78)	0(85)			
	III PR	G:C → T:A	.318(78)	.705(85)	1.01	.93	.92
A46	I FR	A:T → G:C A:T → C:G	.710(38)	.882(34)	.82	.11	.14
	II PR	A:T → T:A	.184(38)	0(34)	.21	0	n/a
	III PR	A:T → G:C	.105(38)	.117(34)	.12	.02	.17
A58	I FR ^c	A:T → G:C	.142(63)	.081(49)	.23	.03	.13
	II PR	A:T → C:G	.206(63)	0(49)	.34	0	n/a
	III PR ^d	G:C → A:T	.650(63)	.918(49)	1.07	.40	.37
A78	I FR	A:T → C:G	.203(123)	.013(145)	.86	.04	.05

- a. Reversion frequencies for the particular base-pair substitutions were calculated by multiplying the distribution proportion times the *Trp*⁺ reversion frequency (Table 2).
- b. In parentheses are the numbers of revertants screened. The assignment of revertants to each revertant class (FR, full revertant; PR, partial revertant;) was as described in Materials and methods.
- c. Distribution for *umuC*-*trpA*58 was taken from the study of Schaaper, et al, 1989.
- d. This class results from intergenic suppression of the *trpA*58 allele (Squires and Carbon, 1971).

from A:T→G:C transitions and A:T→C:G transversions respectively (Yanofsky *et al.*, 1966) while the III PR class results from G:C→A:T transitions at an intergenic suppressor site (Squires and Carbon, 1971). The only revertant class of *trpA78* that has been characterized is I FR which results from A:T→C:G transversions (Yanofsky *et al.*, 1966). This event decreases in proportion in the *umuC* strain compared to *umuC*⁺.

As shown in Table 3 generally transitions and transversions at A:T sites are sharply reduced in *umuC* strains compared to *umuC*⁺ frequencies. In contrast base-pair substitutions at G:C base-pairs show smaller or no reductions in the *umuC* background.

UV-induced reversion of *trpA* alleles in *umuC* and *umuC*⁺ strains

In order to examine the role of the *umuC* allele in UV mutagenesis, UV-induced Trp⁺ reversion frequencies for *trpA* alleles were measured in *umuC* and *umuC*⁺ strains. The results of the experiments are shown in Table 4. Five of the six base-pair substitution alleles displayed large reductions in the frequency of UV-induced Trp⁺ revertants in the *umuC* background compared to *umuC*⁺. Only the *trpA23* allele showed a near-normal Trp⁺ reversion frequency with *umuC*. For the *trpA3* and *trpA78* alleles no UV-induced Trp⁺ revertants were recovered in the *umuC* strain while the *trpA11*, *trpA46* and *trpA58* alleles had low, but measurable,

UV-induced reversion frequencies. The UV-induced Trp⁺ reversion frequencies were partially reduced for both *trpA* frameshift alleles (*A540* and *A9813*) with *umuC* (Table 4).

The characterization and distribution for UV-induced Trp⁺ revertants of *trpA23* and *trpA58* were determined and are shown in Table 5. No obvious differences between *umuC* and *umuC*⁺ strains in distribution of revertants were detected for either *trpA* allele.

Table 4. Effect of *umuC* on the frequency of UV-induced reversion of *trpA* base-pair substitutions and frameshift mutations.

<i>trpA</i> allele	Irradiated (39.0 J/m ²)				ratio of <i>umuC</i> ⁺ / <i>umuC</i>
	Revertant colonies/plate ^a	Induced revertants per 10 ⁸ survivors ^b	<i>umuC</i>	<i>umuC</i> ⁺	
A3	<i>umuC</i> ⁺ 400	2	698	0	n/a
A11	235	6	411.7	8.64	47.6
A23	435	211	758.8	728.2	1.04
A46	347	6.25	602.5	12.1	49.7
A58	768.5	34.2	1341.2	105.2	12.7
A78	458.5	9.25	760.7	0	n/a
A540 ^c	326.5	176.6	536.6	440.4	1.22
A9813 ^c	234.5	49.5	373.9	123.2	3.03

a. Mean number of Trp⁺ revertant colonies/plate, each inoculated with approximately 10⁸ washed cells of the indicated *trpA* strain in the *umuC*⁺ or *umuC* background.

b. The mean number of revertant colonies per irradiated plate was corrected for spontaneous revertants by subtracting the mean number of revertant colonies per nonirradiated plate; the mean number of UV-induced revertants per plate inoculated with approximately 10⁸ bacteria thus calculated was divided by the proportion of bacteria surviving irradiation to calculate the number of UV-induced revertants per 10⁸ survivors.

c. Frameshift mutation

Table 5. Characterization and distribution of UV-induced *trpA* base-pair substitutions in *umuC*⁺ and *umuC* strains.

