Master's Theses   Master's Theses and Graduate Research

2009

The role of dinB in UV survival and UV-induced mutagenesis in Escherichia coli

Mei-Chong Wendy Lee
San Jose State University

Follow this and additional works at: https://scholarworks.sjsu.edu/etd_theses

THE ROLE OF *dinB* IN UV SURVIVAL AND UV-INDUCED

MUTAGENESIS IN *ESCHERICHIA COLI*

A Thesis

Presented to

The Faculty of the Department of Biological Sciences

San José State University

In Partial Fulfillment

of the Requirements for the Degree

Master of Science

by

Mei-Chong Wendy Lee

May 2009

UMI Number: 1470985

INFORMATION TO USERS

The quality of this reproduction is dependent upon the quality of the copy
submitted. Broken or indistinct print, colored or poor quality illustrations
and photographs, print bleed-through, substandard margins, and improper
alignment can adversely affect reproduction.
In the unlikely event that the author did not send a complete manuscript
and there are missing pages, these will be noted. Also, if unauthorized
copyright material had to be removed, a note will indicate the deletion.

UMI®

SAN JOSÉ STATE UNIVERSITY

The Undersigned Thesis Committee Approves the Thesis Titled

THE ROLE OF *dinB* IN UV SURVIVAL AND UV-INDUCED MUTAGENESIS IN *ESCHERICHIA COLI*

by
Mei-Chong Wendy Lee

APPROVED FOR THE DEPARTMENT OF BIOLOGICAL SCIENCES
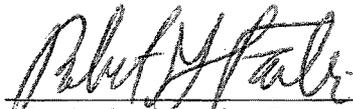
Dr. Robert Fowler, Department of Biological Sciences   Date 4/3/09
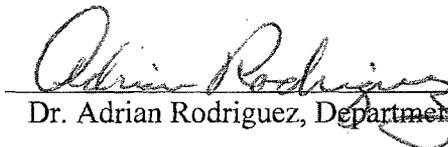
Dr. Adrian Rodriguez, Department of Biological Sciences   Date 4/3/09

Dr. Cleber Ouverney, Department of Biological Sciences   Date 4/3/09

APPROVED FOR THE UNIVERSITY

Associate Dean   Date 5/5/09

ABSTRACT

THE ROLE OF *dinB* IN UV SURVIVAL AND UV-INDUCED MUTAGENESIS IN
ESCHERICHIA COLI

by Mei-Chong Wendy Lee

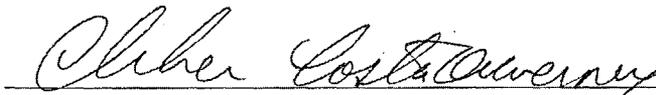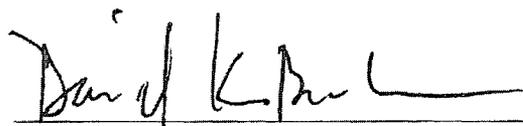This study addresses the possible function of the *Escherichia coli dinB* gene in spontaneous and UV-induced mutagenesis and DNA repair triggered by UV-induced damage. The *trpA* reversion system was used to study spontaneous and UV mutagenesis. This system involved strains carrying specific *trpA* alleles. Revertants were selected that had regained the wild-type phenotype of synthesizing tryptophan. The *trpA* spontaneous and UV-induced revertants were sequenced, and the molecular nature of the original mutations and of the revertants was determined. The *dinB* allele, the *umuC* allele, and the combination of both were examined for their effect on spontaneous and UV mutagenesis. The results show that *dinB* was not significantly involved in spontaneous and UV mutagenesis. The involvement of DinB and UmuC in UV survival was studied through a 20-hour period of growth. It appears that DinB and UmuC were essential for UV repair and cell survival in the log phase of growth. The effect of *dinB* on UV survival and UV mutagenesis was dependent upon an active *umuC* allele. In contrast to the *dinB* strain with a polar deletion mutation that showed a higher level of UV sensitivity, a *dinB* strain with a missense nonpolar mutation showed no UV sensitivity. This suggests that either a downstream gene from *dinB* is actually responsible for protection from UV damage or the function of DinB is not dependent upon its polymerase activity.

ACKNOWLEDGEMENTS

I would like to thank Dr. Robert Fowler for his support and guidance throughout the process of my research and the preparation of this thesis. I am extremely grateful to Magdalena Franco who has worked with me on my research and for always being supportive. I want to thank Mouna Jabri for performing most of the transductions and for preparing the culture media. A special gratitude must also be extended to Nancy Fong, Ricardo Leitão, Vicki Tran, and Paula Matheus for proofreading this thesis. Great appreciation is also due to Dr. Adrian Rodriguez and Dr. Cleber Ouverney for reviewing this thesis. The completion of this thesis would not have been possible without the support from my parents, family, and friends. Last but not least, I would like to thank my loving husband, Dominic Abucejo, for his unconditional love and encouragements.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

1. INTRODUCTION

Ultraviolet (UV) light can damage DNA by causing the formation of pyrimidine (6 − 4) pyrimidone photoproducts and cyclobutane dimer. Photoreactivation and nucleotide excision repair (NER) are the two common mechanisms for repairing UV-induced lesions in *Escherichia coli*. However, if the lesion is not repaired, the replicative DNA polymerase (Pol III) often stalls at the replication fork when it encounters a damaged DNA template base. The SOS regulatory network will then be activated. One repair mechanism in the SOS response involves translesion DNA synthesis where novel DNA polymerases catalyze DNA synthesis past DNA lesions. This is also known as a DNA-tolerance mechanism because the damage is not removed. This is a way to avoid cell death as a consequence of unrepaired or unrepairable damage to genomes. Approximately 40 genes are involved in the SOS regulatory network. There are three SOS genes encoding DNA polymerases: Pol II, Pol IV (DinB), and Pol V (UmuC). Pol II is a high-fidelity polymerase that belongs to the B-family of polymerases. DinB and UmuC are error-prone polymerases that are members of the Y-family polymerases. Little is known about the role of DinB in UV mutagenesis and UV survival. UmuC and Pol II were found to be involved in UV repair in previous studies. In this study, the roles of the *dinB* gene in UV survival and UV and spontaneous mutagenesis, and its relationship with *umuC* are investigated. The effect upon UV survival of the polar deletion mutation in *dinB* is compared with that of a nonpolar *dinB* missense mutation.

1

2. LITERATURE REVIEW

2.1 UV damage and nucleotide excision repair

UV light can cause two adjacent pyrimidine molecules to dimerize. A process called photoreactivation can revert the dimerization. Photoreactivation occurs when a photon activates an enzyme, photolyase, encoded by the *phr* gene and splits the pyrimidine dimer apart (Russell, 2003). Another repair mechanism, nucleotide excision repair (NER), can correct the pyrimidine dimer without the need of light. It can also repair DNA that has serious damage-induced helix distortion. There are four proteins that are involved in NER: UvrA, UvrB, UvrC, and UvrD, encoded by *uvrA, uvrB, uvrC, and uvrD*, respectively (Russell, 2003). First, two UvrA proteins and one UvrB protein form a complex and slide along the DNA. When the complex recognizes a pyrimidine dimer, the UvrA proteins dissociate. UvrC then binds to UvrB at the site of the lesion. UvrC makes a cut four or five nucleotides to the 3' side of the lesion and a cut about eight nucleotides to the 5' side of the lesion. After UvrB is released, UvrD binds to the 5' cut, unwinds the region between the cuts, and releases the short single-stranded DNA. DNA polymerase I fills in the gap in the 5' → 3' direction, and DNA ligase seals the gap. If the UV-induced DNA damage is too severe, some of the lesions can remain unrepaired. The replicative polyermase (Pol III) will arrest when it encounters the UV-induced DNA lesion, and the SOS response will be triggered.

2.2 SOS transcription regulations

SOS-regulated gene expression is under very stringent control. The gene products of the *lexA* gene and the *recA* gene regulate the expression of the SOS-regulated genes. In *E. coli* cells that have not been exposed to DNA damage, the product of the *lexA* gene acts as a repressor of more than 40 genes by binding as a homodimer to similar operator sequences to each operon. The operator sequences that bind to the LexA protein are commonly referred to as SOS boxes (Friedberg *et al.*, 2006). Many of these SOS genes, including *recA* and *lexA*, are expressed at significant levels even in the repressed state. In particular, RecA protein expresses at approximately 7,200 molecules per cell in uninduced cells (Sassanfar and Roberts, 1991). When replication is stalled by DNA damage, an intracellular signal for SOS induction is initiated. The *recA* gene product binds to the single-stranded DNA produced at the replication fork, forming a nucleoprotein filament in the presence of nucleoside triphosphates (Craig and Roberts, 1980). LexA molecules then diffuse to the deep grooves in the RecA-ssDNA filaments. The interaction of LexA with the RecA nucleoprotein filament results in proteolytic cleavage of LexA at a specific Ala-Gly bond near the middle of the protein (Friedberg *et al.*, 2006). Cleavage of LexA protein inactivates LexA as a repressor. The cleaved LexA proteins are targeted for destruction. As LexA cleavage proceeds after an SOS-inducing treatment, the pool of LexA proteins begins to decrease. A number of SOS genes, including *recA*, *dinB*, and *umuDC*, are expressed at increased levels, and SOS-induced DNA repair and tolerance begins. As the cells begin to recover from the SOS-inducing treatment, the region of ssDNA disappears as a consequence of various DNA repair and

tolerance processes (Friedberg *et al.*, 2006). The amount of RecA protein present in RecA-ssDNA filaments declines. Continued synthesis of LexA protein then leads to an increase in the LexA pool, which leads to a repression of the SOS genes.

2.3 Unique features of Y-family polymerases

As mentioned earlier, DinB and UmuC are error-prone Y-family polymerases. Y-family polymerases share no clear sequence homology with replicative polymerases, but their structures reveal a similar right-hand fold consisting of a thumb, palm, and fingers domain (Ling *et al.*, 2001). These polymerases have an additional little finger domain that seems to play an important part in substrate specificity and processivity (Boudsocq *et al.*, 2004). It has been shown that the little finger domain interacts with the processivity clamp known as the β clamp. In the presence of the β clamp, Y-family polymerases exhibit 300-fold enhanced processivity (Wagner *et al.*, 2000). Unlike the tight grip seen in the active sites of replicative DNA polymerases, Y-family polymerases have an open active site that is relatively solvent-accessible (Johnson *et al.*, 2003). It is believed that the open active site is essential for accommodating the bulky adducts on the damaged DNA template (Ling *et al.*, 2001). An α-helix responsible for a geometric check to ensure the fidelity of the incipient base pair in the replicative DNA polymerases is absent in Y-family polymerases (Jarosz *et al.*, 2007). Additionally, Y-family polymerases lack 3' to 5' exonuclease (proofreading) activity. Thus, most translesion syntheses are inherently error prone. Y-family polymerases typically exhibit 10-1000-fold lower fidelity than replicative DNA polymerases when replicating undamaged DNA (Friedberg

et al., 2006). However, they can perform translesion syntheses with high accuracy on certain types of damaged templates (Tang *et al.*, 2000). Y family polymerases such as the human Pol η (XP-V Pol) can replicate UV-induced DNA lesions with relatively high fidelity (Kusumoto *et al.*, 2004).

