Research Article

ImuB and ImuC Contribute to UV-Induced Mutagenesis as Part of the SOS Regulon in Pseudomonas aeruginosa

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DNA damage-induced mutagenesis is a process governed by the SOS system that requires the activity of specialized DNA polymerases. These polymerases, which are devoid of proof-reading activity, serve to increase the probability of survival under stressful conditions in exchange for an error-prone DNA synthesis. As an opportunistic pathogen of humans, Pseudomonas aeruginosa employs adaptive responses that originally evolved for survival in many diverse and often stressful environmental conditions, where the action of error-prone DNA polymerases may be crucial. In this study, we have investigated the role of the polymerases ImuB and ImuC in P. aeruginosa DNA-damage induced mutagenesis. UV irradiation of imuB- and imuC-deletion mutants showed that both genes contribute to UV-induced mutagenesis in this bacterium. Furthermore, we confirmed that UV treatment significantly increase the expression levels of the imuB and imuC genes and that they are co-transcribed as a single transcriptional unit under the control of LexA as part of the SOS regulon in P. aeruginosa. Environ. Mol. Mutagen. 60:594–601, 2019. © 2019 Wiley Periodicals, Inc.

Key words: error-prone DNA polymerases; imuB; imuC; induced mutagenesis; Pseudomonas aeruginosa

INTRODUCTION

DNA damage-induced mutagenesis is, in a major extent, an active process that requires specialized DNA polymerases able to perform translesion synthesis (TLS). These DNA polymerases, which belong to the Y family of DNA polymerases and to a subfamily of the C family, diverge from the typical replicative DNA Pols I and III in their low fidelity and processivity due to the lack of 3’→5’ proof-reading exonuclease activity (Goodman and Woodgate 2013). In bacteria, the expression of these specialized polymerases is usually governed by the SOS system, a stress-inducible response that is activated when cells need to be rescued from severe DNA damage (Goodman and Woodgate 2013). Moreover, it has been reported that the induction of these polymerases increase the probability of survival under stressful conditions in exchange for an error-prone DNA synthesis, which bestowed them the name of “mutagenic polymerases.”

Pols IV and V are the best-known bacterial Y family-DNA polymerases and they had been well characterized in Escherichia coli (Tang et al. 1999; Wagner et al. 1999; Fuchs et al. 2004). These proteins are encoded by genes dinB and umuDC, respectively, and while dinB and its homologs have been identified in all three domains of life, the umuDC genes are confined to a group of prokaryotes (Erill et al. 2006; McHenry 2011). Many bacterial species where Pol V is absent, contain a SOS-responsive cassette composed of four genes, lexA2-imuA-imuB-imuC, which is broadly distributed among Proteobacteria, frequently in the form of incomplete versions containing two or three of its genetic components (Erill et al. 2006; McHenry 2011). The

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first gene, \textit{lexA2}, corresponds to a second copy of the SOS-repressor \textit{lexA} and the second gene, \textit{imuA}, encodes a member of the \textit{recA}superfamily of still unknown function. The other two genes, \textit{imuB} and \textit{imuC} (also referred to as \textit{dnaE2}), encode a Y-family DNA polymerase and a C-family homolog of the alpha subunit of DNA Pol III, respectively (McHenry 2011). Previous studies showed that the biological effects for ImuB and ImuC on mutagenesis can vary among bacterial species. In \textit{Streptomyces}, ImuC was reported to be an error-prone DNA polymerase, involved in damage-inducible TLS (Tsai et al. 2012). In \textit{Myxococcus xanthus} the overexpression of ImuC is able to increase the mutation rate and cause phenotypic deficiencies in development and sporulation (Peng et al. 2017). In \textit{Caulobacter crescentus} and \textit{Mycobacterium tuberculosis}, both proteins act cooperatively in the mitomycin-C- and UV-dependent mutagenic response (Boshoff et al. 2003; Galhardo et al. 2005). Importantly, it has been observed that ImuB lacks the triad of catalytic amino acids, which is conserved in its Y-family polymerase homologs, which means that it is most likely unable to polymerase DNA (Warner et al. 2010). Instead, it has been shown that ImuB bridges the interaction between ImuC and the \( \beta \)-clamp, facilitating its access to the replication fork, which as a consequence implies an interdependent activity of both proteins (Warner et al. 2010). However, this cooperative activity seems not to be an absolute rule. For instance, it has been reported that in \textit{Pseudomonas putida} these two TLS factors have antagonistic effects on stationary-phase and UV-mutagenesis, where ImuB and ImuC seem to play a mutator and an antimutator role, respectively (Koorits et al. 2007).