<i>trpA</i> allele	Revert. class	5MT test	IGP test	Substitution pathway	Distribution of UV-induced revertants		
					<i>umuC</i> ⁺	<i>umuC</i>	<i>umuC</i> ⁺
A23	I FR	R	—	A:T→G:C	0.155 (45) ^b	0.222 (54)	121.9
				A:T→C:G			161.7
	II PR	S	+	A:T→T:A			
	II PR	S	+	G:C→C:G	0.711 (45)	0.772 (54)	539.5
A58	II PR	S	+	G:C→T:A	.177 (45)	.074 (54)	134.3
	I FR	R	—	A:T→G:C	0.02 (111)	0.056 (88)	26.8
	II PR ^c	S	+	A:T→C:G	0.02 (111)	0.022 (88)	26.8
	II PR ^d	S	+	G:C→A:T	0.96 (111)	0.920 (88)	1287.5
							5.89
							2.31
							96.7

a. colonies per 10⁸ survivors

b. In parentheses are the numbers of revertants screened. The assignment of revertants to each revertant class (FR, full revertant; PR, partial revertant;) was as described in Materials and methods.

c. Distribution for *umuC* *trpA* 58 taken from the study of Schaaper, *et al.*, 1989.

d. This class results from intergenic suppression of the *trpA* 58 allele (Squires and Carbon, 1971).

Discussion

The sensitivity to UV lethality was modestly enhanced by the presence of the *umuC* allele in this study (Table 1). This is consistent with previous studies that reported limited UV killing in *umuC* strains (Kato and Shinoura, 1977; Sargentini and Smith, 1984). The experiments of Sargentini and Smith (1984) used the same *umuC* allele, *umuC122::Tn5*, as was used in this study and led to the conclusion that the *umuC* gene product is involved in a minor system for the repair of DNA daughter-strand gaps.

The *umuC* and *umuD* alleles were originally isolated as mutants which were non-mutable by UV (Kato and Shinoura, 1977; Steinborn, 1978). Subsequently *umuC* and *umuD* were found to be organized in an operon that, along with several other loci, was inducible by DNA damage (Kitagawa *et al.*, 1985). The inducible expression of these genes is regulated by the *recA* and *lexA* gene products and has become known collectively as the SOS response (Walker, 1984). Active UmuC and UmuD proteins are required for mutation induction by several DNA-damaging agents such as UV and a variety of chemical mutagens (Kato and Shinoura, 1977; Eisenstadt *et al.*, 1980). The *umuC122::Tn5* allele, used in this study was isolated by Elledge and Walker (1983) and has recently been DNA sequenced and found to result in an in frame termination codon at codon 321 of the *umuC* gene (Koch *et al.*, 1992). This results in a truncated protein that lacks 102 carboxyl-terminal amino acids and is assumed to be completely inactive (Koch *et al.*, 1992).

A complex set of events is necessary for the mutagenic activity of the *umuC* and *umuD* gene products. As an initial part of the SOS response the RecA protein causes autocatalytic cleavage of the LexA repressor that derepresses the *umuCD* operon and other SOS genes (Silaty and Little, 1987). The UmuD protein undergoes a similar cleavage in the presence of RecA (Burckhardt *et al.*, 1988; Shinagawa *et al.*, 1988). The cleavage of UmuD does not require UmuC (Woodgate and Ennis, 1991). Cleavage of UmuD produces a mutagenically active C-terminal fragment termed UmuD'. Biochemical evidence has suggested that the UmuC protein forms a complex with a homodimer of UmuD' (Woodgate *et al.*, 1989). The UmuC protein also interacts with the GroE proteins with the half life of UmuC in the absence of UmuD reduced from 17 minutes in wild-type cells to six minutes in groEL mutants (Donnelly and Walker, 1989). It has been suggested that the GroEL and GroES heat-shock proteins stabilize the labile UmuC protein until UmuD' becomes available and able to form a functional complex with UmuC (Donnelly and Walker, 1992). This proposal is consistent with the observation that the GroE proteins are required for full UV mutagenesis (Donnelly and Walker, 1989).

