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

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

by

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ABSTRACT

THE ROLE OF \textit{dinB} IN UV SURVIVAL AND UV-INDUCED MUTAGENESIS IN \textit{ESCHERICHIA COLI}

by Mei-Chong Wendy Lee

This study addresses the possible function of the \textit{Escherichia coli} \textit{dinB} gene in spontaneous and UV-induced mutagenesis and DNA repair triggered by UV-induced damage. The \textit{trpA} reversion system was used to study spontaneous and UV mutagenesis. This system involved strains carrying specific \textit{trpA} alleles. Revertants were selected that had regained the wild-type phenotype of synthesizing tryptophan. The \textit{trpA} spontaneous and UV-induced revertants were sequenced, and the molecular nature of the original mutations and of the revertants was determined. The \textit{dinB} allele, the \textit{umuC} allele, and the combination of both were examined for their effect on spontaneous and UV mutagenesis. The results show that \textit{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 \textit{dinB} on UV survival and UV mutagenesis was dependent upon an active \textit{umuC} allele. In contrast to the \textit{dinB} strain with a polar deletion mutation that showed a higher level of UV sensitivity, a \textit{dinB} strain with a missense nonpolar mutation showed no UV sensitivity. This suggests that either a downstream gene from \textit{dinB} is actually responsible for protection from UV damage or the function of DinB is not dependent upon its polymerase activity.
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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.
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 unrepaird. The replicative polymerase (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, UmuD₂ 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 \textit{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 \textit{dinB}-dependent $-1$ frameshift events observed \textit{in vivo} (Kim et al., 1997; Wagner et al., 1999; Ohashi et al., 2000).

The level of spontaneous mutagenesis in \textit{E. coli} cell increases when \textit{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 $-1$G event is 10-fold more frequent than a $+1$G 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 $\rightarrow$ T:A (96-fold) transversions as compared to G:C $\rightarrow$ A:T (48-fold) transitions (Kim et al., 1997).

Another study showed that deletion of \textit{dinB} resulted in about a 75% decrease in spontaneous frameshift and base substitution mutations in \textit{dnaE} mutator strains containing an impaired Pol III (Strauss et al., 2000). A 1.5- to 3-fold reduction in \textit{dinB}-
induced spontaneous mutagenesis was also found in a $\text{dinB}^+$ 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 $\text{dinB}$ 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 $\text{dinB}$ allele, in which $\text{dinB}$ and the first few nucleotides of the neighboring yafN gene are deleted, but not by a nonpolar missense $\text{dinB}$ allele. It has been suggested that one or more of the genes downstream of $\text{dinB}$ may affect growth-dependent mutations (McKenzie et al., 2003). However, Kuban et al. (2005) studied the effect of the polar and nonpolar $\text{dinB}$ 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.

$\text{dinB}$ 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 N²-position of deoxyguanosine (dG) (Jarosz et al., 2006). Although DinB has the potential -1 frameshift mutagenic activity, it can perform lesion bypass synthesis past N²-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 βγ-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', RecA, and SSB (single-stranded DNA binding protein) were all present (Reuven et al., 1999). The presence of RecA and SSB increased UmuD'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'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'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'2C 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 $\textit{dinA}$ gene, also known as $\textit{polB}$, 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 $\textit{polB}$ 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'zC (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
tolerance (Sutton et al., 1999). UmuD and UmuD' 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, UmuD₂ 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 UmuD2 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 UmuD2 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