Error-prone DNA replication or incorrect repair of base-pair mismatches may contribute directly to the unexpectedly high acquisition rates of drug resistance during chronic infections (Oliver et al. 2000; Björkholm et al. 2001; Martina et al. 2014). One of the most clear examples of such process is the genetic adaptation that \textit{Pseudomonas aeruginosa} undergoes during respiratory chronic infections in cystic fibrosis (CF) patients (Oliver et al. 2000; Mena et al. 2008; Feliziani et al. 2010; Feliziani et al. 2014). Once established, \textit{P. aeruginosa} can remain persistently associated with its host for decades due to the emergence of different phenotypes that are adapted to the CF lung environment. In this sense, factors which are able to alter the mutation rate such as the activity of mutagenic polymerases, may play an important role in this adaptive process. Inspection of the \textit{P. aeruginosa} genome reveals the presence of a \textit{dinB}-encoded Pol IV and also of a three-gene cassette \textit{imuA-imuB-imuC} (Abella et al. 2004), Pol IV being the best characterized. It has been established \textit{in vitro} that Pol IV constitutes a LexA-dependent error-prone DNA polymerase (Sanders et al. 2006), being involved in DNA oxidative and alkylated induced mutagenesis (Jatsenko et al. 2017) and in the acquisition of prototypic CF adaptive phenotypes such as mucoidy and antibiotic resistance (Moyano et al. 2007). However, data about the role of \textit{imuA-imuB-imuC} cassette in \textit{P. aeruginosa} mutagenesis is still incipient and speckled in the literature. ImuC contributes to DNA alkylation (Jatsenko et al. 2017) and UV-induced mutagenesis (Sanders et al. 2006), and the transcription of both, \textit{imuB} and \textit{imuC}, was shown to be unevenly increased upon exposure of \textit{P. aeruginosa} to the SOS-inducing antibiotic ciprofloxacin (Cirz et al. 2006). In this study, we have investigated the role of ImuB and ImuC in \textit{P. aeruginosa} DNA damage induced mutagenesis. We show that both genes contribute to UV-induced mutagenesis in this bacterium, being expressed as part of a single operon regulated by LexA. The results presented here contribute to the small but growing literature investigating mutagenic DNA polymerases in \textit{P. aeruginosa}.

**MATERIALS AND METHODS**

**Bacterial Strains and Media**

Strains, plasmids, and oligonucleotides used in this study are described in Table I. \textit{P. aeruginosa} PA01 (Holloway et al. 1979) and its \textit{imuB} (PA01B) and \textit{imuC} (PA01C) mutant strains were kindly provided by Dr. Herbert Schweizer from University of Florida (Gainesville, FL). In order to generate PA01B and PA01C, internal fragments of the chromosomal \textit{imuB} (nucleotides 327-1051 in the coding sequence) and \textit{imuC} genes (nucleotides 370-2693 in the coding sequence) were replaced with the \textit{aacI} gentamicin-resistance gene, respectively, as previously described in Choi and Schweizer (2005). To get unmarked mutants, Flp-mediated excision of the gentamicin resistance cassette in PA01B and PA01C strains was performed using the pFLP2 plasmid as described by Choi and Schweizer (2005). PA01B strain was generated from PA01 by replacing an internal fragment of the \textit{dinB} gene with a \textit{Km\(^R\)} gene, as described previously (Moyano et al. 2007). \textit{P. aeruginosa} strains were routinely grown at 37°C in Luria-Bertani (LB) broth or agar as the growth medium.