The exact role of the UmuCD' complex in UV and chemical mutagenesis remains unclear. One popular hypothesis suggests that the UmuCD' complex, together with DNA polymerase III and possibly other proteins, allows the polymerase to bypass DNA lesions (such as UV-induced pyrimidine dimers) which block replication (Bridges

and Woodgate, 1984). This model envisages that a nucleotide is inserted opposite a noncoding lesion in the template strand in a reaction that does not require the UmuCD' proteins. In a second step UmuCD' protein activity is needed for replication to continue from the mispaired primer terminus (Bridges and Woodgate, 1985).

Most previous studies have shown that *umuC* mutations decrease UV mutagenesis by 50-fold or more (Kato and Shinoura, 1977; Steinborn, 1978, 1979; Kato and Nakato, 1981; Sargentini and Smith, 1984). While a variety of test systems were used to obtain these data, including both reversion and forward mutation systems, most of these systems detect mainly base-pair substitutions. In contrast Christensen *et al.* (1988) found that when *lac* mutations were selected on media containing 5-bromo-4-chloro-3-indolyl- β -D-galactoside (Xgal) where all surviving cells can form colonies, up to 25% or more of the normal number of *uvrA umuC*⁺ mutations could be recovered in a coisogenic *uvrA umuC122::Tn5* strain. When phenyl β -D-galactoside (Pgal), which prevents post-irradiation growth, was used in the selective media a low number of UV mutations were produced in the *umuC* mutant strain (Christensen *et al.*, 1988). The authors concluded that the UmuC function is not essential to create UV mutations but rather facilitates recovery. When post-irradiation growth occurs the need for this UmuC-dependent recovery is reduced. According to the above described model of Bridges and Woodgate (1984), this recovery

involves the resumption of chain elongation. Bridges and Woodgate (1984) also found that up to 20% of wild-type yields of UV mutations were found in a *umuC122::Tn5* strain if the cells were exposed to photoreactivation following a period of post-irradiation incubation. Again this post-irradiation treatment was interpreted to facilitate the resumption of chain elongation in the absence of a functional UmuC protein.

The limited number of UV mutagenesis experiments done in this present study measured reversion frequencies in *umuC*⁺ and *UmuC122::Tn5* strains. Four of the six base-pair substitution *trpA* alleles showed a nearly 50-fold or more reduction in mutation yield in the *umuC* strain (Table 4). The *trpA58* allele had a 12-fold reduction while the *trpA23* allele had a nearly normal level of UV mutagenesis in the *umuC* strains. The two frameshift alleles, *trpA540* and *trpA9813*, showed modest reductions in mutagenesis in the *umuC* background. With the experimental protocol used in this study of UV mutagenesis, post-irradiated cells were grown on plates supplemented with limiting tryptophan allowing cell divisions to occur before UV-induced Trp⁺ reversions need to be expressed. If the role of UmuC in UV mutagenesis is to facilitate completion of DNA synthesis in mutagen-damaged cells (Bridges and Woodgate, 1984), then possibly the post-irradiation conditions used in this study enhanced the completion of DNA synthesis and the recovery of Trp⁺ revertants in the *trpA* strains that showed significant levels of UV

mutagenesis with *umuC*. Why some *trpA* alleles had higher levels of UV mutagenesis than others in the *umuC* background is not clear. Possibly the *trpA* strains differ in their ability to utilize the limiting tryptophan on the selective media. Recovery of Trp⁺ revertants may be greater with *trpA* strains that can survive longer after irradiation.

A limited comparison was made of the particular base-pair substitutions induced by UV in *umuC* and *umuC*⁺ strains (Table 5). No obvious differences were detected in the distributions of revertant classes. This result is in agreement with the conclusion of Christensen *et al.* (1988) who found similar kinds of *lacI*⁻ mutations induced by UV in *umuC*⁺ and *umuC* strains.