2.4 Role of DinB in mutagenesis

The Y-family proteins that belong to the DinB family involved in translesion synthesis are the most widespread and conserved. DinB family proteins can be found in all three kingdoms of life: bacteria, archaea, and eukaryotes (Ohmori *et al.*, 2001). The *E. coli* DinB protein, encoded by *dinP*, was first isolated in 1995 (Ohmori *et al.*, 1995). Later researchers discovered that *dinP* is allelic to *dinB*, a damage-inducible gene identified 15 years earlier by Kenyon and Walker in 1980 (Kim *et al.*, 1997). DinB is the fourth DNA polymerase identified in *E. coli*, and was termed DNA polymerase IV (Pol IV) (Wagner *et al.*, 1999). The *dinB* gene is part of an operon of four genes: *dinB, yafN, yafO,* and *yafP* (Blattner *et al.*, 1997). The molecular weight of DinB protein is 32,000 daltons, and its intrinsic polymerase activity has been demonstrated (Wagner *et al.*, 1999). Around 250 DinB molecules are found in each cell, and the number increases by 10-fold following SOS-induction (Kim *et al.*, 2001). As mentioned in the previous section, RecA is a protein that plays an important part in the SOS regulatory network. It appears that the *dinB* pathway does not require the RecA function once it is induced by the SOS response (Kim *et al.*, 1997). However, recent studies showed that DNA damage inducible proteins, $UmuD_2$ and RecA, act in concert to modulate the mutagenic activity of

DinB (Godoy *et al.*, 2007).

The function of DinB is not clearly understood. DinB adds template-directed nucleotides *in vitro* in a distributive manner (Woodgate, 1999). It can efficiently extend a 3' primer terminus that is misaligned with a bulged template (Ohashi *et al.*, 2000). However, the replication product resulting from the translesion synthesis by DinB is one nucleotide shorter than expected. This finding is consistent with the *dinB*-dependent −1 frameshift events observed *in vivo* (Kim *et al.*, 1997; Wagner *et al.*, 1999; Ohashi *et al.*, 2000).

The level of spontaneous mutagenesis in *E. coli* cell increases when *dinB* is overexpressed (Strauss *et al.*, 2000; Lenne-Samuel *et al.*, 2002; McKenzie *et al.*, 2001; Jacob *et al.*, 2007). Overproduction of DinB leads to a dramatic increase in spontaneous −1 G frameshift mutations within a G6 cluster sequence (Kim *et al.*, 1997). The DinB mutator effect shows that a -1G event is 10-fold more frequent than a +1G event (Kim *et al.*, 1997; Wagner *et al.*, 2000). DinB overexpression includes an increase in base substitutions; with an obvious bias for substitutions towards GC base pairs, and about 70% of them occurred in 5'-GX-3' sequences, where X represents the base (T, A, or C) that is mutated to G (Wagner *et al.*, 2000). The base substitutions are predominately G:C → T:A (96-fold) transversions as compared to G:C → A:T (48-fold) transitions (Kim *et al.*, 1997).

Another study showed that deletion of *dinB* resulted in about a 75% decrease in spontaneous frameshift and base substitution mutations in *dnaE* mutator strains containing an impaired Pol III (Strauss *et al.*, 2000). A 1.5- to 3-fold reduction in *dinB*-

induced spontaneous mutagenesis was also found in a *dinE*[+] control strain (Strauss *et al.*, 2000). These findings suggest that some of the spontaneous mutations occurring in cells are due to the action of DinB.

In contrast, other studies suggest that *dinB* does not contribute to spontaneous mutagenesis when it is expressed in basal levels in dividing cells (Tago *et al.*, 2005; Kuban *et al.*, 2005). Kuban *et al.* (2005) found that a lack of functional DinB did not significantly reduce the mutation frequencies in growing mismatch repair-deficient cells. This suggests that DinB does not contribute significantly to the normal chromosomal error rate (Kuban *et al.*, 2005).

The aforementioned findings regarding the role of DinB in spontaneous mutagenesis are conflicting. McKenzie *et al.* (2003) attempted to resolve this conflict by finding that spontaneous mutagenesis is reduced by a polar *dinB* allele, in which *dinB* and the first few nucleotides of the neighboring *yafN* gene are deleted, but not by a nonpolar missense *dinB* allele. It has been suggested that one or more of the genes downstream of *dinB* may affect growth-dependent mutations (McKenzie *et al.*, 2003). However, Kuban *et al.* (2005) studied the effect of the polar and nonpolar *dinB* alleles on spontaneous mutagenesis with the same strains used in McKenzie's study (2003) and found no significant effect of either strain on spontaneous mutagenesis. Hence, the role of DinB in spontaneous mutagenesis remains elusive.

dinB associated -1 frameshift mutagenesis was also found in adaptive mutagenesis (McKenzie *et al.*, 2001; Tompkins *et al.*, 2003), a type of spontaneous mutagenesis that can occur in bacteria during periods of prolonged stress in non-dividing or very slowly

dividing populations (Hall, 1998). Pol IV is required for most adaptive point mutations, but apparently not for mutations in growing cells (McKenzie *et al.*, 2001).

DinB was not found to be involved in UV mutagenesis (Courcelle *et al.*, 2005). In another study, *dinB* was shown to have a modest effect on UV mutagenesis. Deletion of *dinB* slightly reduced UV mutagenesis (Wrzesiński *et al.*, 2005).

The *dinB* gene is required for resistance to some DNA-damaging agents that form adducts at the N^2-position of deoxyguanosine (dG) (Jarosz *et al.*, 2006). Although DinB has the potential -1 frameshift mutagenic activity, it can perform lesion bypass synthesis past N^2-deoxyguanosine adducts with great accuracy (Jarosz *et al.*, 2006). It was shown that by mutating a single amino acid, such as the 'steric gate' residue of DinB (Phe13 →Val), the lesion-bypass polymerase would only replicate an undamaged template (Jarosz *et al.*, 2006). DinB was found to be modestly efficient at replicating past N-guanine-linked DNA-peptide cross-links upon exposure to an α,β-unsaturated aldehyde, acrolein, and the incorporation of the correct nucleotide opposite the lesions is highly favored (Minko *et al.*, 2008).

2.5 Mutagenic function of *umuDC*

Earlier studies found that induction of two SOS genes, *umuC* and *umuD*, is required for UV mutagenesis in *E. coli* (Kato and Shinoura, 1977). The *umuDC* genes encode a translesion DNA polymerase, DNA Pol V, in *E. coli*. The *umuDC* operon encodes both the UmuD and UmuC proteins. The gene less proximal from the *umuDC* operator is *umuC*, and it produces a protein synthesized from an overlapping (1bp) reading frame

(Perry *et al.*, 1985). UmuC has a molecular weight of 45,000 daltons, and the UmuD protein is about 16,000 daltons (Elledge and Walker, 1983). In an uninduced cell, there are approximately 180 UmuD molecules and an undetectable amount of UmuC protein. Once the SOS response is initiated, about 2,400 UmuD molecules and 200 molecules of UmuC are detected in a cell (Godoy *et al.*, 2007). Pol V exhibits low processivity by adding six to eight nucleotides before dissociating from the replication site (Tang *et al.*, 2000). In the presence of the non-hydrolyzable ATPγS, Pol V does not require the Pol III's processivity β,γ-complex for translesion synthesis (Tang *et al.*, 2000). When copying undamaged DNA, Pol V shows low fidelity with error rates of about 10^{-3} to 10^{-4}, while Pol IV is 5- to 10-fold more accurate (Tang *et al.*, 2000).

Unlike DinB, UmuDC requires accessory proteins for translesion synthesis (Friedberg *et al.*, 2006). RecA stabilizes UmuD by mediating the post-translational proteolytic processing that removes 24 amino acids from the N-terminal of UmuD, thereby producing the UmuD' protein (Burckhardt *et al.*, 1988; Nohmi *et al.*, 1988). It was shown that RecA also directly stabilizes UmuD'. It does not appear that UmuC is directly stabilized by RecA, but it is dramatically stabilized in the presence of UmuD' (Frank *et al.*, 1996). UmuD' has an active carboxyl terminal fragment that is required for SOS mutagenesis (Burckhardt *et al.*, 1988; Shinagawa *et al.*, 1988). UmuD'$_2$C adds template-directed nucleotides in a distributive manner (Tang *et al.*, 2000). Studies showed that purified UmuD'$_2$C could not carry out translesion synthesis unless RecA was present (Tang *et al.*, 2000). The effects of RecA protein on Pol V caused a 15,000-fold increase in DNA synthesis efficiency (Tang *et al.*, 2000). In another study, a purified

MalB-UmuC chimeric protein was shown to be capable of carrying out translesion synthesis when UmuD'$_2$, RecA, and SSB (single-stranded DNA binding protein) were all present (Reuven *et al.*, 1999). The presence of RecA and SSB increased UmuD'$_2$C activity by 340- and 1040-fold, respectively (Pham *et al.*, 2001). The MalB-UmuC chimeric protein alone could carry out limited DNA synthesis on undamaged template DNA (Reuven *et al.*, 1999).

Like other Y-family polymerases, UmuD'$_2$C lacks proofreading activity and possesses lower fidelity. The observed mutation rate was 41-fold higher than Pol III holoenzyme (Maor-Shoshani *et al.*, 2000). However, UmuD'$_2$C bypasses a site-directed abasic (loss of pyrimidine) lesion with an efficiency of about 100- to 150-fold higher than the Pol III holoenzyme complex (Tang *et al.*, 1999). Mutations in *umuC* and/or *umuD* reduce UV mutability by more than 100-fold and slightly reduce UV sensitivity (Tang *et al.*, 2000). Additional evidence came from translesion synthesis over three well-studied lesions: a *cis-syn* thymine-thymine photodimer, a thymine-thymine (6-4) photoproduct, and an abasic (apurinic / apyrimidinic) site, suggesting that Pol V is indeed responsible for most SOS mutagenesis (Tang *et al.*, 2000). A mutagenic characteristic of Pol V is that it incorporates guanine opposite the 3'-thymine of a TT (6-4) photoproduct (Tang *et al.*, 2000). UmuD'$_2$C preferentially incorporates an adenine opposite the 3' pyrimidine of a thymine-thymine *cis-syn* photodimer and opposite abasic sites (Tang *et al.*, 2000). The *recA* and *umuC* genes are also involved in spontaneous base-pair substitution mutagenesis (Bhamre *et al.*, 2001).