**UV Mutagenesis Assay**

UV-induced mutagenesis assay was performed as previously described (Le Chatelier et al. 2004) with minor modifications. Briefly, cells of

**TABLE I. Strains and Oligonucleotides Used in This Study**

| Strains | Description | Reference |
|---------|-------------|-----------|
| PA01    | Wild type   | Holloway et al. 1979 |
| PA01B   | In-frame deletion mutant of \textit{imuB}; PA01 derivative | Provided by H. Schweizer |
| PA01C   | In-frame deletion mutant of \textit{imuC}; PA01 derivative | Provided by H. Schweizer |
| PA01B::\textit{dinB::Km\(^R\)} | \textit{P. aeruginosa} derivative | This study |
| PAO1L86 | \textit{lexA} G86 V. SOS-non-inducible | Boles and Singh 2008 |

**Primers**

| Primer | Description | Reference |
|--------|-------------|-----------|
| rpoB F | 5’-AATGCGGAGAACACAGTTCGG-3’ | This study |
| rpoB R | 5’-AAGCCTGGCCAGATGCGTGG-3’ | This study |
| imuC-RT-For | 5’-GTTGATGTCGCCTGCGCGTGG-3’ | This study |
| imuC-RT-Rev | 5’-CGACAGCGACGACGGCG-3’ | This study |
| imuB-RT-For | 5’-CAACCTGGCTCCACGGCAGATAC-3’ | This study |
| imuB-RT-Rev | 5’-GCCATCATCGCCTGGGACTAC-3’ | This study |
| rpoD-RT-For | 5’-TGATCTGGTTCGCGGACCTTG-3’ | This study |
| rpoD-RT-Rev | 5’-CTGCTGGCGAAACCCTGGA-3’ | This study |
| imuC-imuB F | 5’-TGTCGCGGAGCGAAGACA-3’ | This study |
| imuC-imuB R | 5’-GGTCGCGGAGCTACGGTGT-3’ | This study |
P. aeruginosa PAO1 strain as well as its imuB, imuC, and dinB deletion mutants were grown on LB medium to mid-exponential growth phase (OD600 = 0.4). Cells from 2.5 mL aliquots were pelleted by centrifugation and resuspended in 2.5 mL of minimal salts. Each aliquot was transferred to a petri dish (5 cm) and subjected to UV-C irradiation at 10 J/m² dose using a UV Crosslinker 500 (Hoefer, Inc, San Francisco, CA). After irradiation, bacteria were cultivated in LB for 1 hr at 37°C and 220 r.p.m to allow mutation fixation. UV-treated and control cultures (mock-irradiated aliquots) were submitted to serial dilutions and plated on LB agar to quantify total colony forming unit (CFU). Then, the whole culture volume was concentrated by centrifugation and plated in LB agar plates supplemented with 100 µg mL⁻¹ of rifampicin. Rifampicin-resistant (RifR) colonies were scored after 48 h of incubation at 37°C. UV-induced mutant frequencies were calculated by dividing the numbers of RifR mutants by the total number of CFU. At least 40 independent measurements were performed for each strain.

**Analysis of rpoB Sequence in RifR Cells**

rpoB gene was sequenced from independent RifR colonies isolated from UV-irradiated and nonirradiated cultures from PAO1, PAOIB, and PAOIC strains. The region corresponding to cluster I and II of rpoB gene was polymerase chain reaction (PCR) amplified using rpoB F and rpoB R primers (Table I). The PCR products were cleaned with a Gel Purification kit (ermoantas, Waltham, MA) and directly sequenced by the DNA Sequencing Service of the University of Chicago. To identify mutations in the rpoB gene, the sequences obtained from the RifR variants were compared with the rpoB PAO1 reference sequence (Stover et al. 2000) by CLUSTALW analysis (Larkin et al. 2007). For each strain, ~30 independent RifR colonies were analyzed.