<table>
<thead>
<tr>
<th>Strain</th>
<th>Relevant genotype</th>
<th>Source</th>
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<tr>
<td>KD1094</td>
<td><em>trpA</em>223, arg, leu, his, thr</td>
<td>Cox <em>et al.</em>, 1972</td>
</tr>
<tr>
<td>SR1282</td>
<td><em>trpA</em>540</td>
<td>Sargentini and Smith, 1987</td>
</tr>
<tr>
<td>SR1284</td>
<td><em>trpA</em>9813</td>
<td>Sargentini and Smith, 1987</td>
</tr>
<tr>
<td>KD1093</td>
<td><em>trpA</em>78, arg, leu, his, thr</td>
<td>Cox <em>et al.</em>, 1972</td>
</tr>
<tr>
<td>KD1092</td>
<td><em>trpA</em>58, arg, leu, his, thr</td>
<td>Cox <em>et al.</em>, 1972</td>
</tr>
<tr>
<td>KD1117</td>
<td><em>trpA</em>11</td>
<td>Yanofsky and Crawford, 1972</td>
</tr>
<tr>
<td>YG7207</td>
<td>Δ(<em>dinB-yafN</em>)::<em>kan</em>^R^ derivative of AB1157</td>
<td>Kim <em>et al.</em>, 1997</td>
</tr>
<tr>
<td>RW82</td>
<td>Δ<em>umuDC595</em>::cat derivative of AB1157</td>
<td>Woodgate, 1992</td>
</tr>
<tr>
<td>AB1157</td>
<td>F^- thr-1 leuB6 proA2 his4 thi1 argE3 lacY1 galK2 rpsL supE44 ara-14 xyl-15 mtl-1, ttx-33</td>
<td>Sargentini and Smith, 1989</td>
</tr>
<tr>
<td>SMR5830</td>
<td>SMR4562 <em>dinB</em>10 [F', <em>dinB</em>10 proAB', lacI33ΩlacZ]^b^</td>
<td>Rosenberg <em>et al.</em>, 2003</td>
</tr>
<tr>
<td>SMR4562</td>
<td>Δ(<em>lac-proAB</em>)^+^<em>XII</em> thi ara Rif^+^[F' <em>dinB</em>^+^ proAB', lacI33ΩlacZ]</td>
<td>Rosenberg <em>et al.</em>, 2003</td>
</tr>
<tr>
<td>RF01</td>
<td><em>trpA</em>223 <em>dinB</em></td>
<td>KD1094 x P<em>lvirA</em> YG7207</td>
</tr>
<tr>
<td>RF02</td>
<td><em>trpA</em>223 <em>umuC</em></td>
<td>KD1094 x P<em>lvirA</em> RW82</td>
</tr>
<tr>
<td>RF03</td>
<td><em>trpA</em>223 <em>dinB</em> <em>umuC</em></td>
<td>RF02 x P<em>lvirA</em> RW82</td>
</tr>
<tr>
<td>RF04</td>
<td><em>trpA</em>540 <em>dinB</em></td>
<td>SR1282 x P<em>lvirA</em> YG7207</td>
</tr>
<tr>
<td>RF05</td>
<td><em>trpA</em>9813 <em>dinB</em></td>
<td>SR1284 x P<em>lvirA</em> YG7207</td>
</tr>
<tr>
<td>RF06</td>
<td><em>trpA</em>11 <em>dinB</em></td>
<td>KD1117 x P<em>lvirA</em> YG7207</td>
</tr>
<tr>
<td>RF07</td>
<td><em>trpA</em>58 <em>dinB</em></td>
<td>KD1092 x P<em>lvirA</em> YG7207</td>
</tr>
<tr>
<td>RF08</td>
<td><em>trpA</em>78 <em>dinB</em></td>
<td>KD1093 x P<em>lvirA</em> YG7207</td>
</tr>
</tbody>
</table>

^a^ The Δ(*dinB-yafN*)::*kan*^R^ strain (YG7207) will be referred to as *dinBA* 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 dinBA (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 poured 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 CaCl₂, 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 stored at 5 °C or used immediately for transduction.

3.4 Transductions

P1 transductions were carried out to transfer the dinBΔ allele into KD1094 trpA223, KD1093 trpA78, SR1284 trpA9813, SR1282 trpA540, KD1092 trpA58, and KD1117 trpA11. Strains trpA223 dinBΔ 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 MgSO₄ and 15 mM CaCl₂, 200 μl of recipient strain, and 25 μl of lysate were mixed in an Eppendorf 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 dinBΔ 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 dinBΔ

All four strains, dinB⁺ umuC⁺, dinB⁺ umuC, dinBΔ umuC⁺, and dinBΔ umuC, were used in a 20-hour survival study to determine their ability to survive after UV irradiation. The dinBΔ 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/m² 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 \textit{trpA223} and \textit{trpA78}