2.6 The roles of Pol II in replication restart and translesion synthesis

Pol II, a B-family polymerase, was discovered in 1970 (Rangarajan *et al.*, 1999). It synthesizes DNA accurately due to its 3' to 5' exonuclease (proofreading) activity (Cai *et al.*, 1995). Its polymerase activity is increased sevenfold in response to DNA damage induced by UV irradiation (Bonner *et al.*, 1988). Upon SOS induction, the number of Pol II molecules in a cell is increased from 50 to 350 (Qiu and Goodman, 1997). Pol II is encoded by the *dinA* gene, also known as *polB*, that is regulated transcriptionally by the LexA repressor (Qiu and Goodman, 1997). The absence of Pol II in a cell does not exhibit any adverse effect upon UV survival unless pol V is also missing (Rangarajan *et al.*, 1999). Double mutants of Pol II and Pol V show approximately 2- to 3-fold more UV sensitivity than Pol V mutants (Rangarajan *et al.*, 1999). Pol II plays a pivotal role in replication restart. Replication restart is a DNA damage recovery phenomenon. Following DNA damage-induced transient partial inhibition of DNA synthesis, DNA synthesis recovers to its predamage rate in about 30 to 45 min (Friedberg *et al.*, 2006). Deletion of *polB* impairs the ability for the cell to carry out replication restart (Rangarajan *et al.*, 1999). Pol II is induced within one minute upon UV exposure, while the induction of Pol V occurs 45 min after UV irradiation (Sommer *et al.*, 1998; Goodman, 2007). Pol II copies the correct information from the uncoupled nascent lagging strand formed during the RecA nucleoprotein-mediated regression. The lesion is bypassed accurately by Pol II. The post-UV DNA synthesis in cells that lack Pol II is delayed significantly and the synthesis depends on UmuD'$_2$C (Rangarajan *et al.*, 1999). It appears that both Pol II-dependant and Pol V-dependant replication restart also require

Pol III (Rangarajan *et al.*, 1999). Replication restart occurs around 90-100 min post-UV in the double Pol II and Pol V mutant, which indicates that there are at least three genetically independent pathways of replication restart (Rangarajan *et al.*, 1999).

Pol II is used for translesion synthesis in copying abasic lesions in the absence of induction of the GroEL$^+$S$^+$ heat shock proteins (Tessman and Kennedy, 1994). These heat shock proteins are chaperones that help stabilize the UmuC protein needed for SOS repair. This might in turn increase the formation of functional UmuC complexes and eliminate the need for Pol II (Tessman and Kennedy, 1994).

2.7 Modulation of function by protein-protein interactions

It is unclear how the cell manages its numerous DNA polymerases and determines which enzyme gains access to the primer terminus. It appears that in *E. coli*, expression of DNA polymerases II, IV and V is increased after the induction of SOS response due to DNA damage. This indicates that part of the control system is at the transcription level (Friedberg *et al.*, 2006). The activity of Pol V is controlled at the post-translational level. The activity of UmuC also depends on the form of UmuD present in the substrate. UmuD must be cleaved to UmuD' in order for UmuC to function as a DNA polymerase (Friedberg *et al.*, 2006).

The interactions between UmuD and RecA in regulating *umuC*-dependent mutagenesis have been thoroughly studied in the past 30 years (Friedberg *et al.*, 2006). As described in the previous section, *umuD and umuC* share the same operon. The *umuDC* gene products play two unique and temporally distinct roles in DNA damage

12

tolerance (Sutton *et al.*, 1999). UmuD and UmuD'$_2$ proteins interact not only with the β

processivity clamp but also with the α (catalytic) and ε (proofreading) subunits of Pol III

(Sutton *et al.*, 1999). Interestingly, UmuD$_2$ and UmuD'$_2$ interact differently with the β

and α subunits. UmuD, which is associated with the DNA checkpoint, interacts more

strongly with the β subunit than with the α alpha subunit. In contrast, UmuD' protein, the

cleaved form of UmuD, associated with mutagenesis and translesion synthesis, interacts

more strongly with the α subunit than then the β subunit (Sutton *et al.*, 1999). The study

performed by Opperman *et al.* (1999) suggested that UmuD and UmuC delayed the

resumption of DNA replication to allow nucleotide excision repair additional time to

repair the damage accurately before replication is resumed. If the lesion cannot be fixed

in about 20 min, the ssDNA:RecA nucleoprotein filament induces the autoproteolytic

process of UmuD, and UmuD' becomes the predominant form available to interact with

UmuC (Opperman *et al.*, 1999; Burckhardt *et al.*, 1988). UmuD'$_2$ and RecA are required

for UmuC to perform translesion synthesis (Tang *et al.*, 1999; Reuven *et al.*, 1999).

UmuC, UmuD, and RecA are believed to function in concert with the replicative

polymerase, the Pol III holoenzyme (Friedberg *et al.*, 2006).

When Pol IV (Din B) interacts with the β,γ-complex of the DNA Pol III, the

efficiency of translesion synthesis is increased by 3,000-fold (Lenne-Samuel *et al.*, 2002;

Tang *et al.*, 2000). Lenne-Samuel *et al.* (2002) identified the extreme C-terminal peptide

of DinB as being essential for the interaction with the β clamp. If the C-terminal peptide

of DinB is mutated, it cannot participate in error-free or frameshift mutagenic translesion

synthesis (Lenne-Samuel *et al.*, 2002). DinB was found to be responsible for detaching

Pol III from the β-clamp when Pol III idled at the replicative fork *in vitro* (Furukohri *et al.*, 2008). A recent study has shown that Pol IV is able to slow replication fork progression with the authors concluding that this may allow time for lesions to be removed by excision repair before the fork reaches them (Indiani *et al.*, 2009). DinB has a concentration-dependent inhibitory effect on a moving Pol III in catalyzing rapid chain elongation on the template (Uchida *et al.*, 2008). The inhibitory effect of DinB does not seem to require the presence of its β-binding motif and its polymerase activity (Uchida *et al.*, 2008). This suggests that DinB has at least two distinct roles: translesion synthesis and inhibiting Pol III at the replication fork.

Recently, it has also been shown that UmuD, UmuD', and RecA acting together also regulate both the activity and the mutagenic properties of DinB through protein-protein interactions (Godoy *et al.*, 2007). The ternary complex of UmuD, RecA, and DinB was isolated and was stable on the minute timescale (Godoy *et al.*, 2007). The study showed that DinB-dependent -1 frameshift mutagenesis could be suppressed by the co-overproduction of UmuD. A noncleavable UmuD variant completely suppressed DinB-dependent -1 frameshift mutagenesis (Godoy *et al.*, 2007). The DinB-binding deficient UmuD variant can perform RecA-mediated autoproteolysis, but cannot reduce DinB-dependent -1 frameshift mutagenesis (Godoy *et al.*, 2007). When additional RecA and UmuD$_2$ are added to an assay in which DinB replicates a template, it results in a remarkable increase in DinB catalytic proficiency with a properly paired terminus. It appears that UmuD$_2$ and RecA modulate the mutagenic potential of DinB by enclosing its open active site (Godoy *et al.*, 2007).

3. MATERIALS AND METHODS

3.1 Bacterial strains

The *E. coli* strains and *trpA* mutants used in this study are presented in Table 1.

Table 1. *Escherichia coli* strains used in this study

Strain	Relevant genotype	Source
KD1094	*trpA223, arg, leu, his, thr*	Cox *et al.*, 1972
SR1282	*trpA540*	Sargentini and Smith, 1987
SR1284	*trpA9813*	Sargentini and Smith, 1987
KD1093	*trpA78, arg, leu, his, thr*	Cox *et al.*, 1972
KD1092	*trpA58, arg, leu, his, thr*	Cox *et al.*, 1972
KD1117	*trpA11*	Yanofsky and Crawford, 1972
YG7207	Δ(*dinB-yafN*)::kan^R [a] derivative of AB1157	Kim *et al.*, 1997
RW82	Δ*umuDC*595::cat derivative of AB1157	Woodgate, 1992
AB1157	F$^-$ *thr-1 leuB6 proA2 his4 thi1 argE3 lacY1 galK2 rpsL supE44 ara-14 xyl-15 mtl-1, txs-33*	Sargentini and Smith, 1989
SMR5830	SMR4562 *dinB10* [F' *dinB10 proAB$^+$lacI33ΩlacZ*] [b]	Rosenberg *et al.*, 2003
SMR4562	Δ *(lac-proAB)*$_{XIII}$ *thi ara* Rif^r[F' *dinB$^+$ proAB$^+$* lacI33ΩlacZ]	Rosenberg *et al.*, 2003
RF01	*trpA223 dinB*	KD1094 x *P1virA* YG7207
RF02	*trpA223 umuC*	KD1094 x *P1virA* RW82
RF03	*trpA223 dinB umuC*	RF02 x *P1virA* RW82
RF04	*trpA540 dinB*	SR1282 x *P1virA* YG7207
RF05	*trpA9813 dinB*	SR1284 x *P1virA* YG7207
RF06	*trpA11 dinB*	KD1117 x *P1virA* YG7207
RF07	*trpA58 dinB*	KD1092 x *P1virA* YG7207
RF08	*trpA78 dinB*	KD1093 x *P1virA* YG7207

[a] The Δ(*dinB-yafN*)::kan^R strain (YG7207) will be referred to as *dinB*Δ in this study.

[b] The *dinB* and *yafN-yafP* genes are present on both the chromosome and F', therefore, homozygous mutant strains were constructed (McKenzie *et al.*, 2003).

3.2 Media

Minimal medium (MM) plates contain Vogel-Bonner salts (Vogel and Bonner, 1956), solidified with 1.5% agar (Difco Laboratories), and supplemented with 0.2% glucose, vitamin B1 and 50 μg/ml of amino acids. LB (Luria-Bertani) broth consists of 1% tryptone, 0.5% yeast extract and 0.5% NaCl. Tryptone plates consist of 1% tryptone and 0.5% NaCl solidified with 1.5% agar. Transductants were selected on tryptone plates containing kanamycin. The saline for dilutions and washing was 0.85% NaCl. Soft LCTG agar consists of 1% tryptone, 0.5% NaCl, 0.5% yeast extract, 0.1% glucose, and solidified with 0.65% agar. LCTG plates contain 5% tryptone, 2.5% NaCl, 2.5% yeast extract, 0.5% glucose, and solidified with 7.5% agar. The LCTG medium was supplemented with 5 mM CaCl2 and 0.25% of thymidine.