**RNA Harvest and Semi Quantitative Reverse transcriptase PCR**

Reverse transcriptase PCR (RT-PCR) assays were carried out in order to investigate the expression of imuB and imuC genes in response to UV-induced DNA damage. P. aeruginosa PAO1, PAOIB, and PAOIC mid-exponential phase cultures (OD600 = 0.4) were irradiated with UV-C light as previously described. The P. aeruginosa lexA G86V mutant (PAOL86), a mutation that renders cells unable to induce the SOS response (Table I), was used as a negative control. Treated and untreated (PAO1L86), a mutation that renders cells unable to induce the SOS response (Table I), was used as a negative control. Treated and untreated cultures were used to extract total RNA using the RNA Purification Kit (Fermentas, Waltham, MA). RNA was quantified by UV spectrophotometry, and its integrity was checked by electrophoresis in 1.5% (w/v) agarose gels. Then, 1 µg of total RNA was reverse-transcribed using the QuantiTect Reverse Transcription Kit (QIAGEN, Hilden, Germany). PCR primers were manually designed with the assistance of the Netprimer software (PREMIER Biosoft International, Palo Alto, CA) and evaluated for their specificity with the BLAST program at the NCBI Web site. Specific transcripts were semi-quantitatively measured by RT-PCR using primers ImuB-RT-F and ImuB-RT-R (for imuB), ImuC-RT-F and ImuC-RT-R (for imuC). Transcripts of the rpoD gene were amplified with primers RpoD-RT-F and RpoD-RT-R and served as housekeeping controls. All primer sequences are described in Table I. The optimal number of cycles was determined in advance to evaluate expression in the exponential phase of amplification. Final cycling conditions included a hot start at 95°C for 2 min, followed by 28 cycles of 30 sec at 94°C, 30 sec at 56°C, 30 sec at 72°C, and a final extension cycle of 5 min at 72°C. Specificity was verified by agarose gel electrophoresis. Band intensities were then measured with the Gel-Pro Analyzer Software. For the calculation of fold change in gene expression, band intensities of imuB and imuC genes were previously normalized using band intensities of their respective rpoD housekeeping controls. No amplification was observed in PCR reactions containing non-reverse transcribed RNA as template.

**Characterization of imuB and imuC Transcriptional Organization**

Primers were designed to amplify PCR products containing regions of both, imuB and imuC genes, which share the same orientation and are located downstream from imuA. The cDNA to be used as a template for PCR was obtained by reverse transcription of purified total RNA as described above. Thus, primers imuC-ImuB-F and imuC-ImuB-R (Table I) were employed to determine the co-expression of genes imuB and imuC.

**Statistical Analysis**

Statistical significance in UV-induced mutagenesis assays within strains was evaluated using a nonparametric Wilcoxon matched-pairs signed rank test. P values less than or equal to 0.05 were considered statistically significant. The statistical significance of differences between RifR mutational spectra was determined by using the hypergeometric test algorithm previously described (Adams and Skopek 1987; Cariello et al. 1997). All P values were based on 30,000 iterations. A P-value of <0.05 means that the spectra are different in a pairwise comparison.

