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A *dinB* mutation that sensitizes *Escherichia coli* to the lethal effects of UV and X-radiation

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Abstract

The DinB (PolIV) protein of *Escherichia coli* participates in several cellular functions. We investigated a *dinB* mutation, (*dinB-yafN*)883(::kan) [referred to as *dinB883*], which strongly sensitized *E. coli* cells to both UV- and X-radiation killing. Earlier reports indicated *dinB* mutations had no obvious effect on UV radiation sensitivity which we confirmed by showing that normal UV radiation sensitivity is conferred by the *dinB749* allele. Compared to a wild-type strain, the *dinB883* mutant was most sensitive (160-fold) in early to mid-logarithmic growth phase and much less sensitive (twofold) in late log or stationary phases, thus showing a growth phase-dependence for UV radiation sensitivity. This sensitizing effect of *dinB883* is assumed to be completely dependent upon the presence of UmuDC protein; since the *dinB883* mutation did not sensitize the *umuDC* strain to UV radiation killing throughout log phase and early stationary phase growth. The DNA damage checkpoint activity of UmuDC was clearly affected by *dinB883* as shown by testing a *umuC104 dinB883* double-mutant. The sensitivities of the *umuDC* strain and the *dinB883 umuDC* double-mutant strain were significantly greater than for the *dinB883* strain, suggesting that the *dinB883* allele only partially suppresses UmuDC activity. The *dinB883* mutation partially sensitized (fivefold) *uvrA* and *uvrB* strains to UV radiation, but did not sensitize a *recA* strain. A comparison of the DNA sequences of the *dinB883* allele with the sequences of the (*dinB-yafN*)882(::kan) and *dinB749* alleles, which do not sensitize cells to UV radiation, revealed *dinB883* is likely a “gain-of-function” mutation. The *dinB883* allele encodes the first 54 amino acids of wild-type DinB followed by 29 predicted residues resulting from the continuation of the *dinB* reading frame into an adjacent insertion fragment. The resulting

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Conflict of interest

The authors have no conflicts of interests to declare.

polypeptide is proposed to interfere directly or indirectly with UmuDC function(s) involved in protecting cells against the lethal effects of radiation.

Keywords

Escherichia coli; DinB; UmuDC; UV radiation sensitivity; TLS; checkpoint

1. Introduction

Escherichia coli has five DNA polymerases, including two Y-family translesion synthesis (TLS) polymerases, PolIV or DinB, and PolV or UmuD'2C, that are induced as part of the SOS response [1, 2]. The biological functions of UmuDC proteins include roles in cellular survival after UV radiation-induced DNA damage via TLS [3, 4], in cellular checkpoint activity [5] and in induced and spontaneous mutagenesis [1, 6-10].

DinB protein is involved in TLS past template cytotoxic DNA alkylation lesions and adducts at the N2-position of deoxyguanosine [11, 12], replication arrest-stimulated recombination [13], stress-induced mutagenesis [14, 15], survival under conditions of nucleotide starvation [16], and it may possibly function as a cellular checkpoint by inhibiting replication fork progression [17, 18]. Some studies have suggested a role for DinB in spontaneous mutagenesis in growing cells [19], while others have not found evidence for DinB in this process [20, 21].

During experiments in our laboratory designed to evaluate the effect of DinB on UV radiation mutagenesis, it appeared that the *dinB883* mutant strain was significantly more sensitive to the lethal effects of UV radiation than an isogenic *dinB*⁺ strain, and that the degree of this sensitivity was dependent on culture growth phase. This observation was not consistent with previously reported results for *dinB* mutants [3, 22], so we initiated a study to specifically address how the *dinB883* allele affects UV radiation sensitivity, including its interaction with a *umuDC* mutation, which knocks out the other *E. coli* Y-family translesion polymerase, PolV.

2. Materials and methods

2.1. Bacterial strains

The bacterial strains used in this study are listed in Table 1. Strains were constructed by P1 transduction as described [23]. As this project developed, it became apparent that allele numbers would be helpful to our discussion of the three (*dinB*) mutations involved in this study. The Coli Genetic Stock Center assigned numbers for our purposes, and these numbers are explained further in Table 1.

2.2. Media

LB (Luria-Bertani) broth was 1% tryptone, 0.5% yeast extract and 1% NaCl [23]. Tryptone agar was 1% tryptone, 0.5% NaCl and 1.5% agar. Transductants were selected on tryptone

plates supplemented with kanamycin at 50 µg/ml, chloramphenicol at 20 µg/ml, or tetracycline at 15 µg/ml. Saline was 0.85% NaCl. PB was 67 mM NaK phosphate buffer.