3.3 Lysate preparation

P1 lysates were prepared for the *umuC* and *dinB*Δ (YG7207) donor strains. Soft LCTG agar was microwaved for 30 sec and kept in a 60 °C water bath. 3 ml aliquots were made for each donor strain. The donor strain (100 μl) and phage (5 μl) (P1 *virA*) were added to each aliquot, and mixed. The mixture was then pored onto freshly made LCTG plates and kept at 65 °C for 5 min. Control plates were made for each donor strain by spreading 100 μl of the donor strain without phage onto the LCTG plate. The plates were incubated at 37 °C for 24 to 48 hr. If there was bacterial growth on the plates after incubation, the soft agar layer was scraped off, rinsed with 3 ml of LB and 5 mM CaCl$_2$, and transferred to a test tube. Four drops of chloroform was added to each tube. The

tube was then centrifuged for 15 min at full speed. The supernatant was then collected

and transferred into a glass tube, and 70 μl of chloroform was added. The supernatant

was store at 5 °C or used immediately for transduction.

3.4 Transductions

P1 transductions were carried out to transfer the *dinB*Δ allele into KD1094

trpA223, KD1093 *trpA78*, SR1284 *trpA9813*, SR1282 *trpA540*, KD1092 *trpA58*, and

KD1117 *trpA11*. Strains *trpA223 dinB*Δ *umuC* and *trpA223 umuC* were constructed by

P1 transduction using donor strain RF01 *trpA223 dinB* and KD1094 *trpA223*,

respectively. The transductions were done according to the protocol described by

Lennox (1955). The recipient strains were grown to saturation in 5 ml of L-broth. 200 μl

of 30 mM $MgSO_4$ and 15 mM $CaCl_2$, 200 μl of recipient strain, and 25 μl of lysate were

mixed in an Eppendof tube and incubated at 37 °C for 20 min. It was then centrifuged for

1-2 min, and the supernatant was discarded afterwards. The pellet was resuspended with

0.1 ml of 1x Vogel-Bonner salts (Vogel and Bonner, 1956). The mixture was spread onto

kanamycin plates to select for *dinB*Δ transductants, and onto chloramphenicol plates to

select for *umuC* transductants. Control plates were prepared by spreading the lysate and

recipients on the antibiotic plates separately.

3.5 UV survival of *dinB*Δ

All four strains, *dinB⁺ umuC⁺*, *dinB⁺ umuC*, *dinB*Δ *umuC⁺*, *and dinB*Δ *umuC*,

were used in a 20-hour survival study to determine their ability to survive after UV

irradiation. The *dinB*Δ mutant in this study had a polar mutation, where most of the *dinB*

gene and the first part of the downstream *YafN* gene are deleted. An overnight culture was made for each strain in 5 ml L-broth; 1 ml of the overnight culture was then added to 50 ml of fresh L-broth and incubated at 37 °C in a shaker.

For UV survival, a 5 ml aliquot of the bacterial culture was collected every 30 min, washed, resuspended in 500 µl of 0.85% saline, and spread onto two tryptone plates to titer the total number of surviving cells. One of the tryptone plates was exposed to UV irradiation at 25 J/m^2 for 60 sec and then wrapped in aluminum foil, and the other was unexposed. The tryptone plates were incubated at 37 °C overnight and then the resulting total cells were counted. The percent survival was calculated by dividing the average number of cells that survived the UV-irradiation on the tryptone plate by the average number of cells found on the non-irradiated tryptone plate. The growth curves for all four strains (Fig. 1) were constructed by measuring the absorbance of the bacterial culture every 30 min at 550 nm (Jenway 6405 Spectrophotometer, Jenway, England).

3.6 UV survival of *dinB10*

The strain *dinB10* (SMR5830) contains a nonpolar *dinB* missense mutation that does not affect the downstream genes *YafN*, *YafO*, and *YafP* (McKenzie *et al.*, 2003). An overnight culture was made for the *dinB10* strain and an isogenic *dinB*$^+$(SMR4562) strain in 5 ml L-broth; 1 ml of the overnight cultures were then added to 50 ml of fresh L-broth and incubated at 37°C in a shaker. 5 ml aliquots of the bacterial cultures were collected between one to three hours of incubation, washed, re-suspended in 500 µl of 0.85% saline, and spread onto two tryptone plates for each strain. One of the tryptone plates was

exposed to UV irradiation at 25 J/m^2 for 60 sec and then wrapped in aluminum foil, and the other was not exposed. The tryptone plates were incubated at 37 °C for 24 hrs and then the resulting cells were counted. The percent survival was calculated by dividing the number of cells that survived the UV irradiation by the number of cells found on the non-irradiated tryptone plate.

3.7 UV-induced mutagenesis of *trpA223* and *trpA78*

The Trp$^+$ reversion frequency was measured by inoculating individual colonies from each of the *trpA223* and *trpA78* strains in 5ml of L-broth and incubating at 37 °C for 24 or 48 hr. The cells were then concentrated and washed by centrifugation and resuspended with 500 μl of 0.85% saline. Serial dilutions were made and were plated on minimal media without tryptophan to select for Trp$^+$ revertants. Tryptone plates were used to titer for total cells. Three to six plates were exposed to 25 J/m^2 UV light from a Sylvania germicidal lamp at 254 nm. The other plates were not exposed to UV light to detect any reversion due to spontaneous mutagenesis. Irradiated plates were wrapped in aluminum foil to prevent photorepair. The minimal medium plates were incubated for 4 to 5 days at 37°C and the number of revertants was determined by counting the resulting colonies. The tryptone plates were incubated overnight and the resulting colonies counted. The Trp$^+$ revertants were purified by restreaking each revertant colony on the same medium. The colonies that did not grow on the new minimal medium plate were assumed to be not true revertants and subtracted from the original revertant counts. The number of UV-induced revertants per 10^8 surviving cells was calculated by subtracting

the average number of Trp$^+$ revertants on the irradiated plates by the average number of Trp$^+$ revertants per non-irradiated plate and dividing the result by the average titer of cells that survived the irradiation.

3.8 Spontaneous mutagenesis and reversion frequencies

Spontaneous mutation frequencies were determined for each strain in both wild-type (*dinB$^+$*) and *dinB*Δ and *umuC* backgrounds. Overnight cultures were made from a small inoculum and grown to saturation at 37 °C in L-broth. The cells were washed in saline, diluted when appropriate, and 0.1 ml of samples of each culture spread on minimal plates without tryptophan to determine the number of revertants found in each strain. The number of total cells was determined by plating on tryptone plates. The reversion frequencies were calculated by dividing the average number of revertants by the average number of total cells.

3.9 PCR amplification of the *trp$^+$* gene

Direct DNA sequencing was performed to determine the changes in the nucleic acid sequence that gave the *trpA223* revertants the Trp$^+$ phenotype. The DNA of each strain was isolated by suspending a single colony in 30 μl of sterile water in an Ependorff tube. The tubes were placed in a boiling water bath for 2 min and were frozen at -70 °C. The samples were then centrifuged for 2 min. The supernatant was collected which contained the DNA. For each strain, 5 μl of the template DNA and 20 μl of the master mix were mixed in an Ependorff tube. The control for the PCR (Polymerase Chain Reaction) contained 5 μl of H$_2$0 and 20 μl of the master mix. The master mix for PCR

contained 1x PCR buffer, 1.5 mM magnesium ion, 0.2 mM dNTPs, 0.25 µM of forward

primer, 0.25 µM of reverse primer, and 0.04 U/ml of taq polymerase. The forward and

reverse primers specific for the *trpA* alleles and their revertants within the *trpA* gene were

5'-GATCCGGGCATTGAGCAGTCA-3' and 3'-CATGTTGGCTACTTTCGCCGC-5',

respectively. The PCR product of 717 base-pairs was cleaned using ExoZap (USB

Corporation, Cleveland, OH). The concentration and purity of the DNA was measured

using a nanodrop spectrophotometer (NanoDrop, ThermoScientific, Willmington, DE).

The PCR product at 60-80 ng/µl was transferred into a new 96 well plate for DNA

sequencing (Polymorphic DNA Technologies, Alameda, CA). The DNA sequencing

results were analyzed by performing a multiple sequence alignment against the *E. coli*

trpA gene sequence and the *trpA223* sequence using ClustalW.

4. RESULTS

4.1 Growth curves

The 20 hour growth curves for the $dinB^+umuC^+$, $dinB^+umuC$, $dinB\Delta umuC^+$, and $dinB\Delta\ umuC$ strains are shown in Fig. 1. All four strains had similar growth curves and entered log phase before 30 min and then started to enter stationary phase at about 360 to 400 min.

4.2 Cell survival in UV-exposed $dinB\Delta$ and $umuC$ strains in a 20-hour growth period

20 hour UV survival curves were determined for four strains, $dinB^+\ umuC^+$ (wild-type), $dinB^+umuC$, $dinB\Delta\ umuC^+$, and $dinB\Delta\ umuC$ and are shown in Fig. 2. The percentage of surviving bacteria was measured at UV fluences of 25 J/m^2. The wild-type strain maintained a similar level of UV resistance throughout the 20 hours. The $dinB\Delta$ $umuC^+$ stain was highly sensitive to UV damage for the first three hours of growth, and then became more resistant as the growth curve reached seven or eight hours. The $dinB^+umuC$ and $dinB\Delta\ umuC$ strains had very similar low levels of UV survival throughout the 20 hour period (Fig. 2). There was some increase in UV resistance toward the end of the growth period but it remained lower than $umuC^+$ levels.

4.3 The effect of $dinB10$ on UV survival

The UV survival of a $dinB$ strain with a nonpolar mutation, $dinB10$, was determined. The $dinB10$ strain had a similar survival level after UV damage compared to

an isogenic wild-type strain (SMR4562) at the early growth stage (2-3 hr). In contrast, the polar *dinB*Δ allele is 100-fold more sensitive to UV damage compared to the nonpolar *dinB10* strain and the wild-type strain (Fig. 3).

4.4 UV-induced mutagenesis and spontaneous mutagenesis

In order to understand the role of the *dinB* allele in UV mutagenesis, UV-induced Trp$^+$ reversion frequencies for the *trpA223* and *trpA78* alleles were measured in wild-type and *dinB*Δ strains. The results are shown in Table 2. It appears that *dinB* has little or no effect on UV-induced mutagenesis with the *trpA223* allele. There was a small increase in the Trp$^+$ reversion frequency with the *trpA78* allele in the *dinB*Δ strain.