**RESULTS**

**ImuB and ImuC Contribute to UV-Induced Mutagenesis**

As mentioned, it has been previously shown that ImuC is involved in UV-induced mutagenesis in P. aeruginosa (Sanders et al. 2006). However, the role of ImuB in this process remains still unexplored. Thus, we examined the contribution of ImuB and ImuC in DNA-damage induced mutagenesis in P. aeruginosa. To do this, we performed UV mutagenesis assays using the P. aeruginosa PAO1 strain which expresses all five known P. aeruginosa polymerases, and its isogenic PAOIB and PAOIC strains carrying deletions in the imuB and imuC genes, respectively. As a control, we also included a dinB deficient strain (PAO1DB), which, according to the literature, is not involved in UV mutagenesis (Sanders et al. 2006). It is important to note that in-frame non-polar deletion of imuB did not affect the expression of imuC and vice versa, as the expression of the reciprocal transcripts was checked in each of the mutant strains by RT-PCR (Fig. S1). Table II shows the frequencies of RifR mutants for control and UV-induced cultures in all the analyzed strains. Following UV irradiation, PAO1 and PAO1DB displayed a significant increase in its mutant frequency, confirming that P. aeruginosa displays a modest UV-induced mutator phenotype and that DinB is not playing an important role in the generation of these mutants. However, this increase was not further observed in strains PAOIC and PAO1B as determined by their mutant frequency to Rif, indicating that both gene products participate and are necessary for UV-induced mutagenesis in this species. Evaluation of the effect of UV-treatment on cell viability showed ~30% of surviving cells after UV exposure, with no significant differences observed among the different strains (Fig. S2, P = 0.086). Furthermore, UV treatment did not produce any significant filamentation of dividing cells (Fig. S3), which is one potential problem of cells exposed to UV that may generate artifacts in CFU counts (Rudolph et al. 2007). Interestingly, PAOIB showed the highest mutant frequency among
mock-irradiated controls (Table II). However, further mutation fluctuation tests indicated that no significant differences were observed in spontaneous mutagenesis between PAO1 wild type and PAOIB (P = 0.23), PAOIC (P = 0.39), and PAOBD (P = 0.12) mutants (Fig. 1).

**UV-Induced Mutational Spectra in PAO1, PAOIB, and PAO1 Strains**

The rifampicin mutation assay detects point mutations in the *rpoB* gene, which encodes the β-subunit of bacterial RNA polymerase, and is very useful due to the high conservation of the target gene, allowing its utilization in a wide variety of bacterial species (Garibyan et al. 2003). So, we next analyzed the nature of UV-induced mutations in the *rpoB* gene of PAO1 and its isogenic deletion mutants PAOIB and PAOIC by obtaining randomly selected Rif<sup>R</sup> mutants from UV-irradiated cultures and further sequencing of their *rpoB* clusters (Material and Methods section). Rif<sup>R</sup> mutants from mock-irradiated cultures were used as controls to obtain spontaneous mutations in *rpoB*. Interestingly, there were no differences in the distribution of transitions and transversions observed after UV treatment in the different strains (hypergeometric test, pairwise comparisons among UV-treated samples for PAO1 and PAOBD (Wilcoxon matched-pairs signed rank test).

| Strains<sup>a</sup> | Rif<sup>R</sup> mutants/10<sup>6</sup> cells<sup>b</sup> | Ratio of UV-induced/spontaneous | Median of differences (P) |
|--------------------|---------------------------------|-------------------------------|--------------------------|
|                    | Control                        | UV-induced                    |                          |
| PAO1               | 1.76 ± 0.24                    | 4.9 ± 1.04                    | 2.78                     | 0.92 (<0.0001) |
| PAOIB              | 3.16 ± 0.42                    | 2.18 ± 0.42                   | 0.68                     | –0.50 (0.0509) |
| PAOIC              | 1.36 ± 0.20                    | 1.37 ± 0.26                   | 1.00                     | –0.59 (0.3157) |
| PAOBD              | 1.67 ± 0.65                    | 3.04 ± 0.68                   | 1.82                     | 1.93 (0.0273) |

Table II: Role of *imuB* and *imuC* in UV-Mutagenesis in *P. aeruginosa*

<sup>a</sup>*P. aeruginosa* strains are described in Table I.