2.3. UV- and X-radiation survival

Cells were grown to saturation in LB broth, diluted 1:250 into fresh broth, and grown at 37°C with aeration in log phase until the optical density at 600 nm (OD₆₀₀) was 0.6. Cells were harvested by centrifugation (6 min at 6,000 x g), washed twice and resuspended in PB. UV irradiation used a Sylvania germicidal lamp (254 nm) while working under GEF40GO Gold 40 W lamps. In two studies, cells were UV-irradiated on tryptone plates to determine survival. In all other studies, cells were UV-irradiated in PB and then plated to determine survival. X-irradiation used a Polaris 160 kV cabinet irradiator (Kimtron) with a 3000 W, Varian NDI-161 tube running at 160 kV and 15 mA, and a dose rate of 56.6 Gy/min. Cells were bubbled with air during X-irradiation. Irradiated cell samples were plated on tryptone agar, and incubated at 37°C in the dark for 24 h before counting colonies to determine colony-forming units per milliliter (CFU/ml) and cell survival. Statistical analyses relied on SigmaPlot for Windows, v.12.0 (Systat Software, Inc) with significance defined as P 0.05. Statistical comparisons of paired cell survival curves were performed using a nonparametric analysis of covariance (ANCOVA) with the covariate being radiation dose [24]. Other statistical comparisons relied on ANOVA.

2.4. Genomic DNA isolation, PCR amplification and DNA sequencing

Genomic DNA was isolated from overnight LB broth cultures of the *dinB* strains, SR4109, SMR6111 and JW0221-1, and a *dinB*⁺ strain (SR2227) using a commercially available system (Gentra Puregene kit, Gentra Systems Inc), which essentially involves cell lysis using a heated detergent, followed by RNase digestion, protein precipitation via the addition of a concentrated salt solution, centrifugation to pellet the protein and harvest the supernatant. The genomic DNA was then precipitated from the supernatant by the addition of isopropanol. The material was collected by centrifugation and washed with 70% ethanol. The DNA was briefly air dried and resuspended in TE buffer (10 mM Tris –HCL, pH 8.0, 1 mM EDTA). The Gentra protocol is essentially a simple modification of the detergent lysis/salt precipitation protocols of Buffone and Darlington [25] and Miller et al. [26]. The isolated genomic DNA samples were analyzed by NanoDrop spectrophotometry to determine yield and estimate purity. Agarose gel electrophoresis was used to estimate average fragment size and confirm the absence of ethidium bromide-stainable RNA.

DNA sequences of interest from the *dinB* and *dinB*⁺ strains were PCR-amplified for DNA sequencing analysis. The PCR primers used are listed (Table S1, Supplementary Data). The PCR products were separated by electrophoresis in Tris acetate EDTA agarose gels and imaged to confirm product size, determine yield and inspect for evidence of non-specific products. With the absence of any detectable non-specific PCR products confirmed, the target PCR fragment was batch purified by adsorption to silica spin columns and eluted in Tris buffer. The isolated target PCR products were analyzed by NanoDrop spectrophotometry to determine yield and estimate purity. Agarose gel electrophoresis was used to confirm product size and absence of detectable degradation fragments. The purified PCR products were sequenced commercially (Sequetech; Mountain View, CA) using single

primer extension with an ABI 3730XL capillary sequencer. The “*dinB*” sequences determined for strains SR4019, SMR6111, SR2227 and JW0221-1 are shown Fig. S1 (Supplementary Data).

3. Results

3.1. The *dinB883* mutation sensitizes cells to the lethal effects of UV and X-radiation

For log phase cells, the *dinB883* strain was more sensitive to both UV- (Fig. 1A) and X-radiation (Fig. 1B) than the isogenic wild-type strain as measured by colony-forming ability. Sensitivity to X-radiation indicated the protective effect of DinB was not limited to UV radiation-specific DNA lesions, although the sensitization determined at 10^{-3} survival in the *dinB883* strain was less for X-radiation (88-fold, Fig. 1B) than for UV-radiation (130-fold, Fig. 1A).

3.2. The impact of the *dinB883* mutation in UV-irradiated cells is growth phase-dependent and is highly dependent on UmuDC function

To more fully characterize the UV radiation sensitivity conferred by the *dinB883* mutation, we determined cell survival in *umuDC* and wild-type strain backgrounds at multiple time points throughout log and stationary phase growth. Samples were taken at 30-min intervals over a 20-h period to determine culture growth (by OD₅₅₀ values) and capacity for UV radiation survival. To reduce complexity, only hourly sample data are shown in Fig. 2, but the omitted 30-min data are consistent with the data shown. Wild-type cells maintained a small, nearly-constant level of UV radiation sensitivity throughout the time course of this experiment. In contrast, both *dinB883* and *umuDC* strains showed growth-phase dependence and were more sensitive to UV radiation than the wild-type strain at every time point with the effect being most pronounced in early to mid-log phase cells (as much as 386-fold) and declining to as little as 1.7-fold as the culture aged (Table 2). The *dinB883* strain was less sensitive than the *umuDC* strain, which was similar in sensitivity to the *dinB883 umuDC* double-mutant strain (Fig. 2), suggesting DinB protects against lethal DNA damage via direct or indirect interaction with UmuDC.