The spontaneous Trp+ reversion frequencies are shown in Table 3. For both the *trpA* base-pairs substitution and frameshift alleles, there appears to be little effect of the *dinB*Δ allele on spontaneous mutagenesis. There was a very small decrease in *trpA223* Trp$^+$ reversion frequencies for both the *dinB*Δ and *umuC* strains. The *umuC* mutant has the same level of spontaneous mutagenesis as the *umuC dinB* double mutant as shown in Table 3. Neither *umuC* nor *dinB* is contributing significantly to spontaneous mutagenesis in our assay.

4.5 Characterization and distribution of spontaneous *Trp*$^+$ revertants

The characterization and the distribution of the revertants are shown in Table 4. The presence of *dinB*Δ appears to reduce the presence of A:T → G:C transitions while *umuC* eliminated A:T → T:A transversions in the *umuC dinB*$^+$ strain but only slightly

23

reduced them in the *umuC dinB*Δ strain. A:T → G:C transitions occur less frequently

than A:T → C:G transversions in all strains.
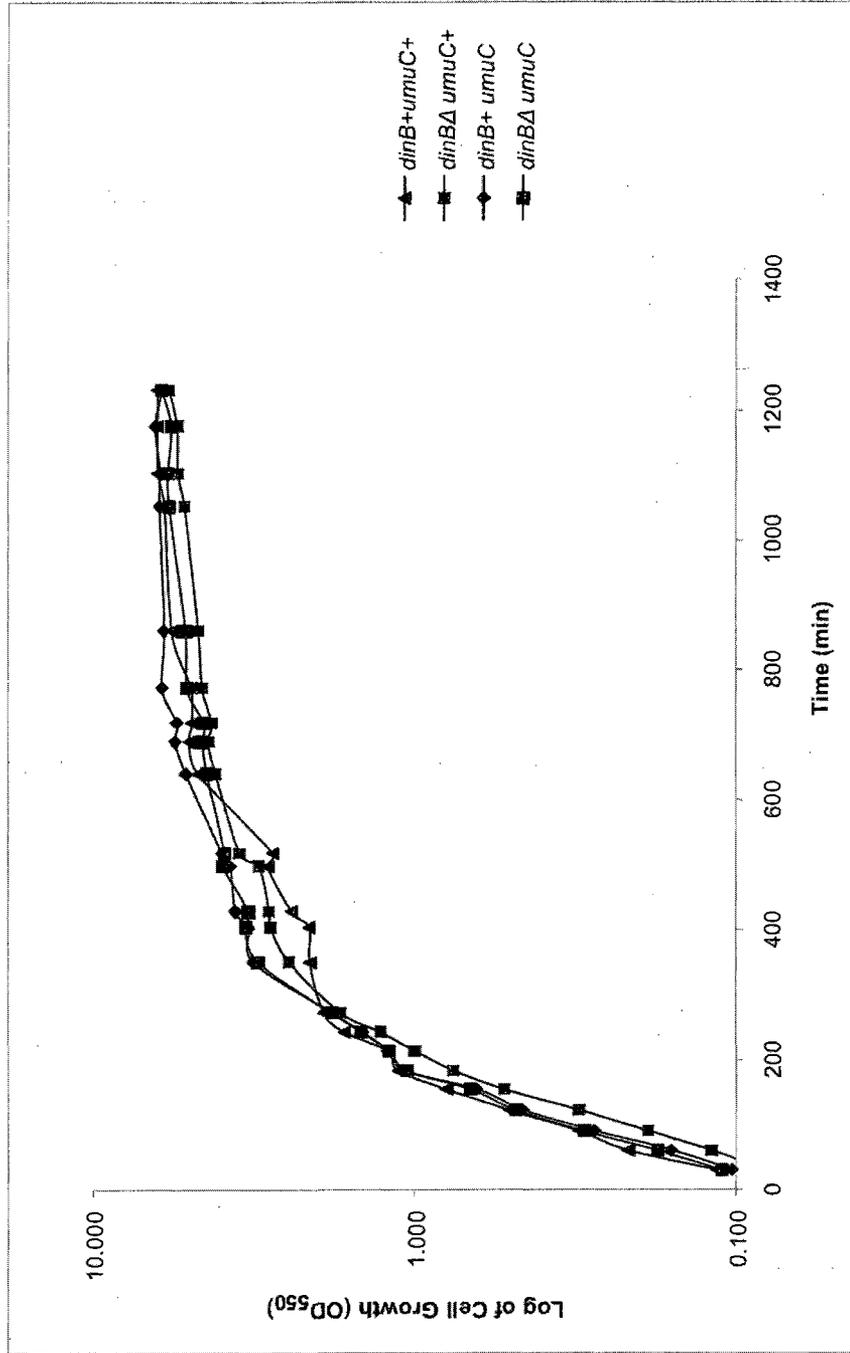
Figure 1. 20-hour growth curves of $dinB^+ umuC^+$, $dinB\Delta umuC^+$, $dinB^+ umuC$, and $dinB\Delta umuC$. All four strains were grown in L-broth and were incubated at 37°C in a shaker. The absorbance of each bacterial culture was measured every 30 min at 550 nm.
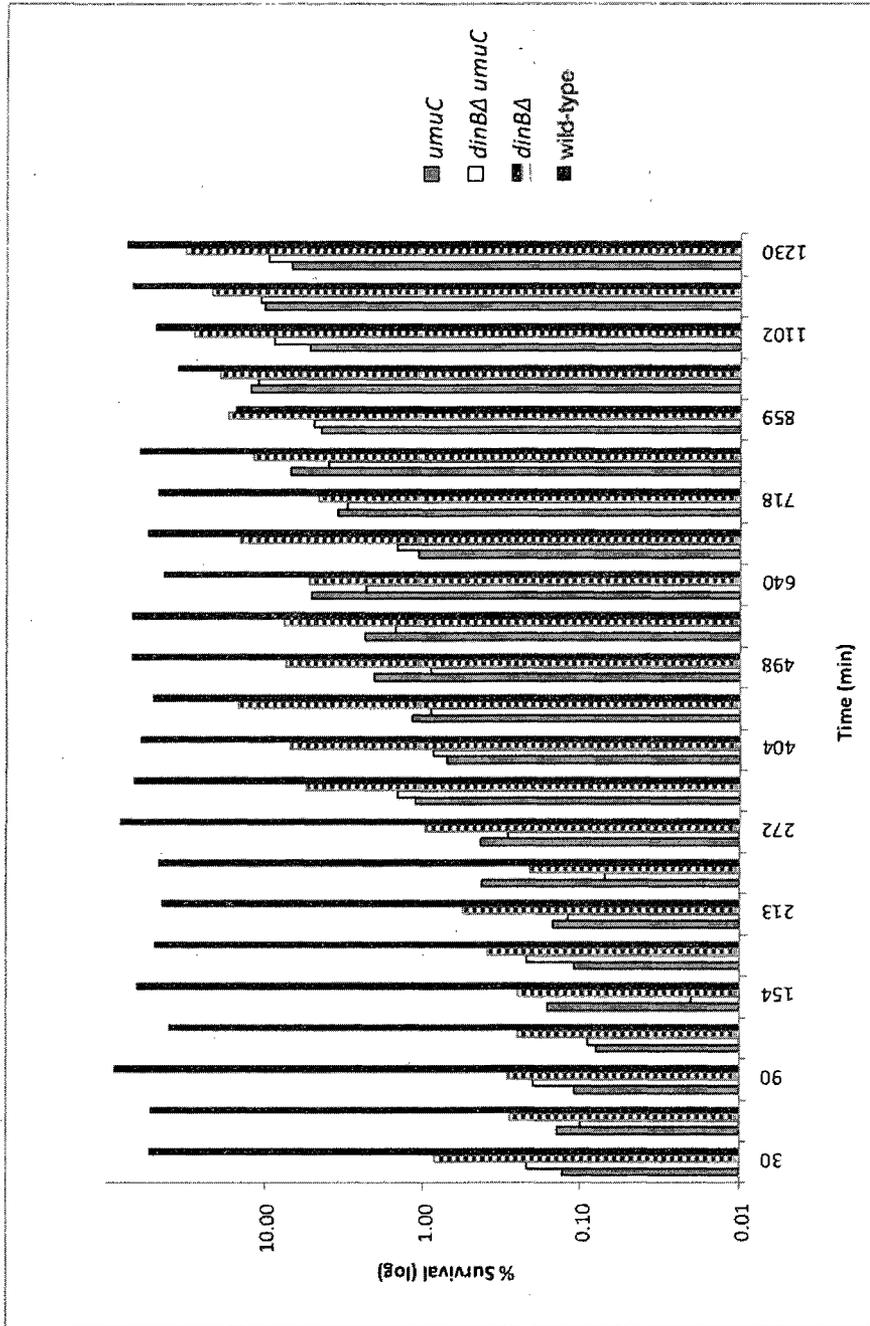
Figure 2. Effect of the *dinBΔ* and *umuC* mutations on the UV radiation survival of *E.coli trpA223* in a 20-hour growth period.
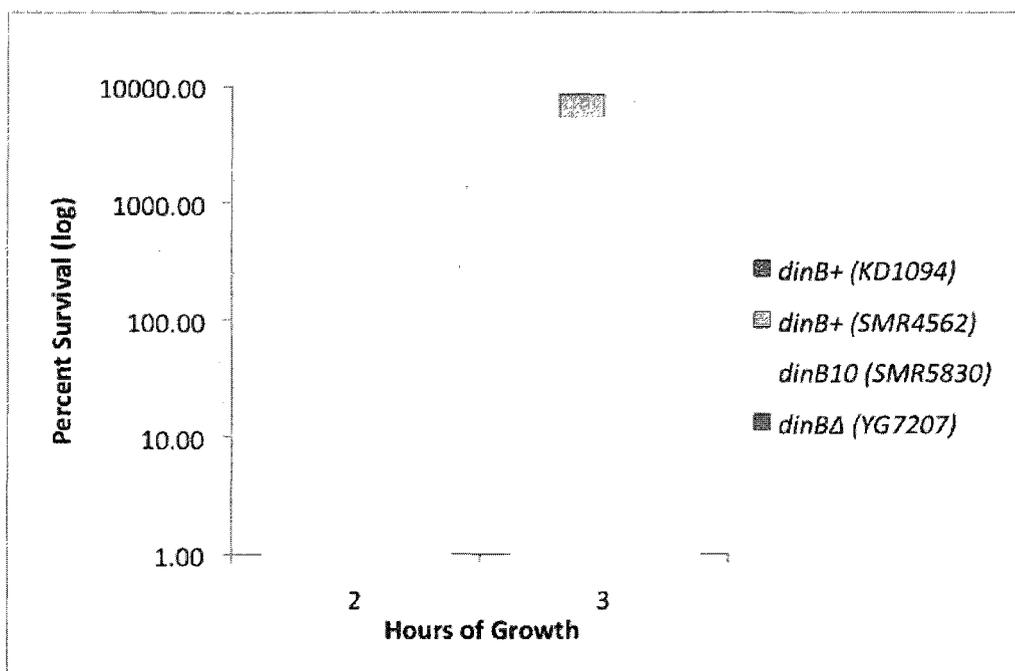
Figure 3. The comparison of UV survival of the polar and nonpolar *dinB* strains with wild-type strains. All strains are exposed to a fluence of 25 J/m^2 of UV.