The Trp\(^+\) reversion frequency was measured by inoculating individual colonies from each of the \textit{trpA223} and \textit{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 \(dinB\Delta\) 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 \(\mu\)l 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 \(\mu\)l of the template DNA and 20 \(\mu\)l of the master mix were mixed in an Ependorff tube. The control for the PCR (Polymerase Chain Reaction) contained 5 \(\mu\)l of H\(_2\)O and 20 \(\mu\)l 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 $\text{dinB}^{+}\text{umuC}^{+}$, $\text{dinB}^{+}\text{umuC}$, $\text{dinB}\Delta\text{umuC}^{+}$, and $\text{dinB}\Delta\text{umuC}$ 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 $\text{dinB}\Delta$ and $\text{umuC}$ strains in a 20-hour growth period

20 hour UV survival curves were determined for four strains, $\text{dinB}^{+}\text{umuC}^{+}$ (wild-type), $\text{dinB}^{+}\text{umuC}$, $\text{dinB}\Delta\text{umuC}^{+}$, and $\text{dinB}\Delta\text{umuC}$ 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 $\text{dinB}\Delta\text{umuC}^{+}$ strain 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 $\text{dinB}^{+}\text{umuC}$ and $\text{dinB}\Delta\text{umuC}$ 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 $\text{umuC}^{+}$ levels.

4.3 The effect of $\text{dinB}10$ on UV survival

The UV survival of a $\text{dinB}$ strain with a nonpolar mutation, $\text{dinB}10$, was determined. The $\text{dinB}10$ 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 $\textit{dinB}\Delta$ allele is 100-fold more sensitive to UV damage compared to the nonpolar $\textit{dinB}\Delta$ strain and the wild-type strain (Fig. 3).

4.4 UV-induced mutagenesis and spontaneous mutagenesis

In order to understand the role of the $\textit{dinB}$ allele in UV mutagenesis, UV-induced Trp$^+$ reversion frequencies for the $\textit{trpA}223$ and $\textit{trpA}78$ alleles were measured in wild-type and $\textit{dinB}\Delta$ strains. The results are shown in Table 2. It appears that $\textit{dinB}$ has little or no effect on UV-induced mutagenesis with the $\textit{trpA}223$ allele. There was a small increase in the Trp$^+$ reversion frequency with the $\textit{trpA}78$ allele in the $\textit{dinB}\Delta$ strain.

The spontaneous Trp$^+$ reversion frequencies are shown in Table 3. For both the $\textit{trpA}$ base-pairs substitution and frameshift alleles, there appears to be little effect of the $\textit{dinB}\Delta$ allele on spontaneous mutagenesis. There was a very small decrease in $\textit{trpA}223$ Trp$^+$ reversion frequencies for both the $\textit{dinB}\Delta$ and $\textit{umuC}$ strains. The $\textit{umuC}$ mutant has the same level of spontaneous mutagenesis as the $\textit{umuC dinB}$ double mutant as shown in Table 3. Neither $\textit{umuC}$ nor $\textit{dinB}$ 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 $\textit{dinB}\Delta$ appears to reduce the presence of A:T $\rightarrow$ G:C transitions while $\textit{umuC}$ eliminated A:T $\rightarrow$ T:A transversions in the $\textit{umuC dinB}^+$ strain but only slightly.

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reduced them in the *umuC* *dinBΔ* strain. $A:T \rightarrow G:C$ transitions occur less frequently than $A:T \rightarrow C:G$ transversions in all strains.
Figure 1. 20-hour growth curves of \( \text{dinB}^+ \text{umuC}^+ \), \( \text{dinB}^\Delta \text{umuC}^+ \), \( \text{dinB}^+ \text{umuC}^- \), and \( \text{dinB}^\Delta \text{umuC}^- \). 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 \textit{dinB}\Delta and \textit{umuC} mutations on the UV radiation survival of \textit{E.coli trpA223} in a 20-hour growth period.
Figure 3. The comparison of UV survival of the polar and nonpolar \textit{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 dinB$\Delta$ strains.