The major focus of this study was to establish whether or not the *umuC* gene has a role in spontaneous mutagenesis in *E. coli*. Compared to the large number of studies to characterize the role of *umuC* in UV mutagenesis, very little is known about a possible *umuC* function in spontaneous mutagenesis. One approach to studying spontaneous mutagenesis is to isolate antimutator alleles which are mutant alleles that lead to a decrease in spontaneous frequencies compared to wild-type values. Subsequent characterization of antimutator strains and identification of the function associated with the mutant gene will elucidate mechanisms that lead to spontaneous mutagenesis. Only a few antimutator alleles have been

recovered in *E. coli* (Quinones and Piechocki, 1985) and they have not been well-studied.

Sargentini and Smith (1981) found an 11-fold decrease in spontaneous mutagenesis in an *uvrB5 umuC36* strain compared to a coisogenic *uvrB5 umuC⁺* strain based upon the reversion of a single *lacZ* nonsense mutation. However the interpretation of this result is complicated by the presence of the *uvrB5* allele in each strain which results in defective excision repair and is itself a weak mutator allele (Sargentini and Smith, 1981). In this study, reversion of several *trpA* alleles and forward mutation to Rif^R and Nal^R were measured in coisogenic *umuC* and *umuC⁺* strains using the nonleaky *umuC122::Tn5* allele. Both strains are assumed to be wild-type for DNA repair and replication genes except for the *umuC* allele.

The *umuC122::Tn5* allele is clearly a weak antimutator for some base-pair substitutions (Table 1 and 2). Forward mutation frequencies to Rif^R and Nal^R were decreased an average of about five-fold in the *umuC* strains. Mutations to Rif^R and Nal^R occur mainly as a result of base-pair substitutions (Miller, 1992). The effect of the *umuC* allele on Trp⁺ reversion frequencies varied among the *trpA* allele (Table 2). For the base-pair substitution alleles *trpA3*, *trpA23* and *trpA78*, the antimutator effect of *umuC* was about two-fold or less. The Trp⁺ reversion frequency for *trpA58* was reduced about four-fold and for *trpA46* about nine-fold. With

the *trpA11* allele *umuC* increased Trp⁺ reversion about two-fold. One of the frameshift *trpA* alleles was decreased about four-fold while the other was enhanced about six-fold by *umuC*.

When individual base-pair substitutions are examined, differential effects of *umuC* at A:T and G:C sites are noted (Table 3). With the exception of A:T→T:A substitutions at the *trpA3* site which only decreased two-fold with *umuC*, transitions and transversions at A:T base pairs decreased five to 20-fold. A decrease in A:T→T:A transversions at the *trpA46* allele and A:T→C:G transversions at *trpA58* could not be calculated since neither of these events occurred in *umuC* strains. In contrast *umuC* did not lower G:C→T:A transversions at *trpA23* and actually caused a two-three-fold increase in the frequency of G:C→C:G transversions with *trpA11*. Only with G:C→A:T transitions that occur at an intergenic suppressor site (Squires and Carbon, 1971) does *umuC* cause a decrease (two-three-fold) in mutation of a G:C base pair. With consideration of the small number of *trpA* alleles used in this study, it appears that the base-pair substitution antimutator activity of *umuC* occurs primarily at A:T base-pairs and suggests the wild-type *umuC*⁺ product may have a mutagenic function at these sites. If the increased frequency of transversions at the *trpA11* site by *umuC* is real, it indicates that *umuC* may act as an antimutator allele at some sites but as a mutator at others. Antimutator alleles in phage

T4 have also been found to have mutator activity for some base-pair substitution pathways (Drake, 1991).

These results indicate that the wild-type *umuC*⁺ gene product contributes toward the production of spontaneous base-pair substitutions at certain sites. Based upon its known involvement in UV mutagenesis and DNA repair, the most obvious hypothesis is that UmuC is part of an error-prone DNA repair system that deals with DNA lesions produced by normal metabolism. There is some evidence of constitutive error-prone repair in *E. coli* B/r (Bridges and Mottershead, 1978). It is also possible that the SOS response is partially or fully induced in a small subset of cells which have suffered some form of DNA lesion from normal metabolism. Mutations are created in the process of repairing this damage. However the *umuC* gene has only been studied in relationship to its role in UV and chemical-induced mutagenesis and DNA repair. It might have an additional function(s) apart from these activities and this may contribute toward the production of spontaneous mutations.

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