<sup>b</sup>Induced mutation rates were determined after UV irradiation (10 J/m<sup>2</sup>) of mid-log phase cultures of *P. aeruginosa* PAO1 (wt), PAOIB (ΔimuB), PAOIC (ΔimuC) and PAOBD (ΔdinB) strains. Control mutation rates were determined in the same manner using mock non-irradiated cultures. Values presented for spontaneous and UV-induced Rif<sup>R</sup> mutant frequencies represent the mean from 40 independent experiments ± SEM. Statistically significant differences UV-treated and non-treated samples for PAO1 and PAOBD (Wilcoxon matched-pairs signed rank test).

**UV-Treatment Induces the Co-Expression of *imuB* and *imuC* Under the Control of *lexA* as Components of a Single Operon**

The genomic organization of *P. aeruginosa* *imuA* (PA0671), *imuB* (PA0670), and *imuC* (PA0669) genes indicate that they constitute an operon (Fig. 3) (operon id: 12177 (Mao et al. 2009)). We subsequently analyzed the transcriptional organization of *imuB* and *imuC* in order to confirm whether these genes behave as a single transcriptional unit, thereby constituting an operon. For that, we carried out PCRs using cDNA as template and primers designed to amplify fragments containing regions of the two neighboring genes which share the same orientation. As shown in Figure 3, we were able to amplify a fragment between *imuB* and *imuC* following UV irradiation suggesting that *imuB* and *imuC* were co-expressed. This result depicts a transcriptional organization of these genes structured in one polycistronic operon, whose expression is increased upon UV-induced DNA-damage.
and 4.5-fold increase in the expression levels of *imuB* and *imuC* transcripts, respectively (Fig. 4, *P* < 0.05), indicating that the stress induced by UV triggered a similar transcriptional response of both genes.

The presence of a LexA binding domain 137 bp upstream from the translational start codon of the *imuA* gene (Cirz et al. 2006) indicates that in *P. aeruginosa* the *imuABC* operon is probably part of the SOS regulon, as it has been described in other Proteobacteria (Abella et al. 2004). In fact, a recent study have shown that the LexA1 protein from *P. putida* is able to bind this regulatory motif in *P. aeruginosa* (Abella et al. 2004).

To confirm whether these genes form part of the SOS regulon, the expression of *imuB* and *imuC* were also analyzed in a *P. aeruginosa* lexA mutant strain (PAOL86) in which the SOS response is impaired (Table I). As shown in Figure 4, the UV-induction of *imuB* and *imuC* transcripts was drastically reduced in the PAOL86 strain, indicating that they are coordinately regulated by the SOS response. In contrast to the PAO1 wild type strain, the lexA mutant showed no significant differences in the transcript levels of *imuB* and *imuC* after UV treatment (Fig. 4, *P* < 0.05). These results confirm the prediction that both *imuB* and *imuC* are expressed in response to DNA damage and are controlled by the SOS regulon in *P. aeruginosa*.

**DISCUSSION**

Bacteria present alternative ways to face stress that can injure DNA. Among these, TLS is carried out by specialized DNA polymerases that allow cell survival at a cost of increasing mutagenesis (Goodman and Woodgate 2013). This mutagenesis may in turn serve as a supply of the genetic resources needed in the adaptation process to new or hostile environments (Rosenberg 2001; Foster 2007). In *E. coli*, this function is fulfilled by three well-known TLS DNA polymerases, Pol II (*polB*), Pol IV (*dinB*), and Pol V (*umuDC*). Particularly in the case of UV-induced DNA damage, Pol V but not Pol IV is able to bypass the UV-lesions with further error-prone synthesis (Kato and Shinoura 1977). Moreover, among *E. coli* TLS polymerases, only Pol V contributes to the survival of UV-irradiated cells (Courcelle et al. 2004). Consistent with this, and with the findings of Sanders et al. (2006), we evaluated a PAO1 *dinB*-deficient mutant and confirmed that Pol IV is not involved in UV-induced mutagenesis in *P. aeruginosa*. Instead, in *P. aeruginosa*, the role of Pol V seems to be replaced by a “mutagenic cassette,” generally constituted by the *imuA-imuB-imuC* genes, which is widely distributed among Proteobacteria (Abella et al. 2004). However, the role of these TLS-associated enzymes in this bacterial species is still poorly known. In order to get insights on their contribution to the mechanisms of adaptive mutagenesis in *P. aeruginosa*, we constructed deletion mutants of the *imuB* and *imuC* genes and evaluated their role in UV-induced mutagenesis. The results presented here suggest that ImuC and ImuB are responsible for most of the induced mutagenesis in...
P. aeruginosa upon UV light exposure, functioning as components of the SOS regulon under the control of the master regulator LexA.