Table 2. UV-induced Trp$^+$ reversion frequencies of trpA base-pair substitution alleles in wild-type and dinBΔ strains.

Irradiated (25.0 J/m^2 for 60 seconds)

trpA allele	Genotype [b]	UV-induced Trp$^+$ revertants per 10^8 survivors [c]	Ratio of dinBΔ / wild-type [d]
A223	Wild-type	13.26 (± 0.83) [a]	
	dinBΔ	14.30 (± 9.31)	1.1
A78	Wild-type	17.61 (± 13.02)	
	dinBΔ	49.99 (± 19.80)	2.8

[a] Figures in parentheses are standard deviations.

[b] Wild-type refers to the dinB$^+$ umuC$^+$ genotype here and the subsequent tables.

[c] The number of UV-induced revertants per 10^8 survivors was calculated by dividing the mean number of UV-induced revertants per plate by the number of bacteria surviving the irradiation. The mean number of revertant colonies per irradiated plate was corrected for spontaneous revertants.

[d] Ratio denotes the increase of UV-induce Trp$^+$ reversion above the wild-type level.

28

Table 3. Spontaneous Trp⁺ reversion frequencies for base-pair substitution and frameshift *trpA* alleles in wild-type, *dinB*Δ, *umuC*, and *dinB*Δ *umuC* backgrounds.

trpA allele	Genotype	Trp⁺ revertants per 10⁸ cells	Ratio of *dinB*Δ / wild-type [a]
A9813 [b]	Wild-type	2.08	1.3
	*dinB*Δ	2.66	
A540 [b]	Wild-type	4.47	1.4
	*dinB*Δ	6.34	
A78	Wild-type	2.21	1.2
	*dinB*Δ	2.61	
A223	Wild-type	0.73	0.7
	*dinB*Δ	0.54	
	*dinB*Δ *umuC*	0.55	
	umuC	0.60	
A58	Wild-type	0.20	1.3
	*dinB*Δ	0.25	
A11	Wild-type	0.08	2.3
	*dinB*Δ	0.18	

[a] Ratio denotes the increase of spontaneous Trp⁺ reversion above the wild-type level

[b] Frameshift mutation

[c] The number of spontaneous revertants per 10⁸ survivors was calculated by dividing the mean number of spontaneous revertants per plate by the mean bacteria titer per tryptone plate.

Table 4. Spontaneous Trp$^+$ reversion frequencies of *trpA223* in wild-type and *dinB*Δ strains.

Genotype	Trp$^+$ revertants per 10^8 cells [b]	Substitution pathway		Trp$^+$ revertants per 10^8 cells for each pathway
Wild-type	0.73	A:T → G:C	(9)[a]	0.16
		A:T → C:G	(16)	0.29
		A:T → T:A	(12)	0.22
		Suppressed	(3)	0.05
umuC	0.60	A:T → G:C	(3)	0.09
		A:T → C:G	(16)	0.51
		A:T → T:A	(0)	–
*dinB*Δ	0.54	A:T → G:C	(6)	0.09
		A:T → C:G	(12)	0.18
		A:T → T:A	(16)	0.23
		Suppressed	(3)	0.04
*dinB*Δ *umuC*	0.55	A:T → G:C	(3)	0.06
		A:T → C:G	(15)	0.31
		A:T → T:A	(8)	0.16
		Suppressed	(1)	0.02

[a] In parentheses are the number of revertants sequenced.

[b] The number of spontaneous Trp$^+$ revertants per 10^8 total cells.

5. DISCUSSION

5.1 The role of DinB in UV damage repair

UV light can form DNA lesions, such as T-T pyrimidine (6 – 4) pyrimidone photoproducts and *cis-syn* cyclobutane T-T dimer, which interfere with DNA replication and transcription. If the damage is left unrepaired, it can cause mutation, malfunction, and cell death. There are DNA repair mechanisms in cells for preventing these deleterious effects. In the presence of light, a specialized UV damage repair pathway in *E. coli* is photoreactivation. An enzyme, photolyase, binds specifically to the photoproduct and directly reverses the damage in an error-free manner by absorbing a photon of the appropriate wavelength (350-450 nm) and using the energy to break the dimer covalent bond. Nuclear excision repair (NER) is an error-free repair mechanism that can correct the pyrimidine dimer without the need of light. NER in *E. coli* removes the damage by generating nicks at a specific distance 5' and 3' from the damaged bases and excises the short single-stranded DNA. Pol I then fills the gaps in the 5' → 3' direction, and DNA ligase seals the nick. When cells are overwhelmed by UV damage and lesions persist in the DNA, the SOS response will be triggered. There are more than 40 genes regulated by the *lexA* and *recA* in the SOS regulatory network, including *dinB* and *umuC*.

Results from this study show that in the 20-hour growth curve experiment on UV survival, *dinB* was found to be essential for full UV resistance. During log phase of growth, there was a significant reduction in the UV survival level of the *dinB*Δ strain

compared to wild-type. As the growth enters stationary phase, the UV repair efficiency of the *dinB*Δ strain improves to the level slightly lower than that of the wild-type. In the stationary phase, perhaps some other proteins are recruited to the site of DNA lesions to assist in UV damage repair when DinB is absent.

The UV survival data here show that DinB's activity in UV survival is absolutely depended on UmuD/C. The two strains, *dinB*Δ and *dinB*Δ*umuC,* show the same level of UV survival (Fig. 2). This suggests that in the absence of umuD/C, DinB does not protect cells against UV damage. DinB and UmuD/C are likely to be working in close association on UV damage repair.

The effect of *dinB*Δ in UV repair found in this study is inconsistent with the results found in some other studies. Courcelle *et al.* (2005) and Wrzesiński *et al.* (2005) found no effect of *dinB* in UV survival. The same *dinB* strain with the *dinB*Δ polar mutation was presumably used in all three studies. One possible explanation for the differences is that the stage of the cell growth cycle at the time of UV exposure could be different between this study and the other two studies. The other two studies examined UV sensitivity at only one time point in the late log phase. It is worth noting that *dinB*Δ has been found to increase both UV and ionizing radiation sensitivity in another study (N. Sargentini, personal communication). It appears that DinB is essential in UV repair at log phase, and has a lesser effect in stationary phase.

The role of DinB in UV repair could be to allow UmuD/C to gain access to the replication fork to perform translesion synthesis where DNA synthesis occurs past the

UV-induced lesions or simply delay the progression of the replication fork. A recent

study showed that the presence of DinB does slow the replication fork (Indiani *et al.*,

2009). The interactions between DinB and UmuD'$_2$ may be disrupted when UmuD/C is

missing (Godoy *et al.*, 2007). Thus, DinB cannot improve the UV survival in the absence

of UmuD/C. When an UV-induced lesion is encounted by the replicative polymerase,

UmuD and UmuC delay the resumption of DNA replication to allow nucleotide excision

repair additional time to repair the damage accurately before replication is resumed

(Opperman *et al.*, 1999). If the lesion could not be fixed in about 20 min, the

ssDNA:RecA nucleoprotein filament induces the autoproteolytic processing of UmuD,

and UmuD' becomes the predominant form available to interact with UmuC which can

lead to translesion synthesis past the lesion (Opperman *et al.*, 1999; Burckhardt *et al.*,

1988; Nohmi *et al.*, 1988). Direct interactions amongst DinB, UmuD, UmuD'$_2$, and

RecA, are found under physiological conditions (Godoy *et al.*, 2007). It was found that

DinB replaces the stalled Pol III holoenzyme on the β-clamp at the replication fork

(Furukohri *et al.*, 2008). By doing so, DinB may improve the accessibility for UmuD/C

to perform translesion synthesis past lesions.

5.2 The effect of polar *dinB*Δ and nonpolar *dinB10* on UV damage repair

In this study, the effects on UV damage repair of two different *dinB* alleles were

compared. The nonpolar *dinB10* allele contains a missense mutation. A previous study

had shown that a nonpolar *dinB10* mutation with a replacement of a highly conserved

amino acid (R49F) does not have an effect on cell survival after UV damage (Mckenzie

et al., 2001). In the polar *dinB*Δ strain, most of the *dinB* gene and the first part of the

downstream *yafN* gene are deleted. The data in Fig. 2 show that only the *dinB*Δ strain

affects UV survival when compared to the wild-type. Its effect seems to depend upon the

stage of the growth the cells are in at the time of plating. In contrast to the polar *dinB*Δ

deletion, the nonpolar *dinB10* allele does not reduce the level of UV survival compared to

the wild-type during log phase of growth. This suggests that DinB may not act as a

polymerase but play another important role during UV repair. It appears that the DinB

protein encoded by *dinB10* is able to displace pol III and allow other repair machinery to

remove the UV damage, but the DinB protein made by the *dinB*Δ allele cannot. As

mentioned previously, the stalled Pol III holoenzyme was found to be replaced with DinB

on the β-clamp at the replication fork (Furukohri *et al.*, 2008). DinB gains access to the

primer terminus and recruits another specialized polymerase to perform the translesion

DNA synthesis, probably UmuC\D (DNA pol V). DinB could also be playing a role in

delaying the resumption of DNA replication to allow nucleotide excision repair time to

repair the damage accurately before replication is resumed.