3.3. The *dinB883* mutation modestly sensitizes strains to UV radiation that are defective in nucleotide excision repair (NER)

To determine if there is an interaction between NER and DinB activity, *dinB883 uvrA* and *dinB883 uvrB* double-mutant strains were constructed and tested for UV radiation sensitivity. As expected, the *uvrA* and *uvrB* strains, being defective in NER, were very sensitive to UV radiation (Fig. 3). However, sensitization of the *uvrA* and *uvrB* strains by the *dinB883* mutation was only about fivefold when determined at the 10^{-3} survival level in *uvr dinB883* double-mutant strains, which compared to the 130-fold sensitization to UV radiation seen for the *dinB883* mutation in wild-type cells at the same survival level (Fig. 1A). Sensitization of *uvr* strains by *dinB883* was only significant ($P = 0.006$) at UV radiation doses of 9-15 J m⁻² and was insignificant ($P > 0.05$, by ANCOVA) at smaller UV radiation doses (Fig. 1A). It is concluded that *dinB883* only modestly sensitizes cells to UV radiation in the absence of NER function.

3.4. The *dinB883* mutation does not sensitize a *recA* strain to UV radiation

Cells without functional RecA are defective in all forms of DNA repair and tolerance mechanisms relevant to UV radiation-induced DNA damage, except for basal levels of NER [27]. We determined the effect of the *dinB883* mutation on the UV radiation sensitivity of a *recA* strain (Fig. 4). The *recA* strain and the *dinB883 recA* double-mutant strain were not statistically different ($P = 0.05$, by ANCOVA) in UV radiation sensitivities. Although the *dinB883 recA* double-mutant strain consistently showed a greater sensitivity than the *recA* strain in multiple experiments, it is concluded that *dinB883* had no effect on UV radiation sensitivity in the absence of RecA function.

3.5. The *dinB883* mutation suppresses the checkpoint function of UmuDC

UmuDC functions as both a DNA polymerase and as a participant in a DNA damage checkpoint [27]. To determine which of these functions is suppressed by *dinB883* we measured the UV radiation sensitivity of an *umuC104 lexA51* strain in the presence and absence of *dinB883* (Fig. 5). The *umuC104* allele is devoid of polymerase activity [28, 29], but retains its checkpoint function [30]. The sensitization of the *umuC104 lexA51* strain by *dinB883*, indicates that *dinB883* does partially suppress UmuDC checkpoint function. The level of *dinB883* suppression of UV radiation resistance in the *umuC104 lexA51* strain is less than the level of *dinB883* suppression in an *umuDC*⁺ strain at similar UV radiation doses (Fig. 1) suggesting that the TLS activity of *umuDC* may also be somewhat impaired by the presence of *dinB883*. However, this comparison is somewhat compromised since the *umuDC*⁺ strain used in Fig. 1 and the *umuC104* strain used in Fig. 5 differ in genotype beyond the *umuDC* locus.

The *lexA51* allele produces a defective LexA protein which allows constitutive expression of LexA-repressed genes [27]. UV radiation sensitization by *dinB883* of the *umuC104 lexA51* strain indicates that *dinB883* does not act by suppressing normal LexA activity.

3.6. The UV radiation sensitizing effect of the *dinB883* mutation is suggested to be due to a novel fusion polypeptide derived from DinB

Given that the UV radiation sensitivity observed for the *dinB883* strain conflicted with previous reports of the UV-radiation phenotype of *dinB* mutants, we compared the UV radiation sensitivity (under our experimental protocol) of our *dinB883* strain with two other *dinB* mutant strains, SMR6111 and JW0221-1, which are reported to carry *dinB* null alleles [31, 32]. We found that strains (SMR6111 and JW0221-1, respectively) carrying the (*dinB-yafN*)882(::*kan*) [referred to as *dinB882*] or *dinB749::kan* [referred to as *dinB749*] alleles were not different in their UV radiation sensitivity from two *dinB*⁺ strains (SMR4562 and SR2227) used for comparison (Fig. 6). In contrast, all four strains were significantly more resistant than the *dinB883* strain (Fig. 6; $P < 0.05$, by Kruskal-Wallis One Way ANOVA on ranks with pair-wise comparison using Dunn's Method).

To test whether another mutation in strain SR4109 might be responsible for its unexpected radiation sensitivity, we moved the *dinB883* and *dinB882* alleles, which are both derived from the original (*dinB-yafN*)::*kan* mutant strain (YG7207), developed by Kim et al. [31] into different *dinB*⁺ strains by P1 transduction, selecting for kanamycin resistance. In every

transductant strain tested, the resulting UV radiation sensitivities remained different for the *dinB882* and *dinB883* alleles (data not shown). These results suggest it is unlikely the phenotypic difference for radiation sensitivity between strains SR4109 and SMR6111 is due to a gene other than *dinB*.