It has been reported that ImuB and ImuC act cooperatively during TLS in several bacterial species (Boshoff et al. 2003; Le Chatelier et al. 2004; Galhardo et al. 2005; Warner et al. 2010; Alves et al. 2017). Based on sequence analyses, Warner et al. (2010) suggested that ImuB is devoid of DNA polymerase activity because it lacks the highly conserved residues of the active site required to polymerize DNA. Instead, ImuB has a conserved peptide sequence motif able to interact with the β-clamp and mediate the access of the bona fide polymerase ImuC to the replication fork (Warner et al. 2010). On the other hand, a study in P. putida described antagonistic functions between ImuB and ImuC during stationary-phase and UV-induced mutagenesis: while ImuB shows an error-prone activity, ImuC seems to play an antimutator role (Koorits et al. 2007). Thus, both factors may probably fulfill different roles in different bacterial species. Particularly in P. aeruginosa, ImuC has been reported to be involved in UV-induced mutagenesis (Sanders et al. 2006) and in coping with DNA alkylation damage, along with ImuA and ImuB (Jatsenko et al. 2017). Consistent with previous reports, our observations indicate that ImuC significantly contribute to the UV-induced mutator phenotype in P. aeruginosa. However, the role of the putative polymerase ImuB, which is part of the same polycistrionic operon than ImuC, remained still unexplored. Here we demonstrate that ImuB plays an essential role in UV-induced mutagenesis in P. aeruginosa. Importantly, it has been described that the P. aeruginosa ImuB harbors a β-clamp binding domain but lacks the active site to polymerize DNA which is highly conserved in the Y-family of DNA polymerases (Warner et al. 2010). Although we have not evaluated any physical interaction between ImuB and ImuC, or with the β-clamp, our results in P. aeruginosa fit well with an interdependency of roles for ImuB and ImuC, as it was observed in other bacteria. Thus, the P. aeruginosa’s mutasome complex resembles that proposed for M. tuberculosis in which ImuB seems to constitute an accessory factor functioning as a nexus between ImuC and the β-clamp. The fact that ImuB and ImuC form part of an operon which has been widely spread by horizontal transfer (Abella et al. 2004) opens the question of whether this reported “bridge” role of ImuB is limited to its operon partner ImuC or is, in turn, able to interact with other DNA polymerases.

Because of its contribution to pathogenesis, understanding the different mechanisms of adaptive mutagenesis in P. aeruginosa is important and timely. Our results constitute a new step to comprehend the adaptive repertory of P. aeruginosa by positioning ImuB and ImuC as relevant contributors to thrive in stressful environments.

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AUTHOR CONTRIBUTIONS

A.M.L. and A.M.S conceived the study. A.M.L., A.J.M and A.M.S designed the experiments, supervised the study and wrote the manuscript. A.M.L., A.J.M., R.A.M., S.F. and
M.U. performed the experiments. All authors discussed, edited, and agreed on the final manuscript.

CONFLICT OF INTEREST

The authors declare no competing interests.

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