The downstream genes of *dinB* could also be involved in UV-induced DNA repair

during the exponential stage of growth. The downstream genes of *dinB* are *yafN*, *yafO*,

and *yafP*. The functions of the *yaf* genes are not clear. The YafN protein, encoded by

yafN gene, is homologous to the anti-toxin of the *relBE* operon of *E. coli* (Grønlund and

Gerdes, 1999). The *yafO* gene is believed to be the toxin gene that produces YafO that

pairs with YafN (Brown and Shaw, 2003). Toxicity was found associated with the

overexpression of *yafO*. However, the antitoxicity of YafN against the YafO toxin has

not been shown (Brown and Shaw, 2003). Mckenzie *et al.* (2003) suggest that *yafN* could be an anti-toxin gene that regulates the function of another yet to be discovered toxin gene. The *yafN* mutant (which would also be the case for the polar *dinB*Δ mutant) might experience killing due to lacking antitoxin in the presence of toxin during SOS induction (Mckenzie *et al.*, 2003). This could be another possible explanation for the polar *dinB*Δ strain being more sensitive to UV irradiation compared to the nonpolar *dinB10* and the wild-type strains.

5.3 The role of *dinB* in UV-induced mutagenesis and spontaneous mutagenesis

The involvement of DinB in UV mutagenesis is not clear. One study shows that *dinB* has a slight effect on UV mutagenesis (Wrzesiński *et al.*, 2005). In contrast, this study suggests that *dinB* does not contribute significantly to UV mutagenesis (Table 2), although a possible small effect cannot be eliminated.

An effect of *dinB* in spontaneous mutagenesis was reported in previous studies. Some studies show that *dinB* is involved in spontaneous mutagenesis, especially when it is overexpressed (Strauss *et al.*, 2000; Lenne-Samuel *et al.*, 2002; McKenzie *et al.*, 2001; Kuban 2005; Jacob *et al.*, 2007). DinB was shown to be involved in the mutagenesis of undamaged bacteriophage λ DNA when grown on a UV-irradiated host (Brotcorne-Lannoye and Maenhaut-Michel, 1986). These studies showed an increase in spontaneous mutagenesis with overexpression of *dinB*. Other studies suggest that *dinB* does not contribute to spontaneous mutagenesis when it is expressed at basal levels in dividing cells (Tago *et al.*, 2005; Kuban *et al.*, 2005). In contrast, Strauss *et al.* (2000) did find a

small reduction in spontaneous mutagenesis in a *dinB* strain. The data in Table 3 show that *dinB*Δ does not have a large effect on spontaneous mutagenesis in growing cells. Another study performed on the *trpA46* strain showed a slight decrease in *dinB*Δ spontaneous mutagenesis compared to the wild-type strain (R. Fowler, unpublished data).

The base-pair substitution mutation of *trpA223* strain is located in a GATC sequence. The adenine at the GATC site is methylated during DNA replication by a methylase encoded by the *dam* gene (Edmonds *et al.*, 1992; Løbner-Olesen *et al.*, 2005). A:T → G:C transitions typically occur more frequently than A:T → C:G transversions in the *trpA* gene (Bhamre *et al.*, 2001). However, the data in Table 4 show that the A:T → C:G transversion is occurring more frequently than the A:T → G:C transition among all strains. The A:T base pair is identical with both events. Since GATC methylation can influence cellular functions such as DNA mismatch repair, gene transcription, initiation of chromosome replication, and nucleoid structure (Løbner-Olesen *et al.*, 2005), the results in this study showing a relatively lower frequency of transitions compared to transversions could be due to the methylation of adenine.

The *umuC* allele has been shown to decrease the spontaneous levels of some base-pair substitutions (Bhamre *et al.*, 2001). With the *trpA223* allele, it appears to reduce A:T → T:A transverions.

LITERATURE CITED

Bhamre, S., Gadea, B. B., Koyama, C. A., White, S. J., & Fowler, R. G. (2001). An aerobic *recA*-, *umuC*-dependent pathway of spontaneous base-pair substitution mutagenesis in *Escherichia coli. Mutat Res.*, 473(2), 229-47.

Blattner, F. R., Plunkett, G., Bloch, C. A., Perna, N. T., Burland, V., Riley, M., Collado-Vides, J., Glasner, J. D., Rode, C. K., Mayhew, G. F., Gregor, J., Davis, N. W., Kirkpatrick, H. A., Goeden, M. A., Rose, D. J., Mau, B., & Shao, Y. (1997). The complete genome sequence of *Escherichia coli* K-12. *Science*, 277(5331), 1453-74.

Bonner, C. A., Randall, S. K., Rayssiguier, C., Radman, M., Eritja, R., Kaplan, B. E., McEntee, K., & Goodman, M. F. (1988). Purification and characterization of an inducible *Escherichia coli* DNA polymerase capable of insertion and bypass at abasic lesions in DNA. *J Biol Chem.*, 263(35), 18946-52.

Boudsocq, F., Kokoska, R. J., Plosky, B. S., Vaisman, A., Ling, H., Kunkel, T. A., Yang, W., & Woodgate, R. (2004). Investigating the role of the little finger domain of Y-family DNA polymerases in low fidelity synthesis and translesion replication. *J Biol Chem.*, 279(31), 32932-40.

Brotcorne-Lannoye, A., & Maenhaut-Michel, G. (1986). Role of RecA protein in untargeted UV mutagenesis of bacteriophage λ: evidence for the requirement for the *dinB* gene. *Proc Natl Acad Sci U S A.*, 83(11), 3904-8.

Brown, J. M., & Shaw, K. J. (2003). A Novel Family of *Escherichia coli* toxin-antitoxin gene pairs. *J Bacteriol.*, 185(22), 6600-08.

Burckhardt, S. E., Woodgate, R., Scheuermann, R. H., & Echols, H. (1988). UmuD mutagenesis protein of *Escherichia coli*: overproduction, purification, and cleavage by RecA. *Proc Natl Acad Sci U S A.*, 85(6), 1811-15.

Cai, H., Yu, H., McEntee, K., & Goodman, M. F. (1995). Purification and properties of DNA polymerase II from *Escherichia coli. Methods Enzymol.*, 262, 13-21.

Courcelle, C. T., Belle, J. J., & Courcelle, J. (2005). Nucleotide excision repair or polymerase V-mediated lesion bypass can act to restore UV-arrested replication forks in *Escherichia coli. J Bacteriol.*, 187(20). 6953-61.

Cox, E. C., Degnen, G. E., & Scheppe, M. L. (1972). Mutator gene studies in *Escherichia coli*: the *mutS* gene. *Genetics*, 72(4), 551-67.

Craig, N. L., & Roberts, J. W. (1980). *E. coli recA* protein-directed cleavage of phage λ repressor requires polynucleotide. *Nature*, 283(5742), 26-30.

Edmonds, P., Hall, B. M., Edwards, W. R., & Hartline, K. M. (1992). Presence of methylated adenine in GATC sequences in chromosomal DNAs from *Campylobacter* species. *J Bacteriol.*, 174(24), 8156-7.

Elledge, S. J., & Walker, G. C. (1983). Proteins required for ultraviolet light and chemical mutagenesis. Identification of the products of the *umuC* locus of *Escherichia coli. Mol Biol.*, 164(2), 175-92.

Frank, E. G., Gonzalez, M., Ennis, D. G., Levine, A. S., & Woodgate, R. (1996). *In vivo* stability of the Umu mutagenesis proteins: a major role for RecA. *J Bacteriol.*, 178(12), 3550-6.

Friedberg, E. C., Walker, G. C., Siede, W., Wood, R. D., Schultz, R. A., & Ellenberger, T. (2006). *DNA Repair and Mutagenesis.* Washington, DC: ASM Press.

Furukohri, A., Goodman, M. F., & Maki, H. (2008). A dynamic polymerase exchange with *Escherichia coli* DNA polymerase IV replacing DNA polymerase III on the sliding clamp. *J Biol Chem.*, 283(17). 11260-9.

Godoy, V. G., Jarosz, D. F., Simon, S. M., Abyzov, A., Ilyin, V., & Walker, G. C. (2007). UmuD and RecA Directly Modulate the Mutagenic Potential of the Y-family DNA Polymerase DinB. *Mol Cell.*, 28(6), 1058-70.

Goodman, M. F. (2007). Error-prone repair DNA polymerases in prokaryotes and eukaryotes. *Annu Rev Biochem.*, 71, 17-50.

Grønlund, H., & Gerdes, K. (1999). Toxin-antitoxin systems homologous with *relBE* of *Escherichia coli* plasmid P307 are ubiquitous in prokaryotes. *J Mol Biol.*, 285(4), 1401-15.

Hall, B. G. (1998). Adaptive mutagenesis: a process that generates almost exclusively beneficial mutations. *Genetica*, 102-103(1-6), 109-25.

Indiani, C., Langston, L. D., Yurieva, O., Goodman, M. F., & O'Donnell, M. (2009). Translesion DNA polymerases remodel the replisome and alter the speed of the replicative helicase. *Proc Natl Acad Sci U S A.*, doi:10.1073/pnas.0901403106

Jacob, K. D., & Eckert, K. A. (2007). *Escherichia coli* DNA polymerase IV contributes to spontaneous mutagenesis at coding sequences but not microsatellite alleles. *Mutat Res.*, 619(1-2), 93-103.

Jarosz, D. F., Godoy, V. G., Delaney, J. C., Essigmann, J. M., & Walker, G. C. (2006). A single amino acid governs enhanced activity of DinB DNA polymerases on damaged templates. *Nature*, 439(7073), 225-8.

Jarosz, D. F., Godoy, V. G., & Walker, G. C. (2007). Proficient and accurate bypass of persistent DNA lesions by DinB DNA polymerases. *Cell Cycle*, 6(7), 817-22.

Johnson, R. E., Trincao, J., Aggarwal, A. K., Prakash, S., & Prakash, L. (2003). Deoxynucleotide triphosphate binding mode conserved in Y family DNA polymerases. *Mol Cell Biol.*, 23(8), 3008-12.

Kato, T., & Shinoura, Y. (1977). Isolation and characterization of mutants of *Escherichia coli* deficient in induction of mutations by ultraviolet light. *Mol Gen Genet.*, 156(2), 121-31.

Kenyon, C. J., & Walker, G. C. (1980). DNA-damaging agents stimulate gene expression at specific loci in *Escherichia coli*. *Proc Natl Acad Sci U S A.*, 77(5), 2819-23.

Kim, S. R., Maenhaut-Michel, G., Yamada, M., Yamamoto, Y., Matsui, K., Sofuni, T., Nohmi, T., & Ohmori, H. (1997). Multiple pathways for SOS-induced mutagenesis in *Escherichia coli*: an overexpression of *dinB/dinP* results in strongly enhancing mutagenesis in the absence of any exogenous treatment to damage DNA. *Proc Natl Acad Sci U S A.*, 94(25):13792-7.