<table>
<thead>
<tr>
<th>Irradiated (25.0 J/m$^2$ for 60 seconds)</th>
<th>Genotype$^b$</th>
<th>UV-induced Trp$^+$ revertants per 10$^8$ survivors$^c$</th>
<th>Ratio of dinB$\Delta$ / wild-type$^d$</th>
</tr>
</thead>
<tbody>
<tr>
<td>trpA allele</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>A223 Wild-type</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>dinB$\Delta$</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>13.26 (± 0.83)$^a$</td>
<td></td>
<td>1.1</td>
</tr>
<tr>
<td></td>
<td>14.30 (± 9.31)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>A78 Wild-type</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>dinB$\Delta$</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>17.61 (± 13.02)</td>
<td></td>
<td>2.8</td>
</tr>
<tr>
<td></td>
<td>49.99 (± 19.80)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

$^a$ 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.
Table 3. Spontaneous Trp* reversion frequencies for base-pair substitution and frameshift trpA alleles in wild-type, dinBΔ, umuC, and dinBΔ umuC backgrounds.

<table>
<thead>
<tr>
<th>trpA allele</th>
<th>Genotype</th>
<th>Trp* revertants per 10^8 cells</th>
<th>Ratio of dinBΔ/wild-type a</th>
</tr>
</thead>
<tbody>
<tr>
<td>A9813</td>
<td>Wild-type</td>
<td>2.08</td>
<td>1.3</td>
</tr>
<tr>
<td>A9813</td>
<td>dinBΔ</td>
<td>2.66</td>
<td></td>
</tr>
<tr>
<td>A540</td>
<td>Wild-type</td>
<td>4.47</td>
<td>1.4</td>
</tr>
<tr>
<td>A540</td>
<td>dinBΔ</td>
<td>6.34</td>
<td></td>
</tr>
<tr>
<td>A78</td>
<td>Wild-type</td>
<td>2.21</td>
<td>1.2</td>
</tr>
<tr>
<td>A78</td>
<td>dinBΔ</td>
<td>2.61</td>
<td></td>
</tr>
<tr>
<td>A223</td>
<td>Wild-type</td>
<td>0.73</td>
<td>0.7</td>
</tr>
<tr>
<td>A223</td>
<td>dinBΔ</td>
<td>0.54</td>
<td></td>
</tr>
<tr>
<td></td>
<td>dinBΔ umuC</td>
<td>0.55</td>
<td></td>
</tr>
<tr>
<td></td>
<td>umuC</td>
<td>0.60</td>
<td></td>
</tr>
<tr>
<td>A58</td>
<td>Wild-type</td>
<td>0.20</td>
<td>1.3</td>
</tr>
<tr>
<td>A58</td>
<td>dinBΔ</td>
<td>0.25</td>
<td></td>
</tr>
<tr>
<td>A11</td>
<td>Wild-type</td>
<td>0.08</td>
<td>2.3</td>
</tr>
<tr>
<td>A11</td>
<td>dinBΔ</td>
<td>0.18</td>
<td></td>
</tr>
</tbody>
</table>

a Ratio denotes the increase of spontaneous Trp* reversion above the wild-type level
b Frameshift mutation
c The number of spontaneous revertants per 10^8 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 \textit{trpA223} in wild-type and \textit{dinB\(\Delta\)} strains.