To compare the *dinB883* and *dinB882* alleles at the molecular level, we amplified and sequenced the *dinB* DNA region from three *dinB* strains, SR4109, SMR6111, and JW0221-1, and from a *dinB*⁺ control strain (SR2227). Strain JW0221-1 is from the Keio collection of single-gene knockout mutants [33], and its *dinB749::kan* [referred to as *dinB749*] allele is unrelated to the alleles in strains SR4109 and SMR6111. As expected from the method of its construction [33], sequencing the *dinB* region of strain JW0221-1 showed only the *dinB* start codon followed by nucleotides consistent with the *kan* insertion sequence (Fig. S1, Supplementary Data), confirming the null allele status of *dinB749*. Sequence analysis of the *dinB* region of strains SR4109 and SMR6111 revealed slightly different *dinB* alleles (Fig. 7 and Fig. S1, Supplementary Data). Comparison of the nucleotide sequences suggests both strains maintain the wild-type *dinB* nucleotide sequence for a stretch of 161 nucleotides (Fig. S1, Supplementary Data), and the DinB amino acid sequence for the first 54 amino acids (Fig. 7). The nucleotide sequence differs starting at position 162, the first nucleotide derived from the DNA fragment from the plasmid pUC4K insert, which carries the *kan* gene [31]. Alleles *dinB883* and *dinB882* have the same proximal insert nucleotide sequences, except for the run of G's starting at position 169. For *dinB883*, the run consists of 12 G's, while *dinB882* shows a run of 11 G's (Fig. 7 and Fig. S1, Supplementary Data). The different lengths of the run of G's creates different reading frames and distal DinB amino acid sequences for the two alleles (Fig. 7). We sequenced 270 nucleotides distal to the run of G's and found the sequence identical in both strains (data not shown). We also sequenced 530 nucleotides proximal to the *dinB* start codon and found the same wild-type DNA sequence in both strains (data not shown). It is concluded that the single-base difference in this run of G's is the cause of the different UV radiation sensitivity phenotypes associated with alleles *dinB883* and *dinB882*. We also sequenced the *dinB* allele of YG7207, the strain from which *dinB883* was derived in this study, and found that it also had a run of 12 G's.

4. Discussion

4.1. *dinB883* is likely a “gain-of-function” mutation

Our initial observation that strain SR4109 (*dinB883*) was sensitive to radiation was surprising, since earlier studies had clearly indicated mutant *dinB* alleles had no effect on UV radiation-induced lethality [3, 22]. We confirmed that two other putative *dinB* strains, SMR6111 and JW0221-1 (*dinB882* and *dinB749*, respectively) were not UV radiation sensitive (Fig. 6).

These disparate results for the UV radiation sensitivity of *dinB* strains led to further experiments. In one approach, we confirmed that the UV radiation sensitivity phenotype (sensitive and resistant, respectively) was tightly linked (by bacteriophage P1 transduction) for both the *dinB883* and *dinB882* alleles (data not shown). More significantly, we sequenced the *dinB* regions in three *dinB* strains, SR4109, SMR6111, and JW0221-1, and a

dinB⁺ control strain (SR2227). This analysis revealed a single-base difference between strains SR4109 and SMR6111 in the run of G's associated with the insertion of the *kan* sequence during construction of the parental YG7207 strain (Fig. 7). We suggest this single base difference is the basis for the different UV radiation sensitivities seen for strains SR4109 and SMR6111.

The single base difference (12 G's vs. 11 G's) associated with *dinB* in strains SR4109 and SMR6111, respectively, is believed to have created different reading frames and different truncated versions of mutant DinB polypeptides (Fig. 7). DinB883 is suggested to be an 83 amino acid fusion polypeptide comprised of 54 wild-type DinB amino acids followed by seven non-wild-type amino acids (shared with DinB882) and then by 22 unique C-terminal amino acids before a stop codon appears in the nucleotide sequence (Fig. 7). The DinB882 fusion polypeptide protein is suggested to be identical to DinB883 protein for its first 61 amino acids, then different in its 14 C-terminal amino acids. DinB882 should be 75 amino acids in length considering the position of its stop codon in the DNA sequence (Fig. 7). Since the *dinB882* strain shows the same lack-of-radiation-sensitivity phenotype (Fig. 6) as two *dinB*⁺ strains (SMR4562 and SR2227) strains and a confirmed complete *dinB* deletion strain (JW0221-1), we conclude that DinB883 has a novel active function, which reduces cell survival after radiation treatment, presumably by interfering with DNA repair or tolerance mechanisms.

4.2. The DinB883 fusion polypeptide causes UV radiation sensitivity by modulating UmuCD activity, which is dependent upon cell growth phase

When replication forks are blocked in *E. coli* cells by DNA lesions, such as those created by UV radiation exposure, the SOS response is induced [27]. This response involves the coordinated enhanced expression of a set of SOS genes whose products increase cell survival. The SOS regulon includes *dinB*, *umuDC* and the NER genes *uvrA* and *uvrB*, among others [27]. In studies that prevent photoreactivation, UV radiation survival in *E. coli* depends upon NER, DNA recombination processes and, to a lesser extent, UmuDC activity [27]. Generally speaking, once NER is blocked by *uvrA* or *uvrB* mutations, additional mutations impacting NER should have little effect. While the effect is modest, the *dinB883* mutation does increase the UV radiation sensitivity of *uvrA* and *uvrB* strains in late log phase cells (Fig. 3), suggesting DinB883, to a small degree, interferes with DNA repair or tolerance mechanisms other than NER.