Kim, S. R., Matsui K., Yamada M., Gruz P., & Nohmi, T. (2001). Roles of chromosomal and episomal *dinB* genes encoding DNA Pol IV in targeted and untargeted mutagenesis in *Escherichia coli*. *Mol Genet Genomics.*, 266(2), 207-15.

Kuban, W., Banach-Orlowska, M., Bialoskorska, M., Lipowska, A., Schaaper, R. M., Jonczyk, P., & Fijalkowska, I. J. (2005). Mutator phenotype resulting from DNA polymerase IV overproduction in *Escherichia coli*: preferential mutagenesis on the lagging strand. *J Bacteriol.*, 187(19), 6862-6.

Kusumoto, R., Masutani, C., Shimmyo, S., Iwai, S., & Hanaoka, F. (2004). DNA binding properties of human DNA polymerase η: implications for fidelity and polymerase switching of translesion synthesis. *Genes Cells,* 9(12), 1139-50.

Lenne-Samuel, N., Wagner J., Etienne H., & Fuchs R. P. (2002). The processivity factor β controls DNA polymerase IV traffic during spontaneous mutagenesis and translesion synthesis *in vivo*. *EMBO Rep.*, 3(1), 45-9.

Lennox, E. S. (1955). Transduction of linked genetic characterization of host by bacteriophage P1. *Virology*, 1(2), 190-206.

Ling, H., Boudsocq, F., Woodgate, R., & Yang, W. (2001). Crystal structure of a Y-family DNA polymerase in action: a mechanism for error-prone and lesion-bypass replication. *Cell*, 107(1), 91-102.

Løbner-Olesen, A., Skovgaard, O., & Marinus, M. G. (2005). Dam methylation: coordinating cellular processes. *Curr Opin Microbiol.*, 8(2), 154-60.

Maor-Shoshani, A., Reuven, N. B., Tomer, G., & Livneh, Z. (2000). Highly mutagenic replication by DNA polymerase V (UmuC) provides a mechanistic basis for SOS untargeted mutagenesis. *Proc Natl Acad Sci U S A.*, 97(2), 565-70.

McKenzie, G. J., Lee, P. L., Lombardo, M. J., Hastings, P. J., & Rosenberg, S. M. (2001). SOS mutator DNA polymerase IV functions in adaptive mutation and not adaptive amplification. *Mol Cell.*, 7(3), 571-79.

McKenzie, G. J., Magner, D. B., Lee, P. L., & Rosenberg, S. M. (2003). The *dinB* operon and spontaneous mutation in *Escherichia coli. J Bacteriol.*, 185(13), 3972-7.

Minko, I. G., Yamanaka, K., Kozekov, I. D., Kozekova, A., Indiani, C., O'Donnell, M. E., Jiang, Q., Goodman, M. F., Rizzo, C. J., & Lloyd, R. S. (2008). Replication bypass of the acrolein-mediated deoxyguanine DNA-peptide cross-links by DNA polymerases of the DinB family. *Chem Res Toxicol.*, 21(10), 1983-90.

Nohmi, T., Battista, J. R., Dodson, L. A., & Walker, G. C. (1988). RecA-mediated cleavage activates UmuD for mutagenesis: mechanistic relationship between transcriptional derepression and posttranslational activation. *Proc Natl Acad Sci U S A.*, 85(6), 1816-20.

Ohashi, E., Bebenek, K., Matsuda, T., Feaver, W. J., Gerlach, V. L., Friedberg, E. C., Ohmori, H., & Kunkel, T. A. (2000). Fidelity and processivity of DNA synthesis by DNA polymerase κ, the product of the human *DINB1* gene. *J Biol Chem.*, 275(50), 39678-84.

Ohmori, H., Hatada, E., Qiao, Y., Tsuji, M., & Fukuda, R. (1995). *dinP*, a new gene in *Escherichia coli*, whose product shows similarities to *UmuC* and its homologues. *Mutat Res.*, 347(1):1-7.

Ohmori, H., Friedberg, E. C., Fuchs, R. P., Goodman, M. F., Hanaoka, F., Hinkle, D., Kunkel, T. A., Lawrence, C. W., Livneh, Z., & Nohmi, T. (2001). The Y-family of DNA polymerases. *Mol Cell.*, 8(1), 7-8.

Opperman, T., Murli, S., Smith, B. T., & Walker, G. C. (1999). A model for a *umuDC*-dependent prokaryotic DNA damage checkpoint *Proc Natl Acad Sci U S A.*, 96(16), 9218-23.

Perry, K. L., Elledge, S. J., Mitchell, B. B., Marsh, L., & Walker, G. C. (1985). *umuDC* and *mucAB* operons whose products are required for UV light- and chemical-induced mutagenesis: UmuD, MucA, and LexA proteins share homology. *Proc Natl Acad Sci U S A.*, 82(13), 4331-5.

Pham, P., Bertram, J. G., O'Donnell, M., Woodgate, R., & Goodman, M. F. (2001). A model for SOS-lesion-targeted mutations in *Escherichia coli. Nature*, 409(6818), 366-70.

Qiu, Z., & Goodman, M. F. (1997). The *Escherichia coli polB* locus is identical to *dinA*, the structural gene for DNA polymerase II. Characterization of Pol II purified from a *polB* mutant. *J Biol Chem.*, 272(13), 8611-7.

Rangarajan, S., Woodgate, R., & Goodman, M. F. (1999). A phenotype for enigmatic DNA polymerase II: a pivotal role for pol II in replication restart in UV-irradiated *Escherichia coli. Proc Natl Acad Sci U S A.*, 96(16), 9224-9.

Reuven, N. B., Arad, G., Maor-Shoshani, A., & Livneh, Z. (1999). The mutagenesis protein UmuC is a DNA polymerase activated by UmuD', RecA, and SSB and is specialized for translesion replication. *J Biol Chem.*, 274(45), 31763-66.

Russell, P. (2003). *Essential iGenetics*. San Francisco: Benjamin Cummings.

Sargentini, N. J., & K. C. Smith. (1987). Ionizing and ultraviolet radiation-induced reversion of sequenced frameshift mutations in *Escherichia coli*: a new role for *umuDC* suggested by delayed photoreactivation. *Mutat Res.*, 179(1), 55-63.

Sargentini, N. J., & K. C. Smith. (1989). Role of *ruvAB* genes in UV- and gamma-radiation and chemical mutagenesis in *Escherichia coli. Mutat Res.*, 215(1), 115-29.

Sassanfar, M., & Roberts, J. (1991). Constitutive and UV-mediated activation of RecA protein: combined effects of *recA441* and *recF143* mutations and of addition of nucleosides and adenine. *J Bacteriol.*, 173(18), 5869-75.

Shinagawa, H., Iwasaki, H., Kato, T., & Nakata, A. (1988). RecA protein-dependent cleavage of UmuD protein and SOS mutagenesis. *Proc Natl Acad Sci U S A.*, 85(6), 1806-10.

Sommer, S., Boudsocq, F., Devoret, R., & Bailone, A. (1998). Specific RecA amino acid changes affect RecA-UmuD'C interaction. *Mol Microbiol.*, 28(2):281-91.

Strauss, B. S., Roberts, R., Francis, L., & Pouryazdanparast, P. (2000). Role of the *dinB* gene product in spontaneous mutation in *Escherichia coli* with an impaired replicative polymerase. *J Bacteriol.*, 182(23), 6742-50.

Sutton, M. D., Opperman, T., & Walker, G. C. (1999). The *Escherichia coli* SOS mutagenesis proteins UmuD and UmuD' interact physically with the replicative DNA polymerase. *Proc Natl Acad Sci U S A.*, 96(22), 12373-78.

Tago, Y., Imai, M., Ihara, M., Atofuji, H., Nagata, Y., & Yamamoto, K. (2005). *Escherichia coli* mutator Δ*polA* is defective in base mismatch correction: the nature of *in vivo* DNA replication errors. *Mol Cell.*, 351(2). 299-308.

Tang, M., Shen, X., Frank, E. G., O'Donnell, M., Woodgate, R., & Goodman, M. F. (1999). UmuD'$_2$C is an error-prone DNA polymerase, *Escherichia coli* Pol V. *Proc Natl Acad Sci U S A.*, 96(16), 8919-24.

Tang, M., Pham, P., Shen, X., Taylor, J. S., O'Donnell, M., Woodgate, R., & Goodman, M. F. (2000). Roles of *E. coli* DNA polymerases IV and V in lesion-targeted and untargeted SOS mutagenesis. *Nature*, 404(6781), 1014-18.

Tessman, I., & Kennedy, M. A. (1994). DNA polymerase II of *Escherichia coli* in the bypass of abasic sites *in vivo*. *Genetics*, 136(2), 439-48.

Tompkins, J. D., Nelson, J. L., Hazel, J. C., Leugers, S. L., Stumpf, J. D., & Foster, P. L. (2003). Error-prone polymerase, DNA polymerase IV, is responsible for transient hypermutation during adaptive mutation in *Escherichia coli*. *J Bacteriol.*, 185(11), 3469-72.

Uchida, K., Furukohri, A., Shinozaki, Y., Mori, T., Ogawara, D., Kanaya, S., Nohmi, T., Maki, H., & Akiyama, M. (2008). Overproduction of *Escherichia coli* DNA polymerase DinB (Pol IV) inhibits replication fork progression and is lethal. *Mol Microbiol.*, 70(3), 608-22.

Vogel, H. J., & Bonner, D. M. (1956). Acetylornithinase of *Escherichia coli*: partial purification and some properties. *J Biol Chem.*, 218(1), 97-106.

Wagner, J., Gruz, P., Kim, S. R., Yamada, M., Matsui, K., Fuchs, R. P., & Nohmi, T. (1999). The *dinB* gene encodes a novel *E. coli* DNA polymerase, DNA Pol IV, involved in mutagenesis. *Mol Cell.*, 4(2), 281-286.

Wagner, J., Fujii, S., Gruz, P., Nohmi, T., & Fuchs, R. P. (2000). The beta clamp targets DNA polymerase IV to DNA and strongly increases its processivity. *EMBO Rep.*, 1(6), 484-88.

Woodgate R. (1992). Construction of a *umuDC* operon substitution mutation in *Escherichia coli*. *Mutat Res.*, 281(3), 221-5.

Woodgate R. (1999). A plethora of lesion-replicating DNA polymerases. *Genes Dev.*, 13(17), 2191-5.

Wrzesiński, M., Nowosielska, A., Nieminuszczy, J., & Grzesiuk, E. (2005). Effect of SOS-induced Pol II, Pol IV, and Pol V DNA polymerases on UV-induced mutagenesis and MFD repair in *Escherichia coli* cells. *Acta Biochim Pol.*, 52(1), 139-47.