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Trp(^+) revertants per 10(^8) cells (^b)</th>
<th>Substitution pathway</th>
<th>Trp(^+) revertants per 10(^8) cells for each pathway</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wild-type</td>
<td>0.73</td>
<td>A:T → G:C (9) (^a)</td>
<td>0.16</td>
</tr>
<tr>
<td></td>
<td></td>
<td>A:T → C:G (16)</td>
<td>0.29</td>
</tr>
<tr>
<td></td>
<td></td>
<td>A:T → T:A (12)</td>
<td>0.22</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Suppressed (3)</td>
<td>0.05</td>
</tr>
<tr>
<td>\textit{umuC}</td>
<td>0.60</td>
<td>A:T → G:C (3)</td>
<td>0.09</td>
</tr>
<tr>
<td></td>
<td></td>
<td>A:T → C:G (16)</td>
<td>0.51</td>
</tr>
<tr>
<td></td>
<td></td>
<td>A:T → T:A (0)</td>
<td>–</td>
</tr>
<tr>
<td>\textit{dinB(\Delta)}</td>
<td>0.54</td>
<td>A:T → G:C (6)</td>
<td>0.09</td>
</tr>
<tr>
<td></td>
<td></td>
<td>A:T → C:G (12)</td>
<td>0.18</td>
</tr>
<tr>
<td></td>
<td></td>
<td>A:T → T:A (16)</td>
<td>0.23</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Suppressed (3)</td>
<td>0.04</td>
</tr>
<tr>
<td>\textit{dinB(\Delta)} \textit{umuC}</td>
<td>0.55</td>
<td>A:T → G:C (3)</td>
<td>0.06</td>
</tr>
<tr>
<td></td>
<td></td>
<td>A:T → C:G (15)</td>
<td>0.31</td>
</tr>
<tr>
<td></td>
<td></td>
<td>A:T → T:A (8)</td>
<td>0.16</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Suppressed (1)</td>
<td>0.02</td>
</tr>
</tbody>
</table>

\(^a\) 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 dinBΔ strain.
compared to wild-type. As the growth enters stationary phase, the UV repair efficiency of the \textit{dinBA} 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, \textit{dinBA} and \textit{dinBΔumuC}, 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 \textit{dinBA} in UV repair found in this study is inconsistent with the results found in some other studies. Courcelle \textit{et al.} (2005) and Wrzesiński \textit{et al.} (2005) found no effect of \textit{dinB} in UV survival. The same \textit{dinB} strain with the \textit{dinBΔ} 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 \textit{dinBΔ} 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 encountered 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; Burekhardt et al., 1988; Nohmi et al., 1988). Direct interactions amongst DinB, UmuD, UmuD', 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 dinBA 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 $\text{dinB}\Delta$ strain, most of the $\text{dinB}$ gene and the first part of the downstream $\text{yafN}$ gene are deleted. The data in Fig. 2 show that only the $\text{dinB}\Delta$ 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 $\text{dinB}\Delta$ deletion, the nonpolar $\text{dinB}10$ 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 $\text{dinB}10$ is able to displace pol III and allow other repair machinery to remove the UV damage, but the DinB protein made by the $\text{dinB}\Delta$ allele cannot. As mentioned previously, the stalled Pol III holoenzyme was found to be replaced with DinB on the $\beta$-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\(\Delta\) (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 $\text{dinB}$ could also be involved in UV-induced DNA repair during the exponential stage of growth. The downstream genes of $\text{dinB}$ are $\text{yafN}$, $\text{yafO}$, and $\text{yafP}$. The functions of the $\text{yaf}$ genes are not clear. The YafN protein, encoded by $\text{yafN}$ gene, is homologous to the anti-toxin of the $\text{relBE}$ operon of $\text{E. coli}$ (Grønlund and Gerdes, 1999). The $\text{yafO}$ 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 $\text{yafO}$. However, the antitoxicity of YafN against the YafO toxin has
not been shown (Brown and Shaw, 2003). Mckenzie et al. (2003) suggest that \textit{yafN} could be an anti-toxin gene that regulates the function of another yet to be discovered toxin gene. The \textit{yafN} mutant (which would also be the case for the polar \textit{dinBA} 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 \textit{dinBA} strain being more sensitive to UV irradiation compared to the nonpolar \textit{dinB10} and the wild-type strains.

5.3 The role of \textit{dinB} in UV-induced mutagenesis and spontaneous mutagenesis

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

An effect of \textit{dinB} in spontaneous mutagenesis was reported in previous studies. Some studies show that \textit{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 \(\lambda\) 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 \textit{dinB}. Other studies suggest that \textit{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 dinBΔ does not have a large effect on spontaneous mutagenesis in growing cells. Another study performed on the trpA46 strain showed a slight decrease in dinBΔ 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 transversions.
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