The *dinB883* allele clearly has no UV radiation sensitizing effect on a *umuDC* strain (Fig. 2, Table 2), and it should be noted that the *umuDC* allele used in this study is a deletion of both the *umuC* and *umuD* genes and is assumed to be a null allele [34]. No significant differences were observed for UV radiation sensitivity between *umuDC* and *umuDC* *dinB883* strains over a range of growth conditions from early log phase to early stationary phase (Fig. 2). Additionally, *umuDC* ± *dinB883* strains were much more UV radiation sensitive in their early log growth phases than in late log or early stationary growth phases. In contrast, the wild-type strain maintained the same high level of UV-irradiated cell survival throughout all growth phases (Fig. 2, Table 2). UmuDC function has generally been assumed to be a minor component among the survival mechanisms after UV radiation

exposure [27], but most studies on UV radiation sensitivity in *E. coli* have used cells in late log or early stationary phase. We suggest that UmuDC function is more important for survival from UV radiation exposure in early log than in later growth phases. One explanation for this observation is that in early log phase, rapidly-dividing cells with multiple replication forks may demand a higher level of overall DNA repair and damage tolerance capacity after UV radiation exposure than in later growth phases. Another possible explanation is that early log phase cells exhibit a metabolic state very different from that of late log or stationary phase cells and may accumulate a greater number of DNA lesions due to endogenous DNA damage. Also, the *dinB883* strain was less sensitive to UV radiation than a *umuDC* strain (Fig. 2, Table 2), which suggests DinB883 incompletely suppresses UmuDC function in UV radiation protection.

4.3. Possible interactions of the DinB883 fusion polypeptide with other proteins

Our genetic and DNA sequence data analyses predict *dinB883* will encode a fusion polypeptide (DinB883) 83 amino acids in length with the first 54 being the N-terminal portion of the wild-type DinB protein. We propose DinB883 reduces the activity of UmuDC, resulting in reduced survival after UV radiation exposure. DinB883 and DinB882, which is suggested to not interfere with DNA repair, have identical sequences for the first 61 amino acids, so presumably their different phenotypes result from C-terminal residues.

Wild-type DinB binds to several proteins including UmuD and RecA [35]. The *umuDC* operon encodes the UmuD and UmuC proteins that function in TLS in a highly-regulated fashion [12]. UmuD forms dimers (UmuD₂), which in conjunction with UmuC, are thought to reduce the rate of DNA replication in the presence of DNA damage, allowing time for NER and other error-free forms of repair and damage tolerance to remove or bypass potentially lethal DNA lesions [5]. Eventually, the interaction of UmuD₂ with RecA results in self-cleavage, which removes N-terminal amino acids and converts UmuD₂ to UmuD'₂ [36]. UmuD'₂ associates with UmuC to become an active DNA polymerase (PolV) which, along with RecA, effects TLS past remaining lesions in DNA [37].

Both UmuD₂ and RecA have been previously shown to bind to DinB resulting in suppression of DinB's induction of frameshift mutations by enclosing its open site [32]. This close association of these three proteins is suggested to occur during the initial SOS response before significant amounts of TLS activity occur. Two surface DinB amino acids, cysteine 66 (C66) and proline 67 (P67), have been recently found to modulate the binding of DinB to RecA and UmuD₂ [38].

DinB883 presumably has the capacity to bind to some protein(s) in a way that inhibits UmuDC activity although this may not involve binding to UmuD or UmuC themselves. The *dinB883* sensitizing of an *umuC104* strain (Fig. 5) indicates that the DNA damage checkpoint function of UmuDC is being suppressed. One possibility is that DinB883 binds to UmuD or some other protein and this interferes with the checkpoint activity. DinB883 may also interfere with the TLS activity of UmuDC although we have no data to strongly support this possibility.

It is not clear why DinB883 has radiation-sensitizing activity, whereas DinB882 lacks activity. DinB883 should be eight amino acids longer than DinB882, which may influence their relative stabilities. No proteins that might preferentially bind or interact with DinB883 are obvious. It is also of interest to evaluate the stability of *dinB883* compared to *dinB882*, given that nucleotide runs have been long recognized as frameshift mutation hotspots [39]. So far, in the sequencing of multiple isolates of both strains, we have not observed sequence changes in either allele.

5. Conclusion

We have described a single base (G) difference in the DNA sequence of two commonly used alleles (*dinB883* and *din882*), where one allele displays a novel *umuDC*-dependent activity that reduces cell survival in the presence of DNA damage, presumably by interfering with repair or tolerance mechanisms. Both alleles are derivatives of the (*dinB-yafN*) allele originally characterized by Kim et al. [31] that has been used in many *dinB* studies and presumably initially had a run of 11 or 12G's. The plasmid pUC4K was used in the construction of the (*dinB-yafN*) allele [31] and it contained a run of 12G's [40]. This would suggest that the (*dinB-yafN*) allele had 12G's and thus was identical to *dinB883*. Our copy of YG7207, the strain containing the (*dinB-yafN*) allele, also has 12G's. However we are reluctant to firmly conclude that the original construction of the *dinB* deletion contained 12G's since the sequence at the time of construction is not known as far as we are aware of. Since it is unclear historically what the original run number was and when the run either gained or lost one G, this event may have impacted the results of earlier published studies on *dinB*.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Highlights

- We describe *(dinB-yafN)883(::kan)*, a novel *dinB* allele, referred to as *dinB883*, a deletion that sensitizes *E. coli* cells to UV irradiation.
- This UV radiation sensitivity is most acute in the early logarithmic phase of culture growth.
- This UV radiation sensitivity is completely dependent upon a functional *umuDC* operon
- Sequencing reveals *dinB883* retains the proximal 161 nucleotides, i.e., 54 amino acids, of the wild-type sequence.
- The *dinB883* mutant is hypothesized to produce a peptide of 83 amino acids, DinB883, that compromises UmuDC function.

Fig 1.

The *dinB883* allele reduces cell survival after exposure to radiation. (A) Survival data are shown for logarithmic phase *E. coli* wild-type (SR2227, ●) and *dinB883* (SR4109, ○) strains after UV radiation exposure in buffer. (B) Same format as in A, except strains were irradiated with 160 kV X-rays, aerobically. Data are means $\pm$ SD from triplicate experiments.

Fig. 2.

The *dinB883* allele does not sensitize a *umuDC* strain to UV radiation. Growth and survival data are shown for *E. coli* wild-type (SR2227), *dinB883* (SR4109), *umuDC* (SR4128), and *dinB883 umuDC* (SR4129) strains incubated overnight, diluted (1:50) into fresh LB broth supplemented with 0.1% glucose and incubated at 37°C with aeration. Samples were taken at times indicated to determine values for OD₅₅₀ and survival following UV radiation exposure at 25 J m⁻². Cells were irradiated on tryptone plates. Survival data are means $\pm$ SD from triplicate experiments. Since OD₅₅₀ values were nearly identical for all four strains, only average values are shown.

Fig. 3.

The *dinB883* allele sensitizes *uvrA* and *uvrB* strains to UV radiation. Survival data are shown for logarithmic phase *E. coli* *uvrA* (SR4172, ●), *uvrA* *dinB883* (SR4173, ○), *uvrB* (SR4175, ▼) and *uvrB* *dinB883* (SR4177, △) strains UV-irradiated in buffer. Data are means ± SD from triplicate experiments. The survival curves for *dinB883* strains are significantly different from those for paired *dinB*⁺ strains over the range of 9-15 J m⁻² ($P < 0.05$).

Fig. 4.

The *dinB883* allele does not sensitize a *recA* strain to UV radiation. Survival data are shown for logarithmic phase *E. coli recA* (SR4178, ●) and *recA dinB883* (SR4179, ○) strains UV-irradiated in buffer. Data are means $\pm$ SD from triplicate experiments. The two survival curves shown are not significantly different ($P > 0.05$).

Fig. 5.

The *dinB883* allele sensitizes an *umuC104* strain to UV radiation. Survival data are shown for logarithmic phase *E. coli umuC104 lexA51* (SR4385, ●) and *umuC104 lexA51 dinB883* (SR4391, ○) strains UV-irradiated in buffer. Data are means $\pm$ SD from triplicate experiments.

Fig. 6.

The *dinB883* allele uniquely has the ability to sensitize wild-type cells to UV radiation at 25 J m⁻². Survival data is shown for *E. coli* strains; *dinB*⁺ (SMR4562 and SR2227), *dinB882* (SMR6111), *dinB749* (JW0221-1) and *dinB883* (SR4109). Cells were prepared and UV-irradiated to determine survival as in Fig. 2. Data are means $\pm$ SD from 5-10 experiments per strain. Strain SR4109 is significantly more sensitive than all other strains ($P < 0.05$).

Common Amino Acid (AA) Sequence for Wild-type (*dinB*⁺), Δ *dinB883*, and Δ *dinB882* Strains

AA positions 1-54	AA position	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
	AA	M	R	K	I	I	H	V	D	M	D	C	F	F	A	A	V	E	M
	DNA codon	ATG	CGT	AAA	ATC	ATT	CAT	GTG	GAT	ATG	GAC	TGC	TTT	TTC	GCC	GCA	GTG	GAG	ATG
	AA position	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36
	AA	R	D	N	P	A	L	R	D	I	P	I	A	I	G	G	S	R	E
	DNA codon	CGC	GAC	AAT	CCC	GCC	CTG	CGC	GAT	ATC	CCT	ATT	GCT	ATT	GGC	GGC	AGC	CGC	GAA
AA positions 37-54	AA position	37	38	39	40	41	42	43	44	45	46	47	48	49	50	51	52	53	54
	AA	R	R	G	V	I	S	T	A	N	Y	P	A	R	K	F	G	V	R
	DNA codon	CGT	CGG	GGG	GTG	ATC	AGC	ACC	GCC	AAT	TAT	CCC	GCG	CGT	AAA	TTT	GGC	GTA	CGT/A

Unique AA Sequences for Wild-type (*dinB*⁺), Δ *dinB883*, and Δ *dinB882* Strains

AA positions 55-69	<i>dinB</i> ⁺	AA: <i>dinB</i> ⁺ ≠ Δ <i>dinB883</i> or Δ <i>dinB882</i>																	
	AA position	55	56	57	58	59	60	61	62	63	64	65	66	67	68	69			
	AA	S	A	M	P	T	G	M	A	L	Y	L	C	P	H	L			
	DNA codon	AGC	GCT	ATG	CCG	ACA	GGG	ATG	GCC	CTC	AAA	TTA	TGC	CCA	CAT	CTC			
	Δ <i>dinB883</i>	AA: Δ <i>dinB883</i> = Δ <i>dinB882</i>									AA: Δ <i>dinB883</i> ≠ Δ <i>dinB882</i>								
	AA position	55	56	57	58	59	60	61	62	63	64	65	66	67	68	69			
AA positions 70-84	AA	P	A	G	G	G	G	K	A	T	L	C	L	K	I	S			
	DNA codon	CCT	GCA	GGG	GGG	GGG	GGG	AAA	GCC	ACG	TTG	TGT	CTC	AAA	ATC	TCT			
	Δ <i>dinB882</i>	AA: Δ <i>dinB882</i> = Δ <i>dinB883</i>									AA: Δ <i>dinB882</i> ≠ Δ <i>dinB883</i>								
	AA position	55	56	57	58	59	60	61	62	63	64	65	66	67	68	69			
	AA	P	A	G	G	G	G	K	P	R	C	V	S	K	S	L			
	DNA codon	CCT	GCA	GGG	GGG	GGG	GGA	AAG	CCA	CGT	TGT	GTC	TCA	AAA	TCT	CTG			
AA positions 70-84	<i>dinB</i> ⁺	AA: <i>dinB</i> ⁺ ≠ Δ <i>dinB883</i> or Δ <i>dinB882</i>																	
	AA position	70	71	72	73	74	75	76	77	78	79	80	81	82	83	84			
	AA	T	L	L	P	G	R	F	D	A	Y	K	E	A	S	N			
	DNA codon	ACC	TTG	CTT	CCG	GGG	CGC	TTT	GAC	GCC	TAC	AAA	GAA	GCC	TCA	AAT			
	Δ <i>dinB883</i>	AA: Δ <i>dinB883</i> ≠ Δ <i>dinB882</i>																	
	AA position	70	71	72	73	74	75	76	77	78	79	80	81	82	83	84			
	AA	D	V	T	L	H	K	I	K	I	Y	H	H	E	Q	STOP			
	DNA codon	GAT	GTT	ACA	TTG	CAC	AAG	ATA	AAA	ATA	TAT	CAT	CAT	GAA	CAA	TAA			
	Δ <i>dinB882</i>	AA: Δ <i>dinB882</i> ≠ Δ <i>dinB883</i>																	
	AA position	70	71	72	73	74	75	76											
	AA	M	L	H	C	T	R	STOP											
	DNA codon	ATG	TTA	CAT	TGC	ACA	AGA	TAA											

Fig. 7.

DNA and amino acid (AA) sequences for wild-type (*dinB*⁺) and *dinB* mutant strains. DNA and predicted fusion polypeptide sequences are shown for *dinB*⁺ (wild-type, SR2227, not UV radiation sensitive), *dinB883* (SR4109, significantly UV radiation sensitive), and *dinB882* (SMR6111, not UV radiation sensitive) strains. The nucleotide sequences are identical for the three strains for the first 53 codons. Codon 54 differs in the *dinB883* and *dinB882* strains from the *dinB*⁺ DNA sequence but the AA remains the same. After codon 54 the *dinB*⁺ DNA sequence and resulting AAs differs from those of *dinB883* and *dinB882*. From codon positions 55 to 61, the AA sequences are identical for *dinB883* and *dinB882* as are the DNA sequences except for codon 61. After codon 61 the DNA and AA sequences both differ in the two *dinB* strains. The nucleotides of the *kan*^r insertion fragment fused to *dinB* sequence begin opposite AA position 54 (3rd position), but the AA sequence is not affected until AA position 55.

Table 1

Escherichia coli K-12 strains used in this study.

Strains	Genotype	Source or derivation
BW25113	(<i>araD-araB</i>)567 <i>lacZ</i> 4787(:: <i>rrnB</i> -3) <i>rph</i> -1 (<i>rhaD-rhaB</i>)568 <i>hsdR</i> 514 F ⁻ ! ⁻	Coli Genetic Stock Center
AB1157	<i>argE</i> 3(oc) <i>hisG</i> 4(oc) <i>leuB</i> 6(amIII) (<i>gpt-proA</i>)62 <i>thr</i> -1 <i>thi</i> -1 <i>ara</i> -14 <i>galK</i> 2 <i>lacY</i> 1 <i>mtl</i> -1 <i>xyl</i> -5 <i>tsx</i> -33 <i>rfbD</i> 1 <i>mgl</i> -51 <i>rpsL</i> 31 <i>supE</i> 44(amSulI) <i>rac</i> F ⁻ ! ⁻	Laboratory stock
EST945	As AB1157, but: (<i>recA-srl</i>)306 <i>srlR</i> 301::Tn10 λ(<i>recA</i> ⁺)	E Tessman
JW0221-1	As BW25113, but <i>dinB</i> 749::kan [referred to as <i>dinB</i> 749]	Coli Genetic Stock Center
RW82	<i>umuDC</i> 595::cat <i>uvrA</i> 6 <i>hisG</i> 4(oc) <i>ilv</i> 323ts <i>leuB</i> 6(amIII) (<i>gpt-proA</i>)62 <i>thr</i> -1 <i>thi</i> -1 <i>araD</i> 14 <i>galK</i> 2(oc) <i>lacY</i> 1 <i>mtl</i> -1 <i>xyl</i> -5 <i>tsx</i> -33 <i>kdgK</i> 51 <i>rfbD</i> 1 <i>rpsL</i> 31 <i>supE</i> 44(amSulI)	[34]
SMR4562	(<i>lac-proAB</i>) _{XIII} <i>thi</i> ara Rif ^r [F ⁺ <i>proAB</i> ⁺ <i>lacI</i> 33! <i>lacZ</i>]	SM Rosenberg
SMR6111	As SM4562, but: [F ⁺ (<i>dinB-yafN</i>)882(::kan) <i>proAB</i> ⁺ <i>lacI</i> 33! <i>lacZ</i>] (<i>dinB-yafN</i>)882(::kan) [referred to as <i>dinB</i> 882]	SM Rosenberg
SR2227	As AB1157, but: <i>trpA</i> 78 <i>rpsE</i> (<i>gpt-proA</i>) ⁺ <i>rpsL</i> ⁺	Laboratory stock
SR2626	<i>uvrB</i> 5 <i>gal</i> ::Tn10 <i>leuB</i> 19 <i>metE</i> 70 <i>thyA</i> 36 <i>deoC</i> 2? <i>lacZ</i> 53 <i>rha</i> -5 <i>rpsL</i> 151 IN(<i>rrnD-rrnE</i>) F ⁻ λ ⁻	Laboratory stock
SR4109	As SR2227, but: (<i>dinB-yafN</i>)883(::kan) [referred to as <i>dinB</i> 883]	SR2227 × P1vir■YG7207, Km ^r
SR4128	As SR2227, but: <i>umuDC</i> 595::cat [Referred to as <i>umuDC</i>]	SR2227 × P1■RW82, Cm ^r
SR4129	As SR2227, but: <i>dinB</i> 883 <i>umuDC</i>	SR4109 × P1■RW82, Cm ^r
SR4172	As SR2227, but: <i>uvrA</i> ::Tn10	SR2227 × P1vir■SY55, Tc ^r
SR4173	As SR2227, but: <i>uvrA</i> ::Tn10 <i>dinB</i> 883	SR4109 × P1vir■SY55, Tc ^r
SR4175	As SR2227, but: <i>uvrB</i> 5 <i>gal</i> ::Tn10	SR2227 × P1vir■SR2626, Tc ^r
SR4177	As SR2227, but: <i>uvrB</i> 5 <i>gal</i> ::Tn10 <i>dinB</i> 883	SR4109 × P1vir■SR2626, Tc ^r
SR4178	As SR2227, but: (<i>recA-srl</i>)306 <i>srlR</i> 301::Tn10	SR2227 × P1vir■EST945, Tc ^r
SR4179	As SR2227, but: (<i>recA-srl</i>)306 <i>srlR</i> 301::Tn10 <i>dinB</i> 883	SR4109 × P1vir■EST945, Tc ^r
SR4385	<i>sulA</i> 211 <i>thi</i> -1 (<i>lac-gpt</i>)5 <i>ilv</i> (Ts) <i>mtl</i> -1 <i>rpsL</i> 31 <i>lexA</i> 51(Def) <i>umuC</i> 104	DE1868, R Woodgate
SSR4391	As SR4385, but <i>dinB</i> 883	SR4385 × P1vir■SR4109, Km ^r
SY55	<i>uvrA</i> ::Tn10	BA Bridges
YG7207	As AB1157, but: (<i>dinB-yafN</i>)::kan	[31]

P1vir indicates strain construction by transduction; *cat*, *kan* or Tn10 indicate genes conferring resistance to chloramphenicol (Cm^r), kanamycin (Km^r) or tetracycline (Tc^r), respectively; other genetic nomenclature have been defined [43]. To simplify discussion, we asked the Coli Genetic Stock Center to assign allele numbers for two of the (*dinB*) mutations discussed in this work. The following designations were assigned as requested: (*dinB-yafN*)882(::kan) for strain SMR6111 and (*dinB-yafN*)883(::kan) for strain SR4109, and its derivatives. Abbreviated terminology for these alleles is defined in this table.

Table 2

Growth phase dependence of UV radiation sensitization due presence of *dinB883* and *umuDC* mutations.

Culture incubation times (min)	Culture OD ₅₅₀ (units)	Relative UV radiation survival for wild type (WT)/mutant strain, mean $\pm$ SD (No. of values averaged)		
		WT/ <i>dinB883</i>	WT/ <i>umuDC</i>	WT/ <i>dinB883 umuDC</i>
60-240	<1.1	167 $\pm$ 57 (4)	334 $\pm$ 125 (4)	386 $\pm$ 186 (4)
300	1.4	20 (1)	71 (1)	104 (1)
360-640	~3	6.4 $\pm$ 2.0 (5)	34 $\pm$ 21 (5)	48 $\pm$ 23 (5)
1102-1230	~5	1.7 $\pm$ 0.3 (2)	11 $\pm$ 3 (2)	7 $\pm$ 1 (2)

Data are derived from Fig. 2. UV radiation survival for the wild-type strain (SR2227) divided by survival for the mutant strain SR4109 (*dinB883*), SR4128 (*umuDC*), or SR4129 (*dinB883 umuDC*) at the same time and averaged for the listed